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CIÊNCIAS CARDIOVASCULARES

Role of Subclinical Endocrine Dysfunction in Cardiovascular Risk and Cardiac Function: Implications of Modulation of Thyroid Function and Glucose Homeostasis

Papel da Disfunção Endócrina Subclínica no Risco Cardiovascular e Função Cardíaca: Implicações da Modulação da Função Tiroideia e Homeostasia Glicémica

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**Role of subclinical endocrine dysfunction in cardiovascular risk
and cardiac function: implications of modulation of thyroid
function and glucose homeostasis**

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função cardíaca: implicações da modulação da função tiroideia e
homeostasia glicémica**

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FOREWORD

This thesis is organized in six main chapters:

Chapter 1 includes a general introduction to the role of endocrine dysfunction on cardiovascular risk, with an overview of the effects of thyroid hormones on the cardiovascular system, of the association of prediabetes with cardiovascular risk and of the role of incretins in cardiovascular risk modulation. In this chapter, an introduction to the concept of residual risk of cardiovascular dysfunction and on the potential contribution of subclinical endocrine dysfunction to the residual risk is also presented. Finally, the potential role of the modulation of endocrine function on cardiovascular risk is discussed.

Chapter 2 defines the main objectives of the thesis.

Chapter 3 describes the results, shown in the form of published or submitted articles. These manuscripts have been grouped into three subchapters: A) Thyroid function, cardiovascular risk, and cardiac function; B) Prediabetes and cardiovascular risk; C) Cardiovascular effects of GLP-1 receptor activation.

Chapter 4 provides a global overview and discussion of the major findings, how they integrate with previously published studies, possible implications to clinical practice and future areas of research. Three review articles that discuss in detail the clinical relevance and implications of the findings of this thesis are included in this section.

Chapter 5 presents the main conclusions of this thesis.

Chapter 6 includes the references used in the introduction and discussion.

ABSTRACT

RESUMO

Resumo

Introdução: As doenças endócrinas, particularmente os distúrbios da função tiroideia e da homeostasia glicémica, são altamente prevalentes na população geral. Embora as associações do hipotireoidismo/hipertireoidismo e da diabetes com o risco cardiovascular estejam bem caracterizadas, o papel da variação da função tiroideia dentro do intervalo de normalidade e dos níveis de glicemia na faixa de pré-diabetes é ainda incerto. Apesar do progresso relevante nas últimas décadas na prevenção de eventos cardiovasculares fatais e não-fatais, as doenças cardiovasculares continuam a ser a principal causa de morte a nível global, sendo necessário o desenvolvimento de novas abordagens para reduzir o risco cardiovascular residual. Entre os fatores que contribuem para este risco residual, a disfunção endócrina subclínica pode ter um papel importante no desenvolvimento de doença cardiovascular aterosclerótica e insuficiência cardíaca.

Objetivos: Avaliar o papel da disfunção endócrina subclínica no risco cardiovascular e na função cardíaca, e avaliar as implicações clínicas da modulação da função tiroideia e da homeostasia glicémica.

População e Métodos: Neste projeto, foram utilizadas múltiplas populações e abordagens metodológicas: A) Para a avaliação dos efeitos da função tiroideia no risco cardiovascular e na função cardíaca analisamos: 1) participantes do estudo EPIPorto com idade igual ou superior a 45 anos, representativos da população geral da cidade do Porto; 2) doentes com obesidade mórbida acompanhados em consulta de Endocrinologia do Centro Hospitalar Universitário de São João; 3) participantes do estudo NHANES (*National Health and Nutrition Examination Survey*) representativos da população adulta dos Estados Unidos da América; e 4) modelo animal de insuficiência cardíaca com fração de ejeção preservada (HFpEF) (rato ZSF1 obesos). B) Para a caracterização da associação entre pré-diabetes e o risco cardiovascular analisamos: 5) participantes do estudo SPRINT (*The Systolic Blood Pressure Intervention Trial*), que incluiu indivíduos com mais de 60 anos e alto risco

cardiovascular; e 6) participantes do estudo CRIC (*Chronic Renal Insufficiency Cohort*), que incluiu doentes com doença renal crónica com 21-74 anos com um amplo espectro de gravidade da doença renal. C) Para a avaliação dos efeitos da ativação do recetor GLP-1, analisamos: 7) participantes do ensaio clínico Harmony Outcomes (*Albiglutide and cardiovascular results in patients with type 2 diabetes and cardiovascular disease*), que incluiu doentes com diabetes tipo 2 e doença cardiovascular aterosclerótica estabelecida; 8) participantes do estudo FIGHT (*Functional Impact of GLP-1 for Heart Failure Treatment*), que incluiu doentes com insuficiência cardíaca com fração de ejeção reduzida recentemente hospitalizados; e 9) realizamos uma meta-análise de 7 ensaios clínicos aleatorizados que avaliaram os efeitos dos agonistas do recetor GLP-1 na diabetes tipo 2.

Principais resultados e discussão: Variações da função tiroideia dentro do intervalo de normalidade associaram-se significativamente ao risco cardiovascular e à função cardíaca. Indivíduos com índice de massa corporal superior, maior perímetro abdominal e níveis de proteína C-reativa superiores apresentaram níveis mais elevados de T3 livre e do rácio T3 livre / T4 livre. Uma menor conversão de T4 em T3 associou-se a maior prevalência de diabetes, a perfil lipídico mais adverso, a taxa de filtração glomerular mais baixa e a um aumento do risco de eventos cardiovasculares. Um aumento da função tiroideia dentro do intervalo de normalidade associou-se a frequência cardíaca mais elevada, pressão arterial diastólica mais baixa e volumes do ventrículo esquerdo superiores. A espessura da parede do ventrículo esquerdo e a fração de ejeção apresentaram uma associação em forma de U com os níveis das hormonas tiroideias. Descrevemos também uma importante interligação entre a função tiroideia e a regulação do peso: níveis mais altos de T3 livre antes da cirurgia bariátrica associaram-se a maior perda de peso após a cirurgia, e a diminuição dos níveis de TSH após a cirurgia bariátrica correlacionaram-se com a perda de peso pós-operatória. No estudo NHANES, mostramos que níveis mais baixos de T3 livre dentro do intervalo de normalidade associaram-se a maior mortalidade cardiovascular. Por fim, no modelo animal de HFpEF demonstramos que a suplementação com baixa dose de T3 melhorou a função

metabólica, a função cardíaca diastólica e sistólica, e a regulação intracelular de cálcio no miocárdio. Em relação à relevância da homeostasia glicémica para o risco cardiovascular e função cardíaca, mostramos que a pré-diabetes (particularmente quando definida pela HbA1c) se associou a um risco aumentado de eventos cardiovasculares e a maior progressão da proteinúria. Caracterizamos também os efeitos cardiovasculares da ativação do recetor GLP-1. No estudo Harmony Outcomes e na meta-análise com o AMPLITUDE-O (*Effect of Efpeglenatide on Cardiovascular Outcomes*), demonstramos que os agonistas do recetor do GLP-1 reduzem o risco de eventos cardiovasculares ateroscleróticos independentemente do uso de inibidores SGLT2. Além disso, os resultados do estudo Harmony Outcomes, do estudo FIGHT e da meta-análise de ensaios clínicos aleatorizados com agonistas do recetor do GLP-1 demonstraram que o efeito dos agonistas do recetor do GLP-1 em eventos de insuficiência cardíaca é modulado pela função cardíaca basal. Em doentes sem insuficiência cardíaca, os agonistas do recetor GLP-1 previnem o desenvolvimento de insuficiência cardíaca; em doentes com diabetes tipo 2 e insuficiência cardíaca estável, os agonistas do recetor do GLP-1 têm um efeito neutro no risco de eventos de insuficiência cardíaca; e em doentes com insuficiência cardíaca com fração de ejeção reduzida, os agonistas do recetor do GLP-1 aumentam o risco de eventos adversos.

Conclusões: A disfunção endócrina subclínica deve ser considerada como um fator importante para a regulação da função cardíaca e do risco cardiovascular. A modulação da função tiroideia e da homeostasia da glicose são abordagens relevantes para melhorar o prognóstico cardiovascular. Esta modulação deve ter em conta a função tiroideia, o estado glicémico, e a existência de eventos cardiovasculares ateroscleróticos prévios ou de insuficiência cardíaca. Em doentes com doença cardiovascular ou alto risco de doença cardiovascular, a modulação da função tiroideia e da homeostasia da glicose poderá contribuir para melhorar a função cardíaca e reduzir o risco cardiovascular.

Abstract

Introduction: Endocrine diseases, particularly thyroid dysfunction and dysglycemia, are highly prevalent in the general population. While the association of hypothyroidism/hyperthyroidism and diabetes with the risk of cardiovascular diseases is well characterized, the role of variations of thyroid function within the euthyroid range and of plasma glucose levels within the range of prediabetes is still unclear. Despite the relevant progress in the last decades to prevent fatal and non-fatal cardiovascular events, cardiovascular diseases remain the leading cause of death globally and new approaches are needed to address the residual cardiovascular risk. Among the factors that contribute to this residual risk, subclinical endocrine dysfunction may have an important role in the development of atherosclerotic cardiovascular disease and heart failure.

Purpose: To evaluate the role of subclinical endocrine dysfunction in cardiovascular risk and cardiac function and to assess the clinical implications of modulation of thyroid function and glucose homeostasis.

Populations and Methods: For this project, multiple populations and approaches were used: A) For the evaluation of the effects of thyroid function on the cardiovascular risk and cardiac function, we evaluated: 1) participants of the EPIPorto study aged 45 years or older, representative of the general population of Porto, Portugal; 2) patients with morbid obesity followed in the outpatient setting at Centro Hospitalar Universitário de São João; 3) participants of the NHANES (National Health and Nutrition Examination Survey), representative of the adult population of the United States; 4) an animal model of cardiometabolic-induced heart failure with preserved ejection fraction (HFpEF) (ZSF1 obese rat). B) For the evaluation of the association of prediabetes with the cardiovascular risk we evaluated: 5) participants of the SPRINT (The Systolic Blood Pressure Intervention Trial),

which included individuals older than 60 years at high risk of cardiovascular events; and 6) participants of the CRIC (Chronic Renal Insufficiency Cohort Study), which included patients with chronic kidney disease aged 21 to 74 years with a broad spectrum of renal disease severity. C) For the evaluation of the effects of GLP-1 receptor activation we evaluated: 7) participants of the Harmony Outcomes (Albiglutide and cardiovascular outcomes in patients with type 2 diabetes and cardiovascular disease) trial, which included patients with type 2 diabetes and established atherosclerotic cardiovascular disease; 8) participants of the FIGHT (Functional Impact of GLP-1 for Heart Failure Treatment) trial, which included patients with Heart Failure with Reduced Ejection Fraction (HFrEF) and a recent hospitalization; and 9) data from 7 placebo-controlled randomized clinical trials of treatment with GLP-1 receptor agonists in patients with type 2 diabetes.

Main results and discussion: Variations of thyroid function within the euthyroid range were significantly associated with cardiovascular risk factors and cardiac function. Higher body mass index, larger waist circumference and higher levels of C-reactive protein were associated with higher levels of free T3 (FT3) and FT3/free T4 ratio. A decreased conversion of T4 to T3 was associated with a higher prevalence of diabetes, a more adverse lipid profile, a lower estimated glomerular filtration rate and an increased 10-year risk of cardiovascular events. Increasing thyroid function within the reference range was associated with a higher heart rate, a lower diastolic blood pressure, and larger left ventricle volumes. Left ventricle wall thickness and left ventricular ejection fraction had a U-shaped association with thyroid hormone levels. We also describe an important interconnection between thyroid function and body weight regulation: higher FT3 levels before bariatric surgery were associated with greater weight loss after surgery, and TSH decrease after bariatric surgery was correlated with post-operative weight loss. In the NHANES study, we showed that lower free triiodothyronine levels within the euthyroid range were associated with higher

cardiovascular mortality. Finally, in the animal model of HFpEF we demonstrated that supplementation with low dose of T3 improved metabolic function, cardiac diastolic and systolic function, and myocardial calcium handling. Regarding the relevance of glucose homeostasis to cardiovascular risk and cardiac function, we showed that prediabetes (particularly when defined by HbA1c levels) was associated with an increased risk of adverse cardiovascular events and progression of proteinuria. Furthermore, we characterized the cardiovascular effects of GLP-1 receptor activation. In the Harmony Outcomes and in a meta-analysis with the AMPLITUDE-O (Effect of Epeglenatide on Cardiovascular Outcomes) trial, we showed that GLP-1 receptor agonists reduced the risk of atherosclerotic cardiovascular events independently of SGLT2 inhibitors use. Furthermore, our results from the Harmony Outcomes, the FIGHT trial, and the meta-analysis of randomized clinical trials of GLP-1 receptor agonists in type 2 diabetes showed that the effect of GLP-1 receptor agonists on heart failure events depends on the baseline cardiac function. In patients without heart failure, GLP-1 receptor agonists prevent new-onset heart failure; in patients with type 2 diabetes and stable heart failure, GLP-1 receptor agonists have a neutral effect on the risk for heart failure events; and in patients with HFrEF, GLP-1 receptor agonists increase the risk of adverse events.

Conclusions: Subclinical endocrine dysfunction must be considered as an important factor that influences cardiac function and cardiovascular risk. Modulation of thyroid function and glucose homeostasis is a relevant approach to improve cardiovascular health. This modulation must consider thyroid function, glycemic status, previous atherosclerotic cardiovascular events, and heart failure status. Significant improvements in cardiovascular health may be achieved by considering and modulating thyroid function and glucose homeostasis in patients with cardiovascular disease or at high risk of cardiovascular events.

Chapter 1

INTRODUCTION



1. Endocrine dysfunction and cardiovascular risk

Endocrine diseases are highly prevalent in the general population.¹ Of the diseases that affect the endocrine system, type 2 diabetes and thyroid disorders are among the most relevant, given their significant impact on the quality of life and prognosis of people affected by them.² Diabetes affects 1 in 8 adults worldwide and its prevalence is still rising.³ Thyroid dysfunction affects about 7% of the general population, with up to 5% having hypothyroidism (0.5% overt hypothyroidism and 4.5% subclinical hypothyroidism) and up to 2% hyperthyroidism (0.5% overt hyperthyroidism and 1.5% subclinical hyperthyroidism).⁴ For both type 2 diabetes and thyroid dysfunction, the main cause of the increased risk of premature death is related to the development of cardiovascular diseases and fatal cardiovascular events.^{5,6}

People with type 2 diabetes have a high prevalence of hypertension and dyslipidemia.⁷ Furthermore, dysglycemia in type 2 diabetes promotes endothelial dysfunction, a procoagulant state and myocardial dysfunction.⁸ The presence of microvascular dysfunction, particularly nephropathy and autonomic neuropathy, further increases the risk of adverse cardiovascular events.^{9,10} In type 2 diabetes, the risk of myocardial infarction or stroke is two-fold increased compared with the general population and the risk of heart failure is two- to fourfold increased.¹¹⁻¹³ Given the high risk of adverse cardiovascular events, current guidelines recommend an intensive approach to cardiovascular risk reduction with intensive goals for cholesterol and blood pressure control, and prioritization of the use of antidiabetic drugs that have proven to reduce the risk of cardiovascular events (sodium-glucose cotransporter 2 [SGLT2] inhibitors or glucagon-like peptide-1 [GLP-1] receptor agonists).¹⁴

Both in hypothyroidism and hyperthyroidism, the risk of cardiovascular diseases is increased.^{15,16} Both hypothyroidism and hyperthyroidism are associated with an increased prevalence of hypertension.^{15,16} In the former, diastolic hypertension predominates, while in the latter systolic hypertension is more common. Also, thyroid hormones increase the expression of LDL receptors in the liver.¹⁷ As such, overt hypothyroidism is frequently associated with severe hypercholesterolemia, while hyperthyroidism may present with decreased LDL levels.¹⁸ Despite the lower LDL levels, patients with hyperthyroidism still have an increased risk of atherosclerotic events, which has been attributed, at least in part, to the prothrombotic effect of thyroid hormones.¹⁹ In hypothyroidism, compared with the general population, the risk of myocardial infarction is about 15% increased, the risk of heart failure is two- to threefold higher, and the cardiovascular mortality is up to twofold higher.^{20,21} For hyperthyroidism, the risk of atherosclerotic events is also 15% higher than in the general population and the risk of heart failure is up to threefold higher.^{20,22} Even in subclinical hypothyroidism and hyperthyroidism (defined as abnormal TSH levels with thyroid hormone levels within the reference range), an increased risk of adverse cardiovascular events is observed.^{15,16} The contribution of thyroid dysfunction to the risk of adverse cardiovascular events has led the current guidelines to recommend evaluating thyroid disorders in patients with heart failure or coronary disease.^{23,24}

Earlier diagnosis and treatment of endocrine dysfunction in people at risk of cardiovascular events has contributed to a significant decrease in the rate of fatal and non-fatal cardiovascular events in developed countries over the last decades.^{25,26} However, despite the progress made so far, cardiovascular diseases remain the leading cause of death globally,²⁷ and new approaches to address the residual cardiovascular risk are needed.

2. Residual risk of cardiovascular dysfunction and potential role of subclinical endocrine dysfunction

Residual risk of cardiovascular events is defined as the risk of adverse events that persists despite adequate treatment of classical risk factors such as dyslipidemia, hypertension and diabetes.²⁸ Among the factors that contribute to this residual risk, subclinical endocrine dysfunction may have an important role in the development of atherosclerotic cardiovascular disease and heart failure.

Despite the global investment in the earlier diagnosis and treatment of diabetes, prediabetes has been largely overlooked.²⁹ Prediabetes is estimated to occur in more than 1 in 5 adults in Europe,³⁰ but most patients with prediabetes are not aware of their diagnosis and receive less preventive interventions than patients diagnosed with diabetes.³¹ In a study from Denmark that evaluated more than 300 000 patients without previous diagnosis of prediabetes or diabetes, those with a HbA1c just below the diagnostic threshold for diabetes (HbA1c 46-47 mmol/mol, 6.4-6.5%) had a higher risk of cardiovascular events and all-cause mortality than those with HbA1c of 48-49 mmol/mol (6.5-6.6%).³² This was mainly explained by a lower prescription of antidiabetic, anti-hypertensive and lipid-lowering drugs to those with prediabetes just below the threshold for diabetes.

The risk of cardiovascular disease with thyroid dysfunction has been well-characterized for overt and subclinical hypothyroidism and hyperthyroidism.^{15,16} However, some studies have suggested that, even within the reference range, thyroid hormone levels are associated with the risk of adverse cardiovascular events.³³⁻³⁷ Furthermore, patients with cardiovascular diseases frequently have changes in thyroid hormone levels that are not primarily related to thyroid gland dysfunction but to the development of nonthyroidal illness (further detailed in the next section).³⁸ Whether these changes contribute to the risk of cardiovascular dysfunction is still unknown (Figure 1).

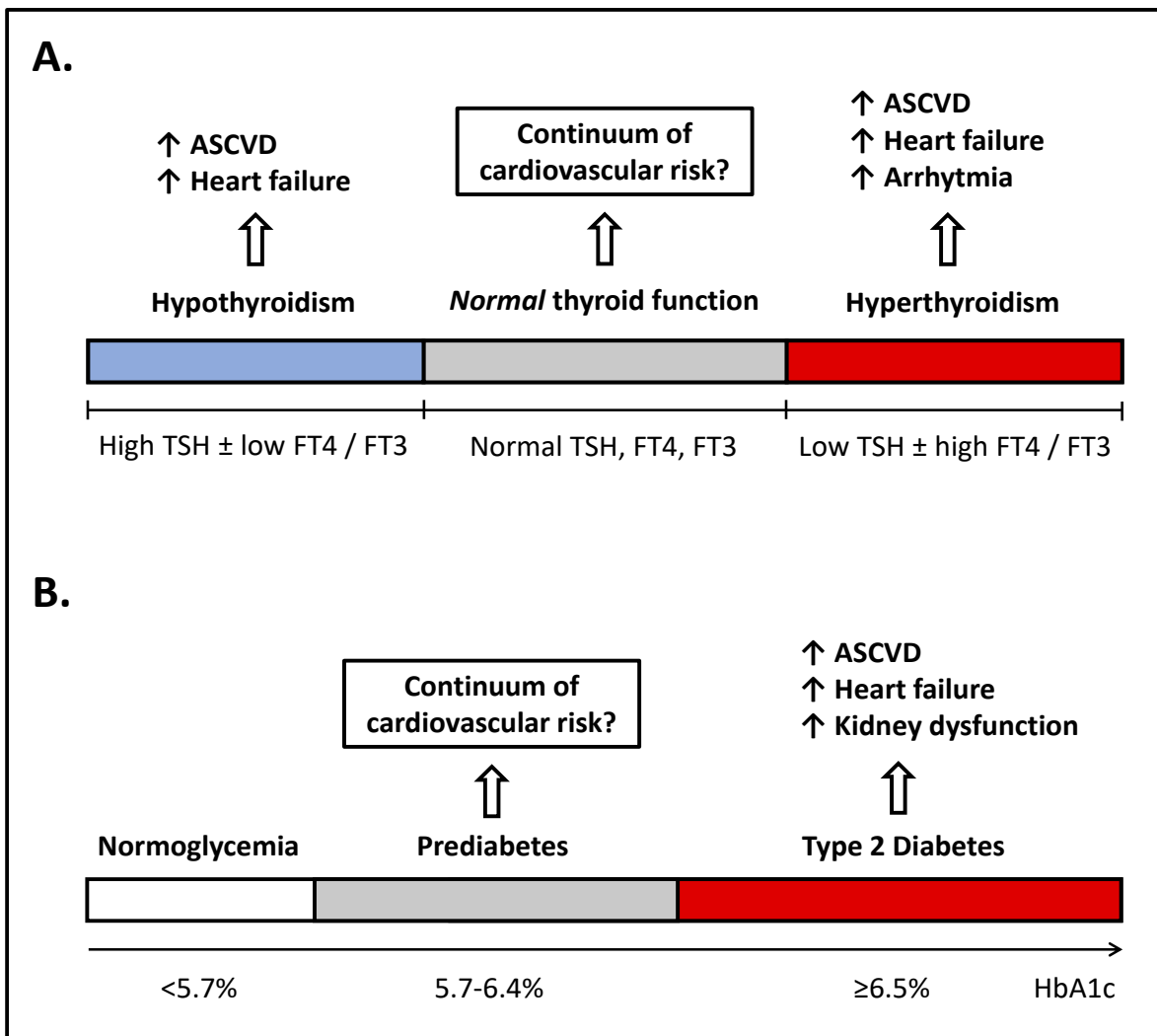


Figure 1. Risk of cardiovascular disease across the continuum of thyroid dysfunction (A) and glycemic homeostasis (B). ASCVD, atherosclerotic cardiovascular disease; TSH, thyroid

3. Thyroid function and cardiovascular system

Thyroid hormones modulate the function of all organs and systems.³⁹ The thyroid gland produces thyroxine (T4) and triiodothyronine (T3) that bind to intracellular receptors and modulate the expression of thousands of genes in virtually all human cells.⁴⁰ The production of T4 and T3 is regulated by a hypothalamus-pituitary-gland feedback loop, with thyrotropin-releasing hormone (TRH) being released from the hypothalamus, stimulating the pituitary to produce thyroid-stimulating hormone (TSH), which activates TSH receptors at thyroid level inducing the production and release of thyroid hormones.⁴¹

At the cardiovascular level, thyroid hormones modulate the activity of myocardial and endothelial cells through direct effects and through indirect effects mediated by activation of the sympathetic nervous system.⁴² Thyroid function also indirectly modulates the cardiovascular system by influencing systemic metabolic function.⁴³ Thyroid hormones are one the main modulators of energy intake and expenditure and, as such, an important regulator of body weight and of the risk of obesity and related metabolic dysfunction.⁴⁴

Hypothyroidism is a highly prevalent disease worldwide. In developed countries, the main cause of hypothyroidism is Hashimoto's thyroiditis.⁴⁵ Other causes of hypothyroidism include iodine deficiency, iatrogenic causes (e.g. thyroidectomy) and hypopituitarism.⁴⁶ At the cardiovascular level, the main pathophysiological effects of hypothyroidism include decreased myocardial contractility, decreased heart rate and increased vascular resistance due impaired endothelial-dependent vasodilation (Table 1).^{47,48} Hypothyroidism also promotes cardiac fibrosis and impairs myocardial relaxation.^{47,48} Diastolic dysfunction appears to be more severe than systolic dysfunction in patients with hypothyroidism.⁴⁷ Several studies have shown that hypothyroidism (both overt and subclinical) increases the risk of heart failure, coronary artery disease and cardiovascular death.^{20,21,49} The

cardiovascular dysfunction in this condition appear to be reversible with thyroid hormones supplementation.^{50,51}

The main causes of hyperthyroidism are Graves’ disease and autonomous nodular goiter.⁵² Hyperthyroidism may also be induced by drugs (e.g. amiodarone), thyroiditis (e.g. subacute thyroiditis) or exogenous thyroid hormone consumption (factitious or iatrogenic thyrotoxicosis).⁵² At the cardiovascular level, hyperthyroidism induces a high cardiac output state with increased heart rate and contractility, and decreased peripheral vascular resistance (Table 1).^{15,16} In short-term hyperthyroidism, thyroid hormones improve myocardial relaxation; however, sustained hyperthyroidism induces myocardial hypertrophy and diastolic dysfunction.^{15,53} Overt and subclinical hyperthyroidism increase the risk of arrhythmias (particularly atrial fibrillation), heart failure, myocardial infarction and cardiovascular death.^{20,22,54,55} As described for hypothyroidism, the adverse effects of hyperthyroidism on the cardiovascular system appear to be reversible with adequate treatment.⁵⁶

Table 1. Cardiovascular effects of hypothyroidism and hyperthyroidism	
Hypothyroidism (subclinical and overt)	Hyperthyroidism (subclinical and overt)
↓ Contractility	↑ Contractility
↓ Heart rate	↑ Heart rate
↓ Cardiac output	↑ Cardiac output
↑ Systemic vascular resistance	↓ Systemic vascular resistance
↑ Diastolic blood pressure	↑ Systolic blood pressure
Cardiac fibrosis	Cardiac hypertrophy
↑ Risk of heart failure	↑ Risk of heart failure
↑ Total and LDL cholesterol	↑ Arrhythmic risk

In patients with cardiovascular disease, thyroid hormone levels may be decreased, not only due to primary dysfunction of the hypothalamus-pituitary-thyroid gland axis, but also due to the development of nonthyroidal illness syndrome (also known as euthyroid sick syndrome or low T3 syndrome).³⁸ Nonthyroidal illness syndrome is defined by the presence of changes in serum thyroid hormone levels observed in critically ill patients in the absence of primary hypothalamic-pituitary-thyroid dysfunction.⁵⁷ The mechanisms of the pathophysiology of the nonthyroidal illness syndrome are still unsettled and may differ between the different causes of non-thyroidal illness syndrome.^{58,59} One of the main mechanisms appears to be mediated by the systemic inflammation, via the inhibitory effect of circulatory cytokines in the activity of deiodinases type 1 and type 2 (that convert T4 into T3) and the stimulatory effect in the activity of deiodinase type 3 (that converts T4 into reverse T3).⁶⁰ As T3 is the main biologically active form of thyroid hormones, non-thyroidal illness syndrome leads to a state of *functional hypothyroidism*. Whether this is adaptive or maladaptive is also unsettled and may depend on the clinical setting.⁶¹ In patients with heart failure, where low thyroid hormone levels may promote further myocardial dysfunction, it has been speculated that this syndrome is maladaptive and that thyroid hormone supplementation could improve myocardial function.^{47,62} However, this has not yet been proved. Given the poor prognosis of heart failure and the shortage of effective therapeutic approaches (particularly in HFpEF), the evaluation of the role of supplementation with thyroid hormones in this population is of major relevance.

4. Prediabetes and cardiovascular risk

Prediabetes is defined as a state of elevated blood glucose levels that are below the diagnostic threshold for diabetes.^{63,64} Prediabetes may be defined by fasting plasma glucose between 100-125 mg/dL, 2h-plasma glucose after an oral glucose tolerance test between 140-199 mg/dL or HbA1c between 5.7-6.4%.^{63,64}

Several of the pathophysiological defects of type 2 diabetes are already observed in prediabetes. People with prediabetes may have insulin resistance, decreased beta-cell function, increased glucagon secretion, increased hepatic glucose production, decreased muscular glucose uptake, increased lipolysis, decreased incretin effect and increased renal glucose reabsorption.⁶⁵ As in type 2 diabetes, different combinations of these pathophysiological defects lead to a heterogeneous pathophysiological and clinical syndrome.^{66,67} This is reflected in the criteria met by patients with prediabetes. Most patients with prediabetes do not meet the 3 diagnostic criteria for prediabetes.⁶⁸ In some, increased fasting plasma glucose may predominate (which has been associated with predominant hepatic insulin resistance). In contrast, in other patients impaired glucose tolerance may predominate (which has been associated with peripheral insulin resistance).⁶⁹ The risk associated with these different phenotypes of prediabetes is still not consensual.⁷⁰

The term prediabetes implies an increased risk of progression to type 2 diabetes. The rate of progression from prediabetes to diabetes is about 5% to 10% annually, and up 70% develop diabetes during their lifetime.^{71,72} It is not of less relevance that prediabetes is also a state of increased cardiovascular risk.⁷³ This is important because at least 30% of the people with prediabetes will never progress to type 2 diabetes. Even among those that

progress, a long period of prediabetes is usually observed before the diagnosis of type 2 diabetes. One of the main unsettled questions in prediabetes field is whether prediabetes is directly promoting an increased cardiovascular risk (which would favor that correcting the dysglycemia of prediabetes could contribute to a reduction of the cardiovascular risk) or whether the increased cardiovascular risk in prediabetes is mediated only through other cardiovascular risk factors (which would favor correcting only other cardiovascular risk factors and not dysglycemia to prevent cardiovascular diseases).

Chronic kidney disease is among the main complications of type 2 diabetes and a growing body of evidence suggests that kidney dysfunction is an important pathophysiological mechanism of cardiovascular dysfunction in type 2 diabetes.^{74,75} The relevance of this association has recently been reinforced by evidence of overlapping mechanisms of kidney and heart dysfunction in diabetes, and by the identification of therapeutic approaches that simultaneously reduce the risk of development and progression of chronic kidney disease and heart failure (e.g. SGLT2 inhibitors and antagonists of mineralocorticoid receptors).^{76,77} Whether prediabetes also promotes kidney dysfunction is controversial, with evidence suggesting that prediabetes increases kidney dysfunction and evidence suggesting a lack of association.⁷⁸ Furthermore, the effect on cardiovascular risk of prediabetes among those with chronic kidney dysfunction is also currently unknown.

5. The role of incretins in cardiovascular risk modulation

Subclinical pathophysiological changes in glucose homeostasis may be observed even in patients with normal glucose levels.⁷⁹ One of the mechanisms that contribute to the progression from normoglycemia to prediabetes and from prediabetes to type 2 diabetes is a reduction in the secretion of incretin hormones from the gastrointestinal tract.^{73,80,81} Of note, the decreased release of incretins may be observed throughout the continuum of glycemic dysfunction – even among treated patients with type 2 diabetes who are meeting all therapeutic goals according to current guidelines, reduced levels of incretins may still be observed.⁸²

Incretins are hormones secreted by the gastrointestinal tract during meals that potentiate glucose-induced insulin secretion.⁸² The main incretins released are the glucagon-like peptide-1 (GLP-1) and the glucose-dependent insulinitropic polypeptide (GIP).⁸³ Despite their initial identification as modulators of glucose homeostasis, incretins, particularly GLP-1, are also important modulators of the cardiovascular system.⁸³

GLP-1 acts through surface receptors coupled to adenylyl cyclase, increasing the intracellular levels of cyclic adenosine monophosphate (cAMP).⁸⁴ At the cardiovascular level, activation of GLP-1 receptor protects the heart against ischemic dysfunction,⁸⁵ increases the heart rate,⁸⁶ and promotes vasodilation.⁸⁷ At the myocardial level, the effects of GLP-1 agonism have been associated with improved energetic efficiency.⁸⁸ From a clinical perspective, this translates into a decreased risk of myocardial infarction and cardiovascular death, which has been documented in several randomized controlled trials.⁸⁹ GLP-1 may also have an antiatherogenic effect at the vascular level, which further protects from myocardial infarction and other atherosclerotic complications of type 2 diabetes, including

stroke and peripheral artery disease.⁹⁰ Given these beneficial effects of GLP-1 receptor agonists, this class of drugs has been recommended as a preferential class for those with type 2 diabetes and cardiovascular disease.¹⁴ GLP-1 receptor agonists are also approved for the treatment of obesity and are among the available therapies with a greater impact on weight reduction.⁹¹

There are two main unanswered questions regarding the use of GLP-1 receptor agonists across the continuum of glycemic dysfunction. First, are the cardiovascular benefits of GLP-1 receptors additive to those of SGLT2 inhibitors in patients with type 2 diabetes and high cardiovascular risk?⁹² From a pathophysiological perspective, correcting low incretin levels in patients with type 2 diabetes, even with good glycemic control, should reduce the risk of atherosclerotic cardiovascular disease.⁹³ However, the evidence for the combination of GLP-1 receptor agonists with SGLT2 inhibitors is scarce.⁹² Second, are GLP-1 receptor agonists safe in people with heart failure (with or without type 2 diabetes)?⁹⁴ GLP-1 receptor agonists increase the intracellular levels of cAMP, and activation of cAMP pathways is considered a central pathophysiological mechanism of heart failure progression, particularly among patients with reduced ejection fraction.^{92,95-100} Two trials (the Heart Failure Network Functional Impact of GLP-1 for Heart Failure Treatment [FIGHT]¹⁰¹ and the Effect of Liraglutide on Left Ventricular Function in Stable Chronic Heart Failure Patients with and without Diabetes [LIVE]¹⁰²) in heart failure have suggested potential detrimental effects of GLP-1 receptor agonists in patients with heart failure with reduced ejection fraction (HFrEF). However, the analysis of both trials had limited power and more data is needed to better understand the effects of GLP-1 receptor agonists on heart failure.

6. Potential role of the modulation of endocrine function on cardiovascular risk

The high burden of cardiovascular dysfunction in endocrine diseases highlights the role of the endocrine system on cardiovascular risk. Whether improvement of subclinical endocrine dysfunction contributes to reducing cardiovascular risk is still uncertain.

The direct effect of thyroid hormones at the cardiovascular level is well established.^{15,16} Furthermore, there is a relevant inter-individual variation in thyroid hormone levels in euthyroid people.¹⁰³ These subclinical variations of thyroid function may contribute to the cardiovascular risk. Also, in patients with cardiovascular diseases, decreases in T3 levels due to the nonthyroidal illness syndrome are common.³⁸ Whether correcting thyroid dysfunction improves cardiac function and the prognosis of cardiovascular disease is still unknown, but this may be a potential approach to reduce cardiovascular risk.

As previously described, prediabetes is a very common state of dysglycemia and several pathogenic mechanisms of diabetes are already observed in prediabetes.^{30,65,104} From a pathophysiological perspective, it is plausible that the risk associated with dysglycemia is a continuum and that prediabetes already contributes to an increased risk of cardiovascular and kidney dysfunction. However, more data is needed to support this hypothesis.

The decreased release of GLP-1 may also be a mediator of increased cardiovascular risk in patients with abnormal glucose metabolism.⁸¹ Given that low GLP-1 levels may be present in those with prediabetes, in those with uncontrolled diabetes, and even in those with “well-controlled” diabetes, there is a great potential to improve cardiovascular risk through the activation of GLP-1 receptors.

In summary, a better understanding of the role of subclinical changes in thyroid function, glucose metabolism and incretin hormones on cardiovascular risk may open new clinical perspectives for improving cardiovascular health in a large group of patients.

Chapter 2

PURPOSE



Purpose

In this thesis, we aimed to evaluate the role of subclinical endocrine dysfunction in cardiovascular risk and cardiac function, and to assess the clinical implications of the modulation of thyroid function and glucose homeostasis. The global overview of the main objectives of this project is shown in Figure 2.

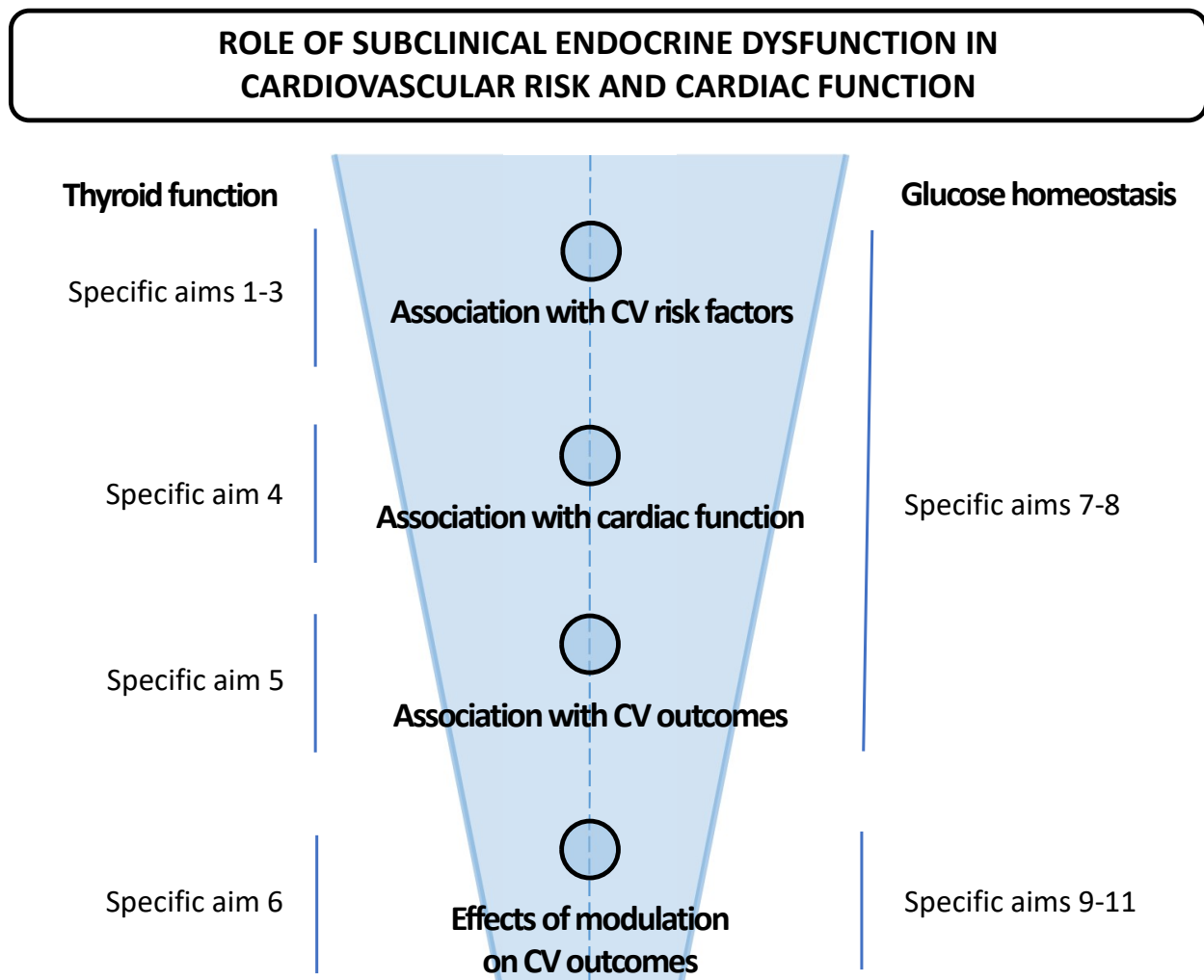


Figure 2. Schematic representation of the main objectives of this project. CV, cardiovascular.

Specific aims were:

- 1) To evaluate the association of thyroid function within the normal range with the cardiovascular risk in the general population.
- 2) To evaluate the association of thyroid function within the normal range with weight loss after bariatric surgery.
- 3) To evaluate the variation of thyroid function after weight loss with bariatric surgery.
- 4) To evaluate the association of thyroid function within the normal range with the cardiac function in the general population.
- 5) To evaluate the association of T3 levels within the normal range with cardiovascular mortality in the general population.
- 6) To evaluate the effects of supplementation with T3 in an animal model of heart failure with preserved ejection fraction.
- 7) To evaluate the association of prediabetes with the risk of kidney dysfunction.
- 8) To evaluate the association of prediabetes with cardiovascular events and progression of kidney disease in patients with chronic kidney disease.
- 9) To evaluate the effects of GLP-1 receptor agonists on cardiovascular events in patients with and without SGLT2 inhibitors use.
- 10) To evaluate the effects of GLP-1 receptor agonists on cardiovascular events in patients with type 2 diabetes with and without heart failure.
- 11) To evaluate the effects of GLP-1 receptor agonists on cardiovascular events in patients with heart failure with reduced ejection fraction.

Chapter 3

RESULTS



The results will be presented in the form of published and submitted articles. The discussion of each individual article is included in the publication. The global discussion is shown in the next chapter. These articles have been organized in 3 sub-chapters:

A) Thyroid function, cardiovascular risk, and cardiac function

1. **Association of thyroid function, within the euthyroid range, with cardiovascular risk: the EPIPorto study.** Neves JS, Fontes-Carvalho R, Borges-Canha M, Leite AR, Von Hafe M, Vale C, Martins S, Guimarães JT, Carvalho D, Leite-Moreira A, Azevedo A. *Front Endocrinol (Lausanne)*. 2022;13:1067801. (Impact factor: 6.055, Q1)
2. **Preoperative thyroid function and weight loss after bariatric surgery.** Neves JS, Souteiro P, Oliveira SC, Pedro J, Magalhães D, Guerreiro V, Costa MM, Bettencourt-Silva R, Santos AC, Queirós J, Varela A, Freitas P, Carvalho D; AMTCO Group. *Int J Obes (Lond)*. 2019;43(2):432-436. (Impact factor: 4.419, Q1)
3. **Effect of Weight Loss after Bariatric Surgery on Thyroid-Stimulating Hormone Levels in Patients with Morbid Obesity and Normal Thyroid Function.** Neves JS, Castro Oliveira S, Souteiro P, Pedro J, Magalhães D, Guerreiro V, Bettencourt-Silva R, Costa MM, Cristina Santos A, Queirós J, Varela A, Freitas P, Carvalho D; AMTCO Group. *Obes Surg*. 2018;28(1):97-103. (Impact factor: 3.603, Q1)
4. **Thyroid Hormones within the Normal Range and Cardiac Function in the General Population: The EPIPorto Study.** Neves JS, Fontes-Carvalho R, Borges-Canha M, Leite AR, Martins S, Oliveira A, Guimarães JT, Carvalho D, Leite-Moreira A, Azevedo A. *Eur Thyroid J*. 2021;10(2):150-160. (Impact factor: 4.084, Q2)
5. **Lower free triiodothyronine levels within the reference range are associated with higher cardiovascular mortality: An analysis of the NHANES.** Neves JS, Leitão L, Baeta Baptista R, Bigotte Vieira M, Magriço R, Viegas Dias C, Oliveira A, Falcão-Pires I, Lourenço A, Carvalho D, Leite-Moreira A. *Int J Cardiol*. 2019;285:115-120. (Impact factor: 3.229, Q2)
6. **Effects of Triiodothyronine Treatment in an Animal Model of Heart Failure with Preserved Ejection Fraction.** Neves JS, Leite AR, Conceição G, Gonçalves A, Borges-Canha M, Vale C, Von-Hafe M, Martins D, Miranda-Silva D, Leite S, Oliveira E, Sousa-Mendes C, Chaves J, Lourenço I, Grijota-Martínez C, Báñez-López S, Miranda IM, Almeida-Coelho J, Vasques-Nóvoa F, Carvalho D, Lourenço A, Falcão-Pires I, Leite-Moreira A. *Thyroid*. 2023. Online ahead of print. (Impact factor: 6.506, Q1)

B) Prediabetes and cardiovascular risk

7. **Impaired Fasting Glucose and Chronic Kidney Disease, Albuminuria, or Worsening Kidney Function: a Secondary Analysis of the SPRINT.** Vieira MB*, Neves JS*, Leitão L, Baptista RB, Magriço R, Dias CV, Oliveira A, Carvalho D, Mc Causland FR. J Clin Endocrinol Metab. 2019;104(9):4024-32. *equally contributing first authors (Impact factor: 5.399, Q1)
8. **Association of Prediabetes with CKD Progression and Adverse Cardiovascular Outcomes: An Analysis of the CRIC Study.** Neves JS, Correa S, Baeta Baptista R, Bigotte Vieira M, Waikar SS, Mc Causland FR. J Clin Endocrinol Metab. 2020 Apr 1;105(4):e1772-80. (Impact factor: 5.958, Q1)

C) Cardiovascular effects of GLP-1 receptor activation

9. **GLP1 Receptor Agonist Therapy with and without SGLT2 inhibitors in Patients with Type 2 Diabetes.** Neves JS, Borges-Canha M, Vasques-Nóvoa F, Green JB, Leiter LA, Granger CB, Carvalho D, Leite-Moreira A, Hernandez AF, Del Prato S, McMurray JJV, Ferreira JP. J Am Coll Cardiol. 2023. Accepted for publication. (Impact factor: 27.206, Q1)
10. **Albiglutide in patients with type 2 diabetes and heart failure: a post-hoc analysis from Harmony Outcomes.** Ferreira JP, Sharma A, Vasques-Nóvoa F, Angélico-Gonçalves A, Leite AR, Borges-Canha M, Carvalho D, Packer M, Zannad F, Leite-Moreira A, Neves JS. Eur J Heart Fail. 2022 ;24(10):1792-1801. (Impact factor: 18.174, Q1)
11. **Glucagon-like peptide 1 receptor agonists in patients with type 2 diabetes with and without heart failure: a meta-analysis of randomized placebo-controlled outcome trials.** Ferreira JP, Saraiva F, Sharma A, Vasques-Nóvoa F, Angélico-Gonçalves A, Leite AR, Borges-Canha M, Carvalho D, Packer M, Zannad F, Leite-Moreira A, Neves JS. Diabetes Obes Metab. 2023;25(6):1495-1502. (Impact factor: 6.408, Q1)
12. **Risk of adverse events with liraglutide in heart failure with reduced ejection fraction: A post hoc analysis of the FIGHT trial.** Neves JS, Vasques-Nóvoa F, Borges-Canha M, Leite AR, Sharma A, Carvalho D, Packer M, Zannad F, Leite-Moreira A, Ferreira JP. Diabetes Obes Metab. 2023;25(1):189-197. (Impact factor: 6.408, Q1)
13. **Increased risk of heart failure hospitalization with GLP-1 receptor agonists in patients with reduced ejection fraction: a meta-analysis of the EXSCEL and FIGHT trials.** Neves JS, Packer M, Ferreira JP. J Card Fail. 2023;S1071-9164(23)00110-0. (Impact factor: 6.592, Q2)

Chapter 3 | Part A

Thyroid function, cardiovascular risk, and cardiac function





Article 1

Association of thyroid function, within the euthyroid range, with cardiovascular risk: the EPIPorto study

Neves JS, Fontes-Carvalho R, Borges-Canha M, Leite AR, Von Hafe M, Vale C, Martins S, Guimarães JT, Carvalho D, Leite-Moreira A, Azevedo A.

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Association of thyroid function, within the euthyroid range, with cardiovascular risk: The EPIPorto study

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Background: Thyroid hormones are important modulators of cardiovascular function. Both hypothyroidism and hyperthyroidism are known to contribute to an increased cardiovascular risk. It remains uncertain whether thyroid hormones level within the euthyroid range are associated with cardiometabolic risk. We aimed to evaluate the association between thyroid function levels within the euthyroid range and cardiovascular risk in a population-based cohort.

Methods: Eight hundred thirty-five subjects aged ≥ 45 years from the EPIPorto population-based cohort were included. We excluded participants with TSH, free T4 (FT4), or free T3 (FT3) outside of the reference range, or with previous cardiovascular or thyroid disease. The associations between thyroid function, cardiovascular risk factors and the 10-year estimated risk of cardiovascular events (using SCORE2 and SCORE2-OP) were evaluated in linear and logistic regression models, crudely and adjusting for age, sex, BMI, diabetes, and smoking.

Results: The mean age of the participants was 61.5 (SD 10.5) years and 38.9% were men. Eleven percent of the participants had diabetes, 47.8% had dyslipidemia, and 54.8% had hypertension. The mean body mass index (BMI) was 27.4 (SD 4.6) kg/m², and the median (percentile25-75) 10-year risk of cardiovascular events was 5.46% (2.92, 10.11). Participants with higher BMI, larger waist circumference and higher hs-CRP had higher levels of FT3 and FT3/FT4 ratio. Lower FT3/FT4 ratio and higher FT4 levels were associated with

higher prevalence of diabetes and more adverse lipid profile. Higher TSH, lower FT3 and lower FT3/FT4 ratio were associated with lower eGFR. Lower FT3, lower FT3/FT4 ratio and higher FT4 were associated with an increased 10-year risk of cardiovascular events.

Conclusions: In a population-based study, variations of thyroid function within the euthyroid range were associated with cardiovascular risk factors. On one hand, individuals with higher BMI, larger waist circumference and higher hs-CRP had higher levels of FT3 and FT3/FT4 ratio. On the other hand, a decreased conversion of T4 to T3 (lower FT3, lower FT3/FT4 ratio and/or higher FT4) was associated with a higher prevalence of diabetes, a more adverse lipid profile, a lower eGFR and an increased 10-year risk of cardiovascular events.

KEYWORDS

thyroid, hypothyroidism, hyperthyroidism, cardiovascular risk, obesity, diabetes, dyslipidemia

Introduction

Thyroid hormones have a central role on cardiovascular homeostasis. Thyroid hormones modulate cardiac and vascular function, basal metabolic rate, glucose tolerance, serum lipids and kidney function (1–3). Both overt hypothyroidism and hyperthyroidism are known to contribute to a higher risk of ischemic heart disease, stroke and heart failure (4–6). Patients with hypothyroidism also have an increased prevalence of several cardiovascular risk factors, such as dyslipidemia, hypertension, obesity and diabetes (5, 7, 8). Patients with hyperthyroidism have an increased prevalence of hypertension and may have a hypercoagulable state (1, 9). A large body of evidence suggests that also subclinical hypo- and hyperthyroidism contribute to increased cardiovascular risk (10, 11). From a physiological perspective, it is likely that there is a continuum of effects across the spectrum of thyroid function, including the euthyroid range. However, while the effects of overt and subclinical thyroid dysfunction on cardiovascular risk factors have been well characterized (1, 12), fewer studies addressed the association of thyroid hormones within the euthyroid range with cardiometabolic risk. In a study of US community-dwelling older adults, lower TSH and higher free thyroxine (FT4) within the euthyroid range were associated with an increased risk of adverse events, including mortality (13). In the Rotterdam study, in elderly subjects, high-normal FT4 and low-normal TSH were associated with an increased risk of atrial fibrillation (14). On the other hand, in a study from Groningen (Netherlands), in people aged 28–75 years, low normal FT4 levels were significantly associated with adverse lipid profile and increased insulin resistance (15). Higher TSH levels and lower FT4 levels were also associated with increased risk of incident diabetes, especially

in individuals with prediabetes (16). Lower free triiodothyronine (FT3) levels have been associated with increased risk of cardiovascular mortality in the general population (17) and in patients with established cardiovascular disease (18–20). In heart failure, low normal FT3 have been associated with higher BNP levels and with increased risk of adverse events (21, 22).

The EPIPorto study is a population-based study (23) that includes detailed data on cardiovascular risk factors. In the second evaluation of the cohort, participants aged 45 years or older were included, an age group that is particularly relevant as it represents the population usually targeted by cardiovascular preventive interventions. Therefore, in this analysis of the EPIPorto study, we aimed to evaluate the association of thyroid function levels within the euthyroid range with cardiovascular risk in middle-aged and elderly individuals.

Methods

Study design and participants

The EPIPorto study is a population-based cohort (23), ongoing for more than 20 years, with the main objective of assessing the determinants of health in the adult population of Porto, Portugal. In the baseline evaluation (1999–2003), 2485 persons were selected at random and have been evaluated over time. Between October 2006 and July 2008, participants with 45 years of age or older over were eligible to an evaluation that included cardiovascular history, physical examination, anthropometric evaluation, collection of fasting blood sample and a transthoracic echocardiogram. Among 2048 cohort members in the eligible age range at this time, 134 (6.5%) had

died, 198 (9.7%) refused re-evaluation and 580 (28.3%) were lost to follow-up. We further excluded 301 patients with history of heart disease (significant valvular heart disease, prior cardiac surgery, previous myocardial infarction, percutaneous or surgical revascularization; $n=136$), history of thyroid disease ($n=50$), treatment with drugs that interfere with thyroid function ($n=17$); without serum available for thyroid function determination ($n=40$); or with TSH, free thyroxine (FT4) or free triiodothyronine (FT3) outside of the reference range (0.35–4.94 $\mu\text{IU/mL}$ for TSH, 0.70–1.48 ng/dL for FT4 and 1.71–3.71 pg/mL for FT3) ($n=58$). Eight hundred and thirty-five participants were included in our analysis. This study was approved by the ethics committee of Centro Hospitalar Universitário São João/Faculdade de Medicina da Universidade do Porto.

Thyroid function measurements

TSH, FT3, and FT4 were measured in serum samples stored at -80°C by an electrochemiluminescence immunoassay using an Abbott Architect i2000 analyzer (Abbott Diagnostics). Previous studies have shown that TSH and thyroid hormones can be analyzed reliably in frozen stored samples (24). The FT3/FT4 ratio, an indicator of peripheral deiodinase activity, was calculated dividing plasma concentrations of FT3 by FT4 levels (25).

Anthropometric and analytical measurements

Anthropometric measures (body mass index, waist circumference and waist-to-hip circumference ratio) were performed after an overnight fast, with the participant wearing light clothing and no footwear. Body weight was measured to the nearest 0.1 kg using a digital scale, and height was measured to the nearest centimeter in the standing position using a wall stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. Body composition was assessed by bioelectrical impedance analysis (Tanita Corp, Arlington Heights, IL) to determine fat mass. Body fat percentage was calculated as (fat mass/body weight) $\times$ 100. Fat mass index was calculated as fat mass in kilograms divided by squared height in meters.

Blood pressure was determined by two measurements separated by at least 5 minutes after a 10-minute rest. When the difference between measurements was larger than 5 mmHg for systolic or diastolic blood pressure a third measurement was taken, and the mean of the 2 closest values was used.

Lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides), glucose profile (fasting glucose and homeostatic model assessment for insulin resistance [HOMA-IR]), adipokines (leptin and adiponectin), creatinine

and high-sensitivity C-reactive protein (hs-CRP) were obtained from a fasting venous blood sample in the morning. Adipokines were only evaluated in a subsample of participants (leptin in 445 participants and adiponectin in 441 participants). HOMA-IR was calculated according to the formula: fasting insulin ($\mu\text{U/L}$) $\times$ fasting glucose (mg/dL)/405 (26). Estimated glomerular filtration rate (eGFR) was estimated using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (27).

Comorbidities and clinical definitions

Obesity was defined as BMI $\geq 30 \text{ kg/m}^2$. Hypertension was defined as systolic blood pressure $\geq 140 \text{ mmHg}$ or diastolic blood pressure $\geq 90 \text{ mmHg}$ at the time of the visit or use of antihypertensive drugs (28). Diabetes was defined as fasting blood glucose $\geq 126 \text{ mg/dL}$, self-reported history of diabetes, or use of diabetes medications (29). Dyslipidemia was defined as low-density lipoprotein cholesterol $\geq 160 \text{ mg/dL}$, triglycerides $\geq 200 \text{ mg/dL}$, high-density lipoprotein cholesterol $< 40 \text{ mg/dL}$, or use of lipid-lowering drugs (30). Chronic kidney disease (CKD) was defined as eGFR $< 60 \text{ mL/min/1.73 m}^2$ (31). Risk of cardiovascular events (10 year risk of fatal and non-fatal events) was calculated using SCORE2 (Systematic Coronary Risk Estimation 2) (32), for participants aged 40–69 years, and SCORE2-OP (Systematic Coronary Risk Estimation 2-Older Persons) (33), for participants aged over 70 years or older. Cardiovascular disease risk categories based on SCORE2 and SCORE2-OP were used according to the European Society of Cardiology (ESC) classification: low-moderate risk (< 50 years: risk $< 2.5\%$; 50–69 years: $< 5\%$; ≥ 70 years: $< 7.5\%$), high risk (< 50 years: 2.5–7.5%; 50–69 years: 5–10%; ≥ 70 years: 7.5–15%) or very high risk (< 50 years: $\geq 7.5\%$; 50–69 years: $\geq 10\%$; ≥ 70 years: $\geq 15\%$) (34).

Statistical analysis

Linear regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and anthropometric parameters, glucose profile, lipid profile, adipokines, hs-CRP and kidney function (dependent variables). The assumptions of normality, homoscedasticity, and linearity were assessed using the Q-Q plot of residuals, plot of residuals against predicted values and plots of residuals against each variable in the regression models. Logistic regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and diabetes, obesity, dyslipidemia, hypertension and CKD (dependent variables). These associations were evaluated crudely and in models adjusted for sex, age and current smoking (model 1). Sex and age were included in the adjustment model due to their known association with thyroid function and their association with cardiovascular risk factors. Smoking is also strongly associated

with several cardiovascular risk factors and influences thyroid hormone levels. Associations were also evaluated in a model adjusted for sex, age, current smoking, BMI and diabetes (model 2). This model was used to evaluate if the association of thyroid function with cardiovascular risk factors was independent of BMI and diabetes. Although thyroid hormones may influence BMI and the risk of diabetes, BMI and diabetes also significantly affect thyroid hormone levels and are potential confounders of the association of thyroid hormones with the cardiovascular risk (35). Models evaluating the association of thyroid function with anthropometric parameters did not include BMI; models evaluating the association of thyroid function with lipid profile and dyslipidemia were restricted to participants not treated with lipid-lowering drugs; and models evaluating the association with glucose profile were restricted to participants not treated with antidiabetic drugs. In models evaluating the association of thyroid function with 10-year risk of cardiovascular events, participants with diabetes were excluded as SCORE2 and SCORE2-OP do not apply to this group of patients. These models were adjusted only for BMI, because sex, age and smoking are part of the model used to calculate SCORE2 and SCORE2-OP. We also evaluated the distribution of cardiovascular disease risk categories according to terciles of TSH, FT4, FT3 and FT3/FT4 ratio. The association of thyroid function parameters (independent variable) with cardiovascular disease risk categories (dependent variable) was evaluated using ordered logistic regression.

Continuous variables are presented as mean (standard deviation) for continuous normally distributed variables, and as median (percentile 25 – percentile 75) for non-normally distributed continuous variables. TSH, triglycerides, HOMA-IR, hs-CRP, adiponectin, leptin and the 10-year risk of cardiovascular events were log-transformed for inclusion in regression models due to skewness. Statistical analyses were performed with Stata software, version 17.0 (StataCorp).

Results

A total of 835 participants were included in this analysis. The mean age of participants was 61.5 (SD 10.5) years, 38.9% were male, the mean BMI was 27.4 (SD 4.6) kg/m², 11.4% had diabetes, 47.8% had dyslipidemia, 54.8% had hypertension, 10.6% had CKD, and 14.7% were current smokers (Table 1). The median 10-year risk of fatal and non-fatal cardiovascular events was 5.5% (percentile 25-75, 2.9-10.1).

Thyroid hormone levels were not significantly associated with the diagnosis of obesity (Table 2). On the other hand, FT3 was positively associated with BMI and fat mass index, and both FT3 and FT3/FT4 ratio were positively associated with waist circumference in adjusted models. Additionally, TSH was positively associated with leptin in unadjusted and adjusted models, and the FT3/FT4 ratio was positively associated with leptin in unadjusted model and in model 1

(adjusted for sex, age and current smoking), but not in model 2 (further adjusted for BMI and diabetes). There were no associations of thyroid function with adiponectin levels (Table 2). FT3 and FT3/FT4 ratio were positively associated with hs-CRP in unadjusted and adjusted models (Table 2).

Regarding the association of thyroid function with diabetes, higher levels of FT4 and lower ratio of FT3/FT4 were associated with a higher odds of diabetes (Table 3). Among participants not treated with antidiabetic drugs, FT3 was positively associated with HOMA-IR in model 1 but not after further adjustment in model 2 (Table 3).

In respect to the lipid profile, thyroid function was not associated with the odds of dyslipidemia (Table 4). Among participants not treated with lipid-lowering drugs, FT4 was positively associated with total cholesterol, LDL cholesterol and triglycerides, and FT3/FT4 ratio was negatively associated with total cholesterol and LDL cholesterol. No significant associations with HDL were observed (Table 4).

Higher levels of FT4 and lower FT3/FT4 ratio were associated with a higher prevalence of hypertension and chronic kidney disease in unadjusted models, but not in the adjusted models (Table 5). Both systolic and diastolic blood pressure were not significantly associated with thyroid hormone levels. On the other hand, a lower TSH, a higher FT3 and a higher FT3/FT4 ratio were associated with higher eGFR in unadjusted and adjusted analysis (Table 5).

Lower FT3, lower FT3/FT4 ratio and higher FT4 were associated with a higher estimated 10-year risk of cardiovascular risk events (Supplementary Table 1) and a higher cardiovascular risk category (Table 6). The proportion of participants in the very high risk category was higher in the lower tercile of FT3 (32.6% vs 21.7% in the higher tercile), in the lower tercile of FT3/FT4 ratio (30.2% vs 17.6% in the higher tercile) and in the upper tercile of FT4 (29.8% vs 21.4% in the lower tercile). An inverse distribution was observed in the low-moderate risk category (Table 6).

Discussion

In a population-based study, we observed a significant association of thyroid function within the euthyroid range with cardiovascular risk factors. On one hand, participants with higher levels of FT3 and/or FT3/FT4 ratio had higher BMI, fat mass and waist circumference, and higher levels of hs-CRP. On the other hand, participants with lower FT3/FT4 ratio and/or higher FT4 levels had higher levels of LDL cholesterol and triglycerides, and higher prevalence of diabetes. Lower FT3 levels and FT3/FT4 ratio, and higher TSH levels were associated with decreased kidney function. Furthermore, lower FT3, lower FT3/FT4 ratio and higher FT4 were associated with an increased 10-year estimated risk of cardiovascular events.

Thyroid hormones play an important role in regulating metabolic rate and body composition (2). Hyperthyroidism

TABLE 1 Characteristics of the study sample (n=835).

Male sex, %	325 (38.9%)
Age, years	61.5 ± 10.5
Hypertension, %	455 (54.8%)
Dyslipidemia, %	396 (47.8%)
Obesity, %	210 (25.2%)
Diabetes, %	95 (11.4%)
CKD, %	88 (10.6%)
Current smoking, %	123 (14.7%)
Body mass index, kg/m ²	27.4 ± 4.6
Waist circumference, cm	92.8 ± 11.4
Waist-to-hip ratio	0.9 ± 0.1
Body fat percentage, %	31.0 ± 8.5
Systolic pressure, mmHg	132.9 ± 19.6
Diastolic pressure, mmHg	78.3 ± 11.3
TSH, μ IU/mL	1.2 (0.9, 1.7)
FT4, ng/dL	1.1 ± 0.1
FT3, pg/mL	2.5 ± 0.4
FT3/FT4 ratio	2.4 ± 0.5
Total cholesterol, mg/dL	219.3 ± 39.0
LDL cholesterol, mg/dL	133.2 ± 34.5
HDL cholesterol, mg/dL	59.7 ± 13.1
Triglycerides, mg/dL	113.0 (84.0, 157.0)
eGFR, mL/min/1.73 m ²	77.8 ± 14.5
Fasting plasma glucose, mg/dL	101.9 ± 23.9
HOMA-IR	1.1 (0.6, 1.8)
Hs-CRP, mg/dL	0.2 (0.1, 0.4)
Adiponectin, ng/mL	6249.5 (3293.1, 10249.5)
Leptin, ng/mL	14.0 (8.0, 22.8)
10-year risk of cardiovascular events, %	5.46 (2.92, 10.11)

Categorical variables are presented as counts (percentages). Continuous variables are presented as mean ± standard deviation or median (25th, 75th percentile). 10-year risk of fatal and non-fatal cardiovascular events was calculated using SCORE2 (Systematic Coronary Risk Estimation 2) and SCORE2-OP (Systematic Coronary Risk Estimation 2-Older Persons). CKD, chronic kidney disease; eGFR, Estimated glomerular filtration rate; Hs-CRP, high sensitivity C-reactive protein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

promotes increased metabolic rate and weight loss, whereas hypothyroidism promotes weight gain (4, 5). Less well-defined is the association between thyroid function and body composition in euthyroid individuals. We observed that participants with higher BMI, fat mass and waist circumference had higher levels of FT3 and FT3/FT4 ratio, which is in agreement with previous studies (36–38). The fat mass directly increases type 1 and 2 deiodinase activity increasing the peripheral conversion of T4 in T3 (39). Higher levels of FT3 can be interpreted as an adaptive process to ameliorate obesity-related morbidities (40, 41), as thyroid hormone effects may limit nutrient overload by inducing tissue thermogenesis and by stimulating metabolic activity (39). The higher FT3/FT4 ratio with increasing BMI may also be a marker of obesity-induced thyroid hormone resistance (42, 43). Laclaustra et al. showed that thyroid hormone resistance is associated with worse

cardiometabolic parameters (44). We also found a positive association between TSH and leptin, even after adjustment for confounders including BMI. The relationship between thyroid axis and leptin is complex (43, 45, 46). On one hand, leptin activates the TRH-TSH-thyroid gland axis at a central level; on the other hand, TSH may stimulate leptin secretion by a direct effect on adipocytes (47, 48). Whether this relationship is adaptative or maladaptive in obesity is unsettled (2, 43).

In our study, higher hs-CRP levels were associated with higher levels of FT3 and FT3/FT4 ratio. Jublanc et al. observed a negative correlation of hs-CRP with FT4 (49), and Roef et al. described higher hs-CRP and IL-6 levels to be associated with higher FT3, lower FT4, and a higher FT3/FT4 ratio (50). Most studies in overt or subclinical hyperthyroidism have not found an association between thyroid function and systemic inflammation (51, 52). Thus, we hypothesize that higher hs-CRP levels in euthyroid individuals with higher levels of FT3 and FT3/FT4 ratio are a marker of thyroid hormone resistance and not a consequence of increased thyroid hormone effects.

Concerning the glycemic and lipid profiles, the association of higher levels of FT4 with diabetes and worse lipid profile in the absence of associations with FT3 and TSH suggests that the increase in FT4 represents a decrease in the conversion to the active form T3, making it unlikely to represent an increased thyroid function. The fact that a lower FT3/FT4 ratio was associated with diabetes and worse lipid profile further reinforces that a decrease of the peripheral conversion of T4 to T3 may contribute to metabolic dysfunction. Despite the known role of overt hypothyroidism in dyslipidemia and diabetes, the effect of subclinical hypothyroidism and thyroid hormones within the euthyroid range remains uncertain (53–55). In a population at high cardiovascular risk, de Vries et al. found no association between TSH levels in the euthyroid range and incident diabetes (55). On the other hand, in a population-based prospective cohort, Chaker et al. showed that low and low-normal thyroid function are risk factors for incident diabetes (16). Garduño-García et al. also showed that low thyroid function, even in the euthyroid range, predisposes to higher cholesterol, glucose and HOMA-IR levels (56). Our results further highlight a potential role of low-normal levels of thyroid hormones in increasing the risk of diabetes and dyslipidemia.

Regarding kidney function, thyroid hormones directly affect renal hemodynamics, and sodium and water homeostasis (57). More specifically, thyroid hormones increase kidney blood flow and activate renin-angiotensin-aldosterone system, therefore, increasing glomerular filtration rate (57). In this study, we found an association between lower thyroid function (higher TSH and lower FT3) and decreased glomerular filtration rate. In agreement with our findings, an observational study of 309 patients with stage 2–4 chronic kidney disease and subclinical hypothyroidism observed a preservation of renal function and decreased risk of adverse renal outcomes in those treated with levothyroxine (58). These results are important because decreased glomerular filtration rate has been recognized as a

TABLE 2 Association of thyroid function with obesity, anthropometric parameters, plasma adipokines and high-sensitivity C-reactive protein.

	TSH (per $\mu\text{IU/mL}$)		FT4 (per ng/dL)		FT3 (per pg/mL)		FT3/FT4 ratio (per unit)	
	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value
Outcomes								
Obesity								
Unadjusted	1.38 (0.99-1.91)	0.057	0.77 (0.23-2.60)	0.671	1.36 (0.90-2.05)	0.140	1.32 (0.93-1.85)	0.116
Model 1	1.16 (0.83-1.63)	0.388	0.81 (0.24-2.81)	0.744	1.38 (0.91-2.11)	0.133	1.31 (0.92-1.87)	0.137
Model 2	1.16 (0.83-1.63)	0.389	0.69 (0.20-2.42)	0.565	1.43 (0.93-2.19)	0.103	1.37 (0.96-1.95)	0.087
	TSH (per $\mu\text{IU/mL}$)		FT4 (per ng/dL)		FT3 (per pg/mL)		FT3/FT4 ratio (per unit)	
	β (95% CI)	P Value	β (95% CI)	P value	β (95% CI)	P Value	β (95% CI)	P Value
Outcomes								
BMI, kg/m^2								
Unadjusted	0.36 (-0.30 to 1.02)	0.289	-0.21 (-2.89 to 2.47)	0.876	0.85 (0.04 to 1.67)	0.041	0.67 (-0.14 to 1.49)	0.104
Model 1	-0.01 (-0.65 to 0.63)	0.985	-0.13 (-2.72 to 2.45)	0.921	0.90 (0.12 to 1.68)	0.024	0.68 (-0.11 to 1.47)	0.092
Model 2	-0.01 (-0.65 to 0.63)	0.978	-0.52 (-3.08 to 2.05)	0.692	0.96 (0.18 to 1.74)	0.016	0.78 (-0.00 to 1.56)	0.051
Waist circumference, cm								
Unadjusted	-0.22 (-1.90 to 1.46)	0.796	0.23 (-6.02 to 6.48)	0.942	1.13 (-0.87 to 3.14)	0.268	0.81 (-0.98 to 2.61)	0.374
Model 1	-0.10 (-1.68 to 1.47)	0.897	-0.64 (-6.83 to 5.56)	0.840	1.89 (0.00 to 3.78)	0.050	1.48 (-0.28 to 3.25)	0.100
Model 2	-0.13 (-1.69 to 1.44)	0.873	-1.83 (-7.89 to 4.23)	0.554	2.07 (0.18 to 3.96)	0.032	1.78 (0.05 to 3.51)	0.044
Waist-to-hip ratio								
Unadjusted	-0.02 (-0.03 to -0.01)	0.004	0.03 (-0.01 to 0.07)	0.211	0.00 (-0.01 to 0.02)	0.760	-0.00 (-0.01 to 0.01)	0.557
Model 1	-0.01 (-0.02 to 0.00)	0.085	0.02 (-0.02 to 0.05)	0.417	0.01 (-0.00 to 0.02)	0.074	0.00 (-0.01 to 0.01)	0.376
Model 2	-0.01 (-0.02 to 0.00)	0.073	0.01 (-0.03 to 0.04)	0.662	0.01 (0.00 to 0.02)	0.046	0.01 (-0.00 to 0.02)	0.206
Fat percentage, %								
Unadjusted	1.97 (0.78 to 3.16)	0.001	0.89 (-3.80 to 5.58)	0.710	0.86 (-0.65 to 2.37)	0.262	0.46 (-0.87 to 1.79)	0.497
Model 1	0.35 (-0.60 to 1.29)	0.472	0.74 (-2.89 to 4.36)	0.690	1.07 (-0.10 to 2.23)	0.073	0.55 (-0.53 to 1.63)	0.316
Model 2	0.34 (-0.61 to 1.29)	0.482	0.38 (-3.24 to 4.00)	0.836	1.13 (-0.04 to 2.30)	0.058	0.64 (-0.43 to 1.72)	0.238
Fat mass index, kg/m^2								
Unadjusted	0.75 (0.24 to 1.27)	0.004	-0.11 (-2.30 to 2.07)	0.918	0.53 (-0.11 to 1.18)	0.103	0.43 (-0.21 to 1.06)	0.191
Model 1	0.19 (-0.27 to 0.65)	0.413	-0.10 (-2.01 to 1.81)	0.920	0.59 (0.04 to 1.14)	0.037	0.44 (-0.13 to 1.01)	0.133
Model 2	0.19 (-0.27 to 0.64)	0.424	-0.35 (-2.26 to 1.57)	0.720	0.63 (0.08 to 1.19)	0.026	0.50 (-0.07 to 1.07)	0.084
Leptin, ng/mL								
Unadjusted	0.39 (0.23 to 0.55)	<0.001	-0.43 (-1.05 to 0.20)	0.180	0.23 (0.02 to 0.44)	0.032	0.25 (0.07 to 0.42)	0.005
Model 1	0.30 (0.17 to 0.43)	<0.001	-0.26 (-0.79 to 0.26)	0.321	0.19 (-0.00 to 0.38)	0.051	0.17 (0.01 to 0.34)	0.039
Model 2	0.25 (0.14 to 0.35)	<0.001	-0.18 (-0.64 to 0.28)	0.434	0.11 (-0.05 to 0.28)	0.181	0.10 (-0.05 to 0.24)	0.191
Adiponectin, ng/mL								
Unadjusted	0.06 (-0.11 to 0.22)	0.501	0.39 (-0.21 to 0.98)	0.201	-0.01 (-0.24 to 0.21)	0.901	-0.10 (-0.31 to 0.11)	0.369
Model 1	0.04 (-0.12 to 0.21)	0.609	0.44 (-0.15 to 1.04)	0.143	-0.03 (-0.26 to 0.20)	0.824	-0.12 (-0.33 to 0.09)	0.258
Model 2	0.06 (-0.10 to 0.23)	0.443	0.48 (-0.11 to 1.08)	0.113	-0.02 (-0.25 to 0.21)	0.869	-0.12 (-0.33 to 0.09)	0.262
Hs-CRP, mg/dL								
Unadjusted	0.08 (-0.07 to 0.24)	0.298	0.19 (-0.39 to 0.78)	0.511	0.28 (0.08 to 0.47)	0.006	0.17 (0.00 to 0.33)	0.048
Model 1	0.07 (-0.09 to 0.23)	0.387	0.09 (-0.49 to 0.67)	0.758	0.34 (0.14 to 0.54)	0.001	0.23 (0.06 to 0.40)	0.007
Model 2	0.07 (-0.08 to 0.22)	0.342	0.06 (-0.48 to 0.60)	0.822	0.27 (0.09 to 0.46)	0.003	0.19 (0.03 to 0.34)	0.020

Model 1: age, sex. Model 2: age, sex, BMI, diabetes, smoking (BMI not included in model for obesity). Linear regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and anthropometric parameters, adipokines, hs-CRP and (dependent variables). Logistic regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and obesity. TSH, hs-CRP, adiponectin, and leptin were log-transformed. BMI, body mass index; Hs-CRP, high sensitivity C-reactive protein; TSH, Thyroid-stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine. Significant associations are highlighted in bold.

TABLE 3 Association of thyroid function with diabetes and glucose profile.

	TSH (per $\mu\text{IU/mL}$)		FT4 (per ng/dL)		FT3 (per pg/mL)		FT3/FT4 ratio (per unit)	
	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value
Outcomes								
Diabetes								
Unadjusted	1.04 (0.67-1.63)	0.857	9.85 (1.91-50.85)	0.006	0.59 (0.33-1.05)	0.071	0.48 (0.29-0.80)	0.005
Model 1	1.02 (0.64-1.62)	0.925	7.05 (1.35-36.67)	0.020	0.71 (0.40-1.28)	0.252	0.58 (0.34-0.97)	0.038
Model 2	1.01 (0.63-1.62)	0.956	6.99 (1.30-37.40)	0.023	0.64 (0.35-1.17)	0.144	0.53 (0.31-0.91)	0.020
	TSH (per $\mu\text{IU/mL}$)		FT4 (per ng/dL)		FT3 (per pg/mL)		FT3/FT4 ratio (per unit)	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
Outcomes								
Fasting plasma glucose, mg/dL								
Unadjusted model	-0.12 (-2.49 to 2.26)	0.922	4.63 (-4.38 to 13.64)	0.314	1.05 (-1.92 to 4.01)	0.489	-0.17 (-2.71 to 2.37)	0.895
Model 1	0.46 (-1.89 to 2.81)	0.702	2.52 (-6.33 to 11.36)	0.577	2.35 (-0.59 to 5.29)	0.117	1.12 (-1.42 to 3.65)	0.387
Model 2	0.71 (-1.48 to 2.90)	0.524	-0.11 (-8.38 to 8.16)	0.979	1.83 (-0.92 to 4.58)	0.192	1.18 (-1.19 to 3.55)	0.329
HOMA-IR								
Unadjusted model	-0.08 (-0.22 to 0.05)	0.223	0.07 (-0.44 to 0.58)	0.788	0.17 (-0.00 to 0.33)	0.053	0.10 (-0.04 to 0.25)	0.158
Model 1	-0.09 (-0.23 to 0.04)	0.174	0.08 (-0.43 to 0.59)	0.764	0.19 (0.02 to 0.36)	0.027	0.12 (-0.02 to 0.26)	0.105
Model 2	-0.07 (-0.20 to 0.05)	0.255	0.06 (-0.41 to 0.53)	0.803	0.11 (-0.04 to 0.27)	0.156	0.07 (-0.06 to 0.20)	0.300

Model 1: age, sex. Model 2: age, sex, BMI, diabetes, smoking (diabetes not included in model for diabetes). Analysis of fasting plasma glucose and HOMA-IR restricted to participants not treated with antidiabetic drugs. Linear regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and glucose profile (dependent variables). Logistic regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and diabetes (dependent variables). TSH and HOMA-IR were log-transformed. HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; TSH, Thyroid-stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine. Significant associations are highlighted in bold.

major risk factor for cardiovascular disease (59) and thyroid hormones may directly modulate it.

Regarding cardiovascular risk, lower FT3, lower FT3/FT4 ratio and higher FT4 levels were associated with an increased 10-year estimated risk of cardiovascular events. This is in agreement with previous studies that showed that individuals with lower FT3 levels within the euthyroid range had increased cardiovascular mortality (17) and all-cause mortality (60, 61). A higher risk of mortality has also been described for individuals with higher FT4 (62) and lower FT3/FT4 ratio (63), supporting our observation of an increased risk of cardiovascular events in these groups. The more adverse lipid profile with lower FT3 and higher FT4 probably contributed to the increased risk calculated by the SCORE2 and SCORE2-OP. These results remained significant even after adjustment for BMI which is not included in the risk score. Furthermore, lower FT3, lower FT3/FT4 ratio and higher FT4 are also associated with a higher prevalence of diabetes and lower eGFR which suggests that the risk of cardiovascular events in these groups may be even higher than that calculated with SCORE2/SCORE2-OP. Whether the risks associated with a lower FT3/FT4 ratio represent a causal effect of decreased deiodinase activity or simply the combination of the risk associated with a low FT3 (marker of frailty and/or pro-inflammatory state) and a higher FT4 (which may have some direct effects and/or represent a better marker of thyroid effects on some tissues) is uncertain; as an example, previous studies have suggested that T4 may be a better marker of thyroid hormone

action on the risk of development of atrial fibrillation (64, 65). Our results suggest that reference values for thyroid hormone levels may have to account for cardiovascular risk. Furthermore, future studies should evaluate if thyroid hormone levels can be used to improve cardiovascular risk prediction.

In our study, most associations of thyroid function with cardiovascular risk factors were observed only for FT4, FT3 or FT3/FT4 ratio, and not for TSH. A recent meta-analysis of 58 studies also described that thyroid hormone levels, particularly FT4 levels, were more strongly associated with clinical parameters than TSH (66). Our results also support that, within the euthyroid range, thyroid hormone levels are more correlated with cardiovascular risk factors than TSH levels.

Our results are clinically relevant as they suggest that mild variations of thyroid hormones within the euthyroid range may modulate cardiovascular risk in the general population. Our study comprised a large sample of individuals aged 45 years or older allowing us to represent the segment of the population usually targeted for cardiovascular preventive interventions. The use of a comprehensive panel of thyroid tests is also a strength, as previous studies evaluating the association of thyroid function with cardiovascular risk factors focused mainly on the impact of TSH and FT4. Also, we included an elaborated panel of cardiometabolic parameters, which allowed us to have an in-depth insight into the association between endocrine and cardiovascular systems. We evaluated not only individual risk factors, but also the global

TABLE 4 Association of thyroid function with dyslipidemia and lipid profile.

	TSH (per $\mu\text{IU/mL}$)		FT4 (per ng/dL)		FT3 (per pg/mL)		FT3/FT4 ratio (per unit)	
	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value
Outcomes								
Dyslipidemia								
Unadjusted	1.10 (0.82-1.46)	0.531	2.45 (0.85-7.09)	0.097	0.97 (0.68-1.39)	0.881	0.81 (0.60-1.09)	0.168
Model 1	1.15 (0.86-1.55)	0.345	1.95 (0.66-5.74)	0.224	1.11 (0.77-1.61)	0.576	0.92 (0.67-1.25)	0.580
Model 2	1.15 (0.85-1.56)	0.353	1.70 (0.56-5.14)	0.348	1.09 (0.75-1.59)	0.654	0.92 (0.66-1.26)	0.593
	TSH (per $\mu\text{IU/mL}$)		FT4 (per ng/dL)		FT3 (per pg/mL)		FT3/FT4 ratio (per unit)	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
Outcomes								
Total cholesterol, mg/dL								
Unadjusted model	2.17 (-4.08 to 8.41)	0.496	33.18 (10.26 to 56.10)	0.005	-3.76 (-11.57 to 4.06)	0.346	-8.07 (-14.51 to -1.63)	0.014
Model 1	0.67 (-5.64 to 6.99)	0.835	31.96 (9.01 to 54.90)	0.006	-2.83 (-10.72 to 5.07)	0.482	-7.54 (-14.06 to -1.01)	0.024
Model 2	0.61 (-5.69 to 6.91)	0.850	32.70 (9.59 to 55.81)	0.006	-3.43 (-11.34 to 4.48)	0.395	-8.03 (-14.59 to -1.46)	0.017
LDL cholesterol, mg/dL								
Unadjusted model	0.28 (-5.19 to 5.75)	0.920	23.51 (3.42 to 43.61)	0.022	-5.55 (-12.39 to 1.29)	0.112	-7.65 (-13.28 to -2.02)	0.008
Model 1	-0.49 (-6.05 to 5.06)	0.862	22.27 (2.07 to 42.47)	0.031	-4.81 (-11.75 to 2.13)	0.174	-7.16 (-12.89 to -1.42)	0.015
Model 2	-0.42 (-5.95 to 5.12)	0.882	24.82 (4.52 to 45.13)	0.017	-5.76 (-12.70 to 1.18)	0.103	-8.15 (-13.90 to -2.39)	0.006
HDL cholesterol, mg/dL								
Unadjusted model	1.93 (-0.21 to 4.07)	0.077	-3.14 (-11.08 to 4.79)	0.437	-2.17 (-4.86 to 0.51)	0.113	-0.75 (-2.98 to 1.47)	0.505
Model 1	0.43 (-1.61 to 2.48)	0.677	-3.22 (-10.73 to 4.30)	0.401	-2.01 (-4.58 to 0.56)	0.125	-0.73 (-2.86 to 1.41)	0.503
Model 2	0.40 (-1.59 to 2.40)	0.691	-1.61 (-9.01 to 5.78)	0.669	-1.77 (-4.29 to 0.74)	0.166	-0.87 (-2.97 to 1.23)	0.414
Triglycerides, mg/dL								
Unadjusted model	-0.01 (-0.09 to 0.06)	0.707	0.50 (0.22 to 0.77)	<0.001	0.06 (-0.04 to 0.15)	0.233	-0.05 (-0.13 to 0.03)	0.190
Model 1	0.00 (-0.07 to 0.08)	0.968	0.49 (0.21 to 0.76)	0.001	0.06 (-0.03 to 0.16)	0.184	-0.05 (-0.13 to 0.03)	0.242
Model 2	0.00 (-0.07 to 0.07)	0.980	0.40 (0.13 to 0.66)	0.004	0.06 (-0.03 to 0.15)	0.176	-0.03 (-0.11 to 0.04)	0.405

Model 1: age, sex. Model 2: age, sex, BMI, diabetes, smoking. Analysis of lipid profile restricted to participants not treated with lipid-lowering therapy drugs. Linear regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and lipid profile (dependent variables). Logistic regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and dyslipidemia (dependent variables). TSH and triglycerides were log-transformed. LDL, low-density lipoproteins; HDL, high-density lipoproteins; TSH, Thyroid-stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine. Significant associations are highlighted in bold.

cardiovascular risk which is more relevant from a clinical perspective. Furthermore, this is one of the first studies to use the recently published SCORE2/SCORE2-OP to evaluate the global cardiovascular risk, which is the most robust cardiovascular risk score currently available for European populations.

There are limitations that should also be acknowledged. First, the cross-sectional nature of the study limits the ability to draw conclusions on causation. Second, thyroid function has an important inter-individual variation that may have underestimated the associations we found (67); in addition, thyroid function was assessed at a single time point for each participant, and acute illness or other interfering factors may transiently affect thyroid hormone levels. Third, despite the differences in TSH distribution according to age, we used a single TSH reference range since these alterations are highly variable among individuals (68). We only evaluated the free thyroid hormones concentration, which are considered to represent the biologically active fraction of thyroid hormones (69). Different associations could have been found if we had evaluated total

thyroid hormones concentration. Furthermore, we did not evaluate the association of surrogate markers of low thyroid hormone tissue levels (22, 70) with cardiovascular risk factors. Also, since it was not possible to measure anti-thyroid antibodies in frozen serum samples, we cannot evaluate the influence of thyroid autoimmunity on cardiovascular risk factors. We evaluated the association of thyroid function with multiple outcomes which increases the probability of obtaining significant results due to chance; given the exploratory nature of our study, no adjustment for multiple comparison was performed. Our results on the association of thyroid function with cardiovascular risk were based on a prediction model and not on cardiovascular events. Finally, despite the adjustment for main known confounders, we cannot exclude the presence of residual confounding (e.g., related to diet or exercise).

In conclusion, in a population-based study of individuals aged 45 years or older, variations of thyroid function within the euthyroid range were associated with cardiovascular risk factors. On one hand, individuals with higher BMI, larger waist circumference and higher

TABLE 5 Association of thyroid function with hypertension, systolic and diastolic blood pressure, kidney function, and chronic kidney disease.

	TSH (per $\mu\text{IU/mL}$)		FT4 (per ng/dL)		FT3 (per pg/mL)		FT3/FT4 ratio (per unit)	
	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value
Outcomes								
Hypertension								
Unadjusted	1.12 (0.84-1.49)	0.453	4.33 (1.48-12.68)	0.008	0.84 (0.59-1.20)	0.339	0.68 (0.51-0.93)	0.014
Model 1	1.03 (0.76-1.40)	0.732	2.94 (0.94-9.17)	0.064	1.13 (0.77-1.67)	0.532	0.88 (0.64-1.22)	0.450
Model 2	1.04 (0.75-1.43)	0.828	2.93 (0.89-9.62)	0.077	1.06 (0.70-1.58)	0.794	0.83 (0.59-1.17)	0.299
CKD								
Unadjusted	1.36 (0.86-2.16)	0.191	9.73 (1.79-52.95)	0.009	0.49 (0.27-0.90)	0.021	0.39 (0.23-0.68)	<0.001
Model 1	1.47 (0.88-2.46)	0.145	4.14 (0.66-25.88)	0.128	0.75 (0.39-1.44)	0.386	0.62 (0.35-1.10)	0.100
Model 2	1.52 (0.90-2.56)	0.115	4.07 (0.64-25.92)	0.137	0.73 (0.37-1.41)	0.342	0.61 (0.34-1.09)	0.093
	TSH (per $\mu\text{IU/mL}$)		FT4 (per ng/dL)		FT3 (per pg/mL)		FT3/FT4 ratio (per unit)	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
Outcomes								
Systolic pressure, mmHg								
Unadjusted model	0.74 (-2.07 to 3.56)	0.604	9.08 (-1.28 to 19.44)	0.086	-0.29 (-3.80 to 3.22)	0.871	-1.73 (-4.68 to 1.22)	0.251
Model 1	0.27 (-2.40 to 2.93)	0.844	3.37 (-6.40 to 13.14)	0.499	2.98 (-0.36 to 6.31)	0.080	1.42 (-1.42 to 4.25)	0.327
Model 2	0.26 (-2.33 to 2.86)	0.843	3.06 (-6.50 to 12.62)	0.530	2.31 (-0.95 to 5.57)	0.165	0.96 (-1.81 to 3.73)	0.497
Diastolic pressure, mmHg								
Unadjusted model	1.57 (-0.04 to 3.18)	0.056	0.97 (-4.98 to 6.92)	0.750	1.07 (-0.94 to 3.09)	0.294	0.62 (-1.08 to 2.31)	0.475
Model 1	1.45 (-0.17 to 3.07)	0.079	2.00 (-3.94 to 7.94)	0.510	0.60 (-1.43 to 2.63)	0.560	0.10 (-1.62 to 1.83)	0.906
Model 2	1.46 (-0.09 to 3.01)	0.066	2.31 (-3.42 to 8.05)	0.429	-0.05 (-2.00 to 1.91)	0.964	-0.42 (-2.08 to 1.24)	0.620
eGFR, mL/min/1.73 m^2								
Unadjusted model	-3.28 (-5.35 to -1.22)	0.002	-9.21 (-16.88 to -1.54)	0.019	6.63 (4.07 to 9.19)	<0.001	6.05 (3.90 to 8.20)	<0.001
Model 1	-2.70 (-4.43 to -0.97)	0.002	-2.12 (-8.52 to 4.28)	0.516	2.87 (0.69 to 5.05)	0.010	2.41 (0.56 to 4.25)	0.011
Model 2	-2.72 (-4.45 to -0.99)	0.002	-1.85 (-8.28 to 4.57)	0.571	2.76 (0.57 to 4.94)	0.013	2.30 (0.44 to 4.15)	0.015

Model 1: age, sex. Model 2: age, sex, BMI, diabetes, smoking (diabetes not included in model for diabetes). Analysis of fasting plasma glucose and HOMA-IR restricted to participants not treated with antidiabetic drugs. Linear regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and blood pressure, kidney function (dependent variables). Logistic regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) and hypertension and CKD (dependent variables). TSH was log-transformed. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; TSH, Thyroid-stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine. Significant associations are highlighted in bold.

TABLE 6 Association of thyroid function with cardiovascular risk categories.

Cardiovascular risk category	TSH			Cardiovascular risk category	FT4		
	Lower tercile (0.36-1.02 $\mu\text{IU/mL}$)	Middle tercile (1.03-1.52 $\mu\text{IU/mL}$)	Higher tercile (1.53-4.86 $\mu\text{IU/mL}$)		Lower tercile (0.74-1.01 ng/dL)	Middle tercile (1.02-1.12 ng/dL)	Higher tercile (1.13-1.46 ng/dL)
Low-moderate risk	40.5%	40.8%	44.4%	Low-moderate risk	47.7%	42.0%	35.1%
High risk	31.9%	33.2%	32.1%	High risk	30.9%	31.5%	35.1%
Very high risk	27.6%	26.0%	23.5%	Very high risk	21.4%	26.5%	29.8%
Cardiovascular risk category	FT3			Cardiovascular risk category	FT3/FT4 ratio		
	Lower tercile (1.71-2.33 pg/mL)	Middle tercile (2.34-2.70 pg/mL)	Higher tercile (2.71-3.69 pg/mL)		Lower tercile (1.35-2.15)	Middle tercile (2.16-2.55)	Higher tercile (2.56-4.18)
Low-moderate risk	38.1%	41.0%	46.9%	Low-moderate risk	37.3%	37.4%	50.8%
High risk	29.3%	36.2%	31.4%	High risk	32.5%	33.1%	31.6%
Very high risk	32.6%	22.8%	21.7%	Very high risk	30.2%	29.5%	17.6%

Cardiovascular disease risk categories were based on SCORE2 and SCORE2-OP according to the European Society of Cardiology classification: low-moderate risk (<50 years: risk <2.5%; 50–69 years: <5%; ≥ 70 years: <7.5%), high risk (<50 years: 2.5–7.5%; 50–69 years: 5–10%; ≥ 70 years: 7.5–15%) or very high risk (<50 years: $\geq 7.5\%$; 50–69 years: $\geq 10\%$; ≥ 70 years: $\geq 15\%$). P for trend across terciles - TSH: $p=0.278$; FT4: $p=0.004$; FT3: $p=0.009$; FT4/FT3 ratio: $p<0.001$. TSH, Thyroid-stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine. Significant associations are highlighted in bold.

hs-CRP had higher levels of FT3 and FT3/FT4 ratio. On the other hand, a decreased conversion of T4 to T3 (lower FT3, lower FT3/FT4 ratio and/or higher FT4) was associated with a higher prevalence of diabetes, a more adverse lipid profile, a lower eGFR and an increased 10-year estimated risk of cardiovascular events. Further studies, ideally randomized clinical trials, are needed to evaluate if the modulation of thyroid hormones axis may be a therapeutic target to decrease cardiovascular risk.

Data availability statement

The datasets presented in this article are not readily available because the dataset used in this analysis is managed by Instituto de Saúde Pública da Universidade do Porto (ISPUP). Requests to access the datasets should be directed to <https://ispup.up.pt/en/cohorts>.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics committee of Centro Hospitalar Universitário São João/Faculdade de Medicina da Universidade do Porto. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JN, MB-C, AL, MH and CV contributed to the conception and design of this work, the statistical analysis, and interpretation of data. RF-C, SM, JG, DC, AA and AL-M contributed to interpretation of data. JN, MB-C, MH, CV, and AL prepared the first draft of this paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2022.1067801/full#supplementary-material>

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Supplementary Material

Supplementary Table 1. Association of thyroid function with 10-year risk of cardiovascular disease (SCORE2 and SCORE2-OP)

	TSH (per $\mu\text{IU/mL}$)		FT4 (per ng/dL)		FT3 (per pg/mL)		FT3/FT4 ratio (per unit)	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
Outcomes								
10-year risk of cardiovascular disease								
Unadjusted	-0.07 (-0.20 to 0.06)	0.277	0.78 (0.30 to 1.26)	0.002	-0.28 (-0.44 to -0.12)	0.001	-0.33 (-0.46 to -0.19)	<0.001
Adjusted	-0.07 (-0.20 to 0.06)	0.287	0.77 (0.29 to 1.25)	0.002	-0.29 (-0.45 to -0.14)	<0.001	-0.34 (-0.47 to -0.20)	<0.001

Adjusted model: BMI. Linear regression models were used to evaluate the associations of TSH, FT3, FT4 and FT3/FT4 ratio (independent variables) with 10-year risk of cardiovascular disease (dependent variables). Patients with diabetes were excluded from these analyses. 10-year risk of fatal and non-fatal cardiovascular events was calculated using SCORE2 and SCORE2-OP. TSH and the 10 year risk of cardiovascular events were log-transformed. TSH: Thyroid-stimulating hormone; FT4: free thyroxine; FT3: free triiodothyronine. SCORE2: Systematic Coronary Risk Estimation 2; SCORE2-OP: Systematic Coronary Risk Estimation 2-Older Persons.



Article 2

Preoperative thyroid function and weight loss after bariatric surgery

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Preoperative thyroid function and weight loss after bariatric surgery

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Abstract

Thyroid function has an important role on body weight regulation. However, the impact of thyroid function on weight loss after bariatric surgery is still largely unknown. We evaluated the association between preoperative thyroid function and the excess weight loss 1 year after surgery, in 641 patients with morbid obesity who underwent bariatric surgery. Patients with a history of thyroid disease, treatment with thyroid hormone or antithyroid drugs and those with preoperative evaluation consistent with overt hypothyroidism or hyperthyroidism were excluded. The preoperative levels of TSH and FT4 were not associated with weight loss after bariatric surgery. The variation of FT3 within the reference range was also not associated with weight loss. In contrast, the subgroup with FT3 above the reference range (12.3% of patients) had a significantly higher excess weight loss than patients with normal FT3. This difference remained significant after adjustment for age, sex, BMI, type of surgery, TSH and FT4. In conclusion, we observed an association between high FT3 and a greater weight loss after bariatric surgery, highlighting a group of patients with an increased benefit from this intervention. Our results also suggest a novel hypothesis: the pharmacological modulation of thyroid function may be a potential therapeutic target in patients undergoing bariatric surgery.

Introduction

Obesity affects more than 600 million adults worldwide, being associated with several comorbidities and a

significant increase in mortality [1]. From the available treatment options, bariatric surgery is the treatment that is associated with greater weight loss [2], being recommended in patients with a body mass index (BMI) of 40 kg/m² or greater and in patients with >35 kg/m² with obesity-related comorbidities who have not met weight loss goals with diet and exercise [1]. One of the important gaps in our knowledge about bariatric surgery is the prediction of weight loss after the procedure [3]. Given the great variability of weight loss after bariatric surgery [4], the ability to predict those patients that will better respond to the surgical procedure may improve the clinical management of patients with obesity.

Thyroid hormone has a central role in energy expenditure and body weight balance [5]. Thyroid hormone promotes an increase in basal metabolic rate and adaptive thermogenesis promoting a decrease of adiposity and body weight. Despite the relevant role of thyroid function on weight regulation, the impact of preoperative thyroid function on weight loss after bariatric surgery remains largely unknown. Therefore, our aim was to evaluate the impact of baseline thyroid function on weight loss after bariatric surgery.

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Methods

We performed a retrospective observational study evaluating the effect of preoperative thyroid function on weight loss in patients with morbid obesity submitted to bariatric surgery in our institution between January 2010 and June 2015. All study participants were treated according to the usual clinical care. No blinding procedures were used for patients or clinicians. Patients were excluded if they had history of thyroid disease, treatment with thyroid hormone or antithyroid drugs or if they presented preoperative thyroid evaluation consistent with overt hypothyroidism (elevated thyroid-stimulating hormone (TSH) with low free thyroxine (FT4)) or overt hyperthyroidism (low TSH with high FT4 and/or high triiodothyronine (FT3)). Patients missing preoperative TSH, FT4 or FT3 were also excluded.

The following preoperative parameters were evaluated: age, sex, body mass index (BMI), blood pressure (BP), thyroid function (TSH, FT4, FT3); history of diabetes, dyslipidemia and hypertension; and the type of bariatric surgery performed (laparoscopic adjustable gastric band, Roux-en-Y gastric bypass or sleeve gastrectomy). Excess weight loss in percentage (EWL) 12 months after surgery was evaluated using the formula: $[(\text{preoperative weight} - \text{current weight}) / (\text{preoperative weight} - \text{ideal weight to produce BMI } 25 \text{ kg/m}^2)] \times 100$, as previously described [6].

To evaluate the effect of variations of thyroid function within the normal range, we divided the study subjects in tertiles of normal reference range interval of TSH, FT4 and FT3 (TSH 0.35–4.94 mIU/L, FT4 0.70–1.48 ng/dL or FT3 1.71–3.71 pg/mL). We also evaluated this effect outside the reference range. Given that <2% of the patients presented TSH below or above the reference range, FT4 below or above the reference range and FT3 below the reference range, only the effect of FT3 above the reference range (12.3% of the population) was evaluated. Patients were subdivided into two groups: normal FT3 group (FT3 between 1.71 and 3.71 pg/mL) and high FT3 group (FT3 superior to 3.71 pg/mL).

To evaluate the effect of preoperative thyroid function on EWL after surgery we used simple linear regression and multiple linear regression models. We built two different models: one model including sex, age, baseline BMI and type of surgery (model 1) and another model including all the covariates in model 1 and preoperative thyroid function (TSH and FT4 in the model of high FT3 and FT3 tertiles; TSH and FT3 in the model of FT4 tertiles; FT3 and FT4 in the model of TSH tertiles) (model 2). For comparison between two groups, independent t-tests and chi-square test were performed. Differences between multiple groups were analyzed by one-way ANOVA. Statistical assumptions for

these tests were evaluated and no violations were detected. Results are presented as mean $\pm$ SD for continuous variables and as percentages for categorical variables.

We calculated that to detect a difference of EWL of 10% between two groups (with the smaller group having 10% of the sample size) with a power of 80% and a significance level of 5%, a sample size of 550 patients would be needed. Statistical analysis was performed with Stata 14.2 (Stata-Corp). We considered a two-sided *P* value <0.05 to be statistically significant.

All procedures performed in this study were in accordance with the ethical standards of the institution, the national research committee and with the Helsinki Declaration and its later amendments.

Results

Six hundred forty-one patients met the inclusion and exclusion criteria and were included in our analysis. Eighty-eight percent of the patients were female, the mean age was 41.8 ± 10.7 years and the mean preoperative BMI was $44.6 \pm 5.9 \text{ kg/m}^2$ (Table 1). Thirty-three percent of the patients had diabetes, 46.7% dyslipidemia and 62.2% hypertension. Fifty-nine percent of the patients were submitted to Roux-en-Y gastric bypass, 24.6% to laparoscopic adjustable gastric band and 16.9% to sleeve gastrectomy. Regarding the preoperative thyroid function, the mean serum TSH level was $2.00 \pm 1.01 \text{ mIU/L}$, FT4 was $1.05 \pm 0.14 \text{ ng/dL}$ and FT3 was $3.21 \pm 0.46 \text{ pg/mL}$. Four patients (0.6%) had TSH below the reference range and 11 (1.7%) had TSH above the reference range. No patient presented low levels of FT4 or FT3 and only 4 patients (0.6%) had FT4 above the reference range. On the other hand, 79 patients (12.3%) of the patients had FT3 above the reference range ($>3.71 \text{ ng/dL}$).

The group of patients with high FT3 was not significantly different regarding sex distribution, type of surgery, BMI or the prevalence of comorbidities (Table 1). The high FT3 group was significantly younger than the normal FT3 group (36.7 ± 10.5 vs 42.6 ± 10.5 years, $p < 0.001$). Patients with high FT3 also had higher serum levels of TSH (2.46 ± 1.20 vs $1.94 \pm 0.96 \text{ mIU/L}$, $p < 0.001$) and FT4 (1.11 ± 0.14 vs 1.04 ± 0.14 , $p < 0.001$).

Twelve months after the surgery, the mean EWL was $66.7 \pm 25.7 \%$ with a mean BMI decrease of $12.9 \pm 5.7 \text{ kg/m}^2$. There were no significant differences in the EWL 12 months after bariatric surgery according to TSH tertiles within the normal range, FT4 tertiles or FT3 tertiles (Table 2), even after adjustment for covariates.

Patients with high FT3 levels presented a significantly greater weight loss after bariatric surgery compared with patients with normal FT3 levels (75.0 ± 24.4 vs 65.5 ± 25.7

Table 1 Baseline characteristics of the study population in the total group and divided in normal FT3 and high FT3 groups

	Total population (n = 641)	Normal FT3 (n = 562; 87.7%)	High FT3 (n = 79; 12.3%)	P value
Sex				0.639
Male (%)	12.3%	13.7%	12.1%	
Female (%)	87.7%	86.3%	87.9%	
Age, years	41.8 ± 10.7	42.6 ± 10.5	36.7 ± 10.5	<0.001
Type of surgery				0.090
Adjustable gastric band	24.6%	25.8%	16.5%	
Roux-en-Y gastric bypass	58.5%	56.9%	69.6%	
Sleeve gastrectomy	16.9%	17.3%	13.9%	
BMI, kg/m ²	44.6 ± 5.9	44.5 ± 5.9	45.5 ± 6.0	0.133
Diabetes, %	33.1%	34.0%	26.6%	0.190
Dyslipidemia, %	46.7%	45.9%	51.9%	0.534
Hypertension, %	62.2%	63.1%	55.7%	0.202
Systolic BP, mm Hg	133.1 ± 16.9	133.1 ± 16.9	133.3 ± 16.9	0.923
Diastolic BP, mm Hg	83.5 ± 11.3	83.2 ± 11.2	85.7 ± 11.8	0.078
Waist circumference, cm	124.7 ± 13.7	124.3 ± 13.3	127.1 ± 16.2	0.100
Hip circumference, cm	133.3 ± 11.5	133.1 ± 11.4	134.9 ± 11.7	0.133
Waist to hip ratio	0.93 ± 0.09	0.94 ± 0.09	0.94 ± 0.08	0.537
FT4, ng/dL	1.05 ± 0.14	1.04 ± 0.14	1.11 ± 0.14	<0.001
TSH, mIU/L	2.00 ± 1.01	1.94 ± 0.96	2.46 ± 1.20	<0.001

Statistically significant p values are presented in bold

Table 2 Excess weight loss in percentage 12 months after bariatric surgery according to preoperative thyroid function

	Excess weight loss (%) 12 months after bariatric surgery	Additional excess weight loss (%) 12 months after bariatric surgery		
		Unadjusted	Model 1	Model 2
Normal FT3 (1.74–3.71 pg/mL)	65.5 ± 25.7	(Reference category)	(Reference category)	(Reference category)
High FT3 (3.72–4.68 pg/mL)	75.0 ± 24.4	9.5 (3.5 to 15.5)*	4.9 (0.2 to 9.6)*	5.5 (0.7 to 10.4)*
<i>Normal FT3 tertiles</i>				
Lowest tertile (1.74–2.97 pg/mL)	64.4 ± 25.6	(Reference category)	(Reference category)	(Reference category)
Middle tertile (2.98–3.31 pg/mL)	66.3 ± 26.4	1.9 (−3.3 to 7.1)	−0.5 (−4.4 to 3.5)	−0.4 (−4.4 to 3.6)
Higher tertile (3.32–3.71 pg/mL)	65.8 ± 25.1	1.4 (−3.9 to 6.6)	−2.4 (−6.4 to 1.6)	−2.3 (−6.4 to 1.9)
<i>Normal FT4 tertiles</i>				
Lowest tertile (0.71–0.98 ng/dL)	65.5 ± 25.1	(Reference category)	(Reference category)	(Reference category)
Middle tertile (0.99–1.10 ng/dL)	68.4 ± 27.7	2.9 (−2.0 to 7.7)	1.9 (−1.9 to 5.6)	1.7 (−2.1 to 5.3)
Higher tertile (1.11–1.47 ng/dL)	66.6 ± 24.0	1.1 (−3.8 to 6.0)	0.5 (−3.3 to 4.3)	0.3 (−3.6 to 4.3)
<i>Normal TSH tertiles</i>				
Lowest tertile (0.46–1.43 mIU/L)	65.4 ± 27.7	(Reference category)	(Reference category)	(Reference category)
Middle tertile (1.44–2.19 mIU/L)	67.2 ± 27.3	1.8 (−3.1 to 6.7)	0.1 (−3.7 to 3.9)	−0.1 (−3.9 to 3.8)
Higher tertile (2.20–4.91 mIU/L)	68.3 ± 21.6	2.9 (−2.1 to 7.8)	0.2 (−3.7 to 4.0)	0.0 (−3.9 to 3.9)

Model 1: adjusted for sex, age, baseline BMI and type of surgery. Model 2: adjusted for covariates in Model 1 and preoperative thyroid function (TSH and FT4 in the model of high FT3 and FT3 tertiles, TSH and FT3 in the model of FT4 tertiles, and FT3 and FT4 in the model of TSH tertiles)

* $p < 0.05$

%, $p = 0.002$). High FT3 levels remained significantly associated with EWL even after adjustment for sex, age, baseline BMI and type of surgery (additional EWL in the

presence of high FT3: 4.9% (0.2–9.6%), $p = 0.042$) and after adjustment for sex, age, baseline BMI, type of surgery and preoperative TSH and FT4 levels (additional EWL in

the presence of high FT3: 5.5% (0.7–10.4%), $p = 0.026$; Table 2).

Discussion

Our study showed a greater weight loss after bariatric surgery among patients with high preoperative FT3 levels compared with patients with normal FT3 levels. On the other hand, variations of FT3, FT4 and TSH within the normal range were not associated with weight loss after surgery.

Several studies have evaluated the effect of bariatric surgery on thyroid function [7–13], however, few evaluated the association between preoperative thyroid function and weight loss after surgery. Both Janssen et al [11]. and Moulin de Moraes et al [12]. evaluated the effect of high TSH levels and reported no significant differences in subsequent weight loss.

Recently, Liu G et al [14]. and Wolters et al [15]. also described, in patients submitted to lifestyle interventions, a greater weight loss among those with higher FT3 levels suggesting a relevant role of thyroid hormone axis in the response to weight loss interventions.

The observation that a significant proportion of patients (12.3%) presented high FT3 levels is also of interest. An increase of FT3 has been described previously in patients with morbid obesity [16, 17]. Furthermore, patients with morbid obesity have also been described to present an increased TSH levels [18, 19], suggesting a compensatory activation of hypothalamus-pituitary-thyroid axis in obese patients [20]. Accordingly, in our study, patients with high FT3 levels also presented higher TSH levels compared with patients with normal FT3 level. While the higher FT3 levels may not be sufficient to promote significant weight loss alone in patients with morbid obesity, our study suggests that higher FT3 levels may be a predictor of a greater weight loss after bariatric surgery.

We must acknowledge limitations of our work. As we only evaluated thyroid function before surgery, we cannot evaluate the effect of variation of thyroid function after bariatric surgery on weight loss. Further studies evaluating the correlation between postoperative FT3 levels and EWL are needed to confirm if FT3 levels are directly correlated with EWL. Furthermore, we only evaluated the association with weight loss 1 year after bariatric surgery and so, our results must not be extrapolated to the long-term prediction of weight loss after bariatric surgery.

From a clinical perspective, the main finding of our study is that high FT3 levels are associated with a greater weight loss after bariatric surgery. This is of relevance as it highlights a group of patients with an increased benefit from this intervention. Furthermore, our results also suggest a novel

hypothesis: the pharmacological modulation of the thyroid function may be a potential therapeutic target in patients submitted to bariatric surgery.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Article 3

Effect of Weight Loss after Bariatric Surgery on Thyroid- Stimulating Hormone Levels in Patients with Morbid Obesity and Normal Thyroid Function

Neves JS, Castro Oliveira S, Souteiro P, Pedro J, Magalhães D, Guerreiro V, Bettencourt-Silva R, Costa MM, Cristina Santos A, Queirós J, Varela A, Freitas P, Carvalho D; AMTCO Group.

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Effect of Weight Loss after Bariatric Surgery on Thyroid-Stimulating Hormone Levels in Patients with Morbid Obesity and Normal Thyroid Function

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Abstract

Background Several studies have reported that morbid obesity is associated with increased thyroid-stimulating hormone (TSH) levels. However, it is not clear what is the impact of bariatric surgery on postoperative thyroid function. The aim of this study was to evaluate the effect of weight loss after bariatric surgery on TSH levels in euthyroid patients with morbid obesity.

Methods We performed a retrospective observational study of 949 euthyroid patients (86.1% female; age 42.0 ± 10.3 years, BMI 44.3 ± 5.7 kg/m²) with morbid obesity submitted to bariatric surgery (laparoscopic adjustable gastric band, Roux-en-Y gastric bypass, or sleeve gastrectomy). Patients were subdivided in two groups: normal TSH group (TSH <2.5 mU/L) and high-normal TSH group (TSH ≥ 2.5 mU/L). The impact of anthropometric parameters, comorbidities,

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TSH, free thyroxine (FT4), free triiodothyronine (FT3), type of surgery, and excessive body weight loss (EBWL) on TSH variation 12 months after surgery was evaluated.

Results The high-normal TSH group (24.3% of patients) included more women, presented a higher BMI, higher systolic blood pressure, and higher FT3 levels. There was a significant decrease of TSH 12 months after surgery that was more marked in the high-normal TSH group (normal TSH group: 1.57 ± 0.49 to 1.53 ± 0.69 mIU/L, $p = 0.063$; high-normal TSH group: 3.23 ± 0.59 to 2.38 ± 0.86 mIU/L, $p < 0.001$). In a multivariate analysis, after adjusting for relevant covariates, EBWL, baseline BMI, and baseline FT3 were significantly associated with TSH decrease 12 months after bariatric surgery.

Conclusion Bariatric surgery promotes a decrease of TSH that is significantly greater in patients with high-normal TSH and is independently associated with EBWL after surgery.

Keywords Morbid obesity · Thyroid function · Thyroid-stimulating hormone · Bariatric surgery

Introduction

Obesity is one of the most common diseases in developed countries, affecting more than 600 million adults worldwide [1]. It is associated with several comorbidities and endocrine abnormalities, including thyroid dysfunction [2]. The most effective treatment of obesity to date is bariatric surgery [3, 4]. Several comorbidities and endocrine abnormalities are known to reverse after bariatric surgery [3–6].

Thyroid hormone axis plays an important role in body weight balance, with hypothyroidism being associated with weight gain and hyperthyroidism promoting weight loss [2, 7]. Even within the normal range, variations of thyroid function have been associated with significant weight variation [8, 9]. On the other hand, obesity, particularly morbid obesity, has been associated with significant alterations of thyroid function [10, 11]. Most studies reported increased serum thyroid-stimulating hormone (TSH) concentrations in patients with morbid obesity [10, 11]. The mechanism and the clinical implications of this alteration remains uncertain. Furthermore, the effect of bariatric surgery on postoperative thyroid function remains incompletely understood with previous studies showing contradictory results regarding the variation of TSH after bariatric surgery and the relation of TSH variation with postoperative weight loss [12–19].

Therefore, our aim was to evaluate the effect of weight loss after bariatric surgery on TSH variation in patients with morbid obesity and normal thyroid function.

Materials and Methods

Study Design and Participants

We performed a retrospective observational study evaluating patients with morbid obesity and normal preoperative thyroid function submitted to bariatric surgery in our institution between January 2010 and June 2015. Patients were excluded if they had a history of thyroid disease, treatment with thyroid hormone, antithyroid drugs, amiodarone or lithium, or if TSH or free thyroxine (FT4) was not within the normal reference range before the surgery (TSH <0.35 or >4.94 mIU/L, or FT4 <0.70 or >1.48 ng/dL). Patients missing preoperative TSH or FT4 and those missing TSH 1 year after surgery were also excluded. Of the 1450 patients submitted to bariatric surgery in our institution during the study period, after applying the exclusion criteria, 949 patients were included in our analysis.

Patients were subdivided in two groups: normal TSH group that included patients with serum TSH levels below 2.5 mIU/L and high-normal TSH group including those with a serum TSH level equal or superior to 2.5 mIU/L. This cutoff was selected based on previous reports that indicate that more than 95% of healthy individuals have TSH levels below 2.5 mIU/L [20, 21].

Clinical Parameters Evaluated

The following preoperative parameters were evaluated: age, sex, body mass index (BMI), waist to hip ratio (WHR), blood pressure (BP), TSH, FT4, free triiodothyronine (FT3), history of diabetes, dyslipidemia and hypertension, and the type of bariatric surgery performed [laparoscopic adjustable gastric band (LAGB), Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy (SG)]. TSH serum levels 12 months after surgery and the excessive body weight loss in percentage (EBWL) 12 months after surgery were also evaluated.

Diabetes was defined by fasting plasma glucose ≥ 126 mg/dL, glycated hemoglobin $\geq 6.5\%$, 2-h plasma glucose after a 75-g oral glucose tolerance test ≥ 200 mg/dL, or the use of antihyperglycemic drugs. Hypertension was defined as systolic BP ≥ 140 mmHg, diastolic BP ≥ 90 mmHg or the use of antihypertensive drugs. Dyslipidemia was defined by the use of lipid-lowering agents, serum low-density lipoprotein (LDL) cholesterol ≥ 160 mg/dL, serum high-density lipoprotein (HDL) cholesterol <40 mg/dL, or serum triglycerides ≥ 200 mg/dL.

EBWL was calculated using the formula: [(preoperative weight–current weight) / (preoperative weight–ideal weight to produce BMI 25 kg/m^2)] $\times 100$.

TSH, FT4, and FT3 were measured on serum, obtained from blood samples during clinical evaluations, by

chemiluminescence immunoassay on the Abbott Diagnostics Architect system (Abbott Diagnostics).

Statistical Analysis

For continuous variables, independent *t* tests (for comparison between groups) or paired *t* tests (for comparisons within the same group at different times) were performed. Differences between groups regarding categorical variables were evaluated with chi-squared test. To evaluate the effect of preoperative parameters on TSH variation after surgery, we used simple linear regression and multiple linear regression models. Results are presented as mean $\pm$ standard deviation for continuous variables and as percentages for categorical variables. Statistical analyses were performed with Stata software, version 14.1 (StataCorp). We considered a two-sided *P* value less than 0.05 to be statistically significant.

Results

Baseline Population Characteristics

Among the 949 patients analyzed, 86.1% were female and the mean age was 42.0 ± 10.3 years. The patients presented a mean preoperative weight of 116.4 ± 18.6 kg, a mean preoperative BMI of 44.3 ± 5.7 kg/m², and a waist circumference of 123.7 ± 13.3 cm (Table 1). Thirty percent of the patients had diabetes, 44.1% dyslipidemia, and 60.6% hypertension. The baseline serum TSH level was 1.97 ± 0.88 mIU/L, FT4 was 1.05 ± 0.13 ng/dL, and FT3 was 3.20 ± 0.45 pg/mL. The surgical procedure most performed was RYGB (58.6% of patients), followed by LAGB (20.9%) and SG (20.6%).

Seven hundred twenty-three patients (75.7%) had a TSH level below 2.5 mIU/L and were included in the normal TSH group. Two hundred thirty-two (24.3%) were included in the high-normal TSH groups. The groups were not significantly different regarding most clinical and laboratorial parameters (Table 1), with exception of sex (92.6% female in high-normal TSH group vs 84.0% in normal TSH group, $p = 0.001$), BMI (45.1 ± 6.0 kg/m² in high-normal TSH group vs 44.1 ± 5.5 kg/m² in normal TSH group, $p = 0.016$), systolic blood pressure (135.9 ± 17.8 mmHg in high-normal TSH group vs 132.4 ± 17.3 mmHg in normal TSH group, $p = 0.011$), and FT3 (3.35 ± 0.47 pg/mL in high-normal TSH group vs 3.15 ± 0.44 pg/mL in normal TSH group, $p < 0.001$).

Weight Loss 12 months After Bariatric Surgery

The mean EBWL 12 months after bariatric surgery was $69.5 \pm 25.6\%$ and the mean BMI decrease after surgery was 13.19 ± 5.38 kg/m². The weight loss after bariatric surgery was not significantly different between groups (EBWL,

$69.6 \pm 26.4\%$ in normal TSH group vs $69.0 \pm 23.0\%$ in high-normal TSH group, $p = 0.736$; BMI decrease, 13.00 ± 5.42 kg/m² in normal TSH group vs 13.75 ± 5.21 kg/m² in high-normal TSH group, $p = 0.073$).

Regarding the weight loss per type of bariatric surgery, RYGB was associated with significantly higher EBWL and BMI decrease than SG and LAGB ($p < 0.001$), and SG was associated with significantly higher EBWL and BMI decrease than LAGB ($p < 0.001$). The mean EBWL was $79.1 \pm 19.4\%$ for RYGB, $70.8 \pm 22.8\%$ for SG, and $40.4 \pm 22.1\%$ for LAGB; and mean BMI decrease was 15.13 ± 4.26 kg/m² for RYGB, 13.46 ± 5.05 kg/m² for SG, and 7.30 ± 4.23 kg/m² for LAGB.

TSH Variation 12 months After Bariatric Surgery

We observed a significant decrease of TSH levels 12 months after surgery in the group of all patients. The mean TSH decreased from 1.97 ± 0.88 to 1.73 ± 0.82 mIU/L ($p < 0.001$). When evaluating by baseline TSH subgroup, the TSH decrease was significantly greater in the high-normal TSH group (0.85 ± 0.84 mIU/L decrease in the high-normal TSH group vs 0.05 ± 0.62 mIU/L in the normal TSH group), and only in the high-normal TSH group this decrease was statistically significant (normal TSH group 1.57 ± 0.49 – 1.53 ± 0.69 mIU/L, $p = 0.063$; high-normal TSH group 3.23 ± 0.59 – 2.38 ± 0.86 mIU/L, $p < 0.001$) (Fig. 1).

Predictors of TSH Decrease After Bariatric Surgery

In univariate analysis, younger age ($\beta = 0.007$, $p = 0.004$), higher BMI ($\beta = -0.011$, $p = 0.011$), higher baseline weight ($\beta = -0.004$, $p = 0.004$), and higher EBWL ($\beta = -0.036$, $p < 0.001$) were significantly associated with greater decrease of TSH after bariatric surgery. Higher baseline TSH ($\beta = -0.439$, $p < 0.001$) and higher FT3 levels ($\beta = -0.325$, $p < 0.001$) were also associated with greater decrease of TSH after bariatric surgery. In comparison with RYGB, LAGB was associated with a smaller TSH decrease ($\beta = 0.286$, $p < 0.001$), while no significant effect was observed comparing SG and RYGB. On the other hand, sex, the presence of diabetes, dyslipidemia or hypertension, or the systolic and diastolic pressure was not associated with the TSH variation after bariatric surgery (Table 2A).

In a multivariate analysis, after adjusting for relevant covariates, EBWL, baseline BMI, and baseline FT3 remained significantly associated with TSH decrease 12 months after bariatric surgery (Table 2B).

Discussion

In a population of patients with morbid obesity and normal baseline thyroid function, we observed a significant

Table 1 Baseline clinical characteristics of the total study population and by TSH groups

	Total population (<i>n</i> = 949)	Normal TSH (<i>n</i> = 718)	High-normal TSH (<i>n</i> = 231)	<i>p</i> value
Sex, %				0.001
Male	13.9%	16.0%	7.4%	
Female	86.1%	84.0%	92.6%	
Age, years	42.0 ± 10.3	41.9 ± 9.7	42.1 ± 12.0	0.852
Type of surgery				0.746
Adjustable gastric band	20.9%	21.0%	20.3%	
Roux-en-Y gastric bypass	58.6%	58.0%	60.6%	
Sleeve gastrectomy	20.6%	21.0%	19.1%	
Diabetes, %	30.1%	30.0%	31.6%	0.577
Dyslipidemia, %	44.1%	43.9%	44.8%	0.818
Hypertension, %	60.6%	59.4%	64.4%	0.183
Weight, kg	116.4 ± 18.6	116.3 ± 18.9	116.5 ± 17.5	0.867
BMI, kg/m ²	44.3 ± 5.7	44.1 ± 5.5	45.1 ± 6.0	0.016
Systolic BP, mmHg	133.3 ± 17.4	132.4 ± 17.3	135.9 ± 17.8	0.011
Diastolic BP, mmHg	83.0 ± 11.3	82.9 ± 11.0	83.4 ± 12.3	0.575
Waist circumference, cm	123.7 ± 13.3	123.2 ± 13.2	125.3 ± 13.3	0.059
Waist-to-hip ratio	0.93 ± 0.09	0.93 ± 0.09	0.93 ± 0.09	0.661
TSH, mIU/L	1.97 ± 0.88	1.57 ± 0.49	3.23 ± 0.59	<0.001
FT4, ng/dL	1.05 ± 0.13	1.05 ± 0.14	1.04 ± 0.13	0.115
FT3, pg/mL	3.20 ± 0.45	3.15 ± 0.44	3.35 ± 0.47	<0.001

Normal TSH group: TSH level superior or equal to 0.35 mIU/L and below 2.5 mIU/L; high-normal TSH group: TSH level superior or equal to 2.5 mIU/L and below or equal to 4.94 mIU/L

BMI body mass index, *BP* blood pressure, *TSH* thyroid-stimulating hormone, *FT4* free thyroxine, *FT3* free triiodothyronine

decrease in TSH levels after bariatric surgery. This TSH decrease was associated with postoperative weight loss and was significantly greater in the subgroup of patients with TSH >2.5 mIU/L.

Most [12–16], but not all [17–19], studies evaluating the variation of TSH after bariatric surgery have also shown a decrease of TSH after the procedure. Moulin de Moraes et al. [13] reported that in 54 euthyroid patients submitted to

Fig. 1 TSH variation 12 months after bariatric surgery. Normal TSH group: TSH level superior or equal to 0.35 mIU/L and below 2.5 mIU/L; high-normal TSH group: TSH level superior or equal to 2.5 mIU/L and below or equal to 4.94 mIU/L. *TSH* thyroid-stimulating hormone

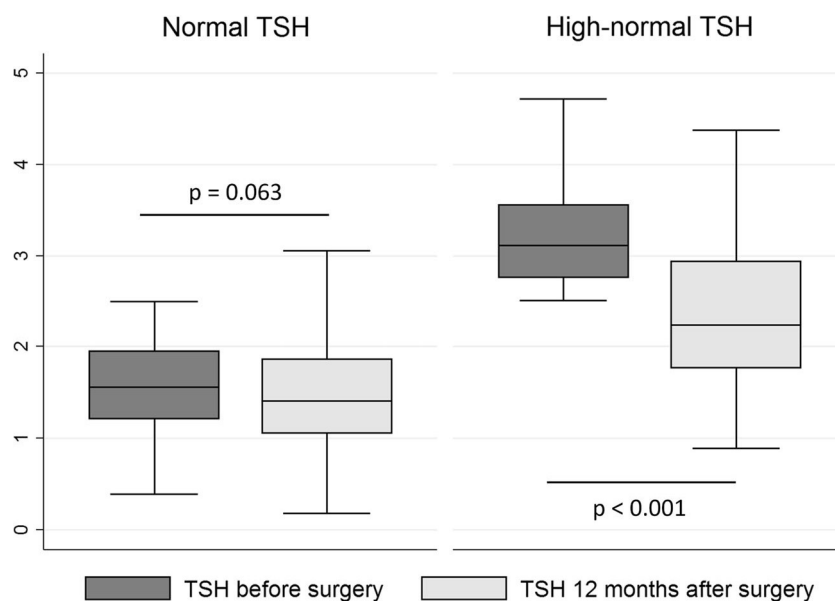


Table 2 TSH variation at 12 months after bariatric surgery

TSH variation 12 months after bariatric surgery		
A. Univariate analysis		
	β coefficient	<i>p</i> value
Excessive body weight loss (10%)	−0.036 (−0.055 to −0.017)	<0.001
Sex (female)	0.09 (−0.047 to 0.233)	0.193
Age, years	0.007 (0.002 to 0.012)	0.004
Type of surgery		
Roux-en-Y gastric bypass	(reference category)	
Sleeve gastrectomy	−0.068 (−0.191 to 0.055)	0.278
Adjustable gastric band	0.286 (0.164 to 0.408)	<0.001
Diabetes	0.014 (−0.092 to 0.120)	0.795
Dyslipidemia	−0.080 (−0.178 to 0.018)	0.111
Hypertension	0.015 (−0.086 to 0.116)	0.769
Preoperative weight, kg	−0.004 (−0.006 to −0.001)	0.004
Preoperative BMI, kg/m ²	−0.011 (−0.020 to −0.003)	0.011
Systolic blood pressure, mmHg	−0.001 (−0.003 to 0.003)	0.831
Diastolic blood pressure, mmHg	0.003 (−0.001 to 0.008)	0.178
Waist-to-hip ratio	−0.270 (−0.914 to 0.374)	0.410
Preoperative TSH, mIU/L	−0.439 (−0.487 to −0.391)	<0.001
Preoperative FT4, ng/dL	0.035 (−0.328 to 0.399)	0.848
Preoperative FT3, pg/mL	−0.325 (−0.464 to −0.187)	<0.001
B. Multivariate analysis		
	β coefficient	<i>p</i> value
Excessive body weight loss (10%)	−0.026 (−0.051 to −0.002)	0.036
Sex (female)	0.127 (−0.050 to 0.304)	0.160
Age, years	0.003 (−0.003 to 0.009)	0.328
Preoperative BMI, kg/m ²	−0.012 (−0.023 to −0.002)	0.024
Type of surgery		
Roux-en-Y gastric bypass	(reference category)	–
Sleeve gastrectomy	−0.066 (−0.244 to 0.113)	0.471
Adjustable gastric band	0.049 (−0.137 to 0.235)	0.605
Preoperative FT3, pg/mL	−0.246 (−0.386 to −0.107)	0.001

A: Univariate analysis of TSH variation at 12 months after bariatric surgery. B: Multivariate analysis of TSH variation at 12 months after bariatric surgery
BMI body mass index, *TSH* thyroid-stimulating hormone, *FT4* free thyroxine, *FT3* free triiodothyronine

RYGB there was a significant decrease in TSH that was not correlated to percent change in BMI and a significant decrease in FT3 but not in FT4. In a study of 38 euthyroid patients submitted to SG, there was also significant decrease in TSH 6–12 months after surgery that was directly related to baseline TSH and not related to EBWL, without significant variation of FT4 [12]. In agreement with our results, a study of 86 patients, not taking medication that could affect the thyroid function, submitted to RYGB or LAGB found a significant decrease of TSH that correlated with the BMI variation [14]. On the other hand, MacCuish et al. (55 patients submitted to RYGB) [17], Alagna et al. (38 patients submitted to biliopancreatic diversion) [18], and Dall'Asta et al. (258 patients submitted to

LAGB) [19] reported an absence of TSH variation after bariatric surgery. The differences between studies may be related to the type of surgery evaluated, the baseline characteristics of the patients or to the statistical power of the studies to detect differences in TSH and to detect associations with EBWL.

Although it was not evaluated in the current study, the variations of FT3 and FT4 levels after bariatric surgery are also relevant to understand the impact of this intervention on the hypothalamus-pituitary-thyroid axis. Most studies [13, 18, 19, 22] evaluating the effect of bariatric surgery on thyroid hormones showed a significant decrease of FT3. On the other hand, the impact of bariatric surgery on FT4 levels is more controversial with different studies reporting decreasing [22],

stable [12–14, 18], or increasing [17, 19] levels after bariatric surgery.

We must highlight that this is the largest study to date evaluating the effect of bariatric surgery on TSH variation in euthyroid patients with morbid obesity and that our evaluation was not restricted to a type of surgery, including three of the most common surgeries for treatment of morbid obesity. According to our results, the decrease of TSH after bariatric surgery is related to the EBWL, and this effect is independent of the type of surgery. As expected, patients submitted to RYGB and SG presented significantly higher EBWL than LAGB. Accordingly, the decrease in TSH levels was significantly smaller in the LAGB group comparing to RYGB and SG (Table 2A). After adjusting to EBWL, the type of surgery was no longer significantly associated with TSH variation (Table 2B), highlighting a more relevant role of weight loss than the type of surgery per se.

In our study, one fourth of the patients had high-normal TSH levels (>2.5 mU/L). This is in accordance with previous reports of an increase of TSH with increasing body weight [23–25]. Also, in agreement with this, the subgroup with high-normal TSH levels had a higher baseline BMI. The mechanism of TSH elevation in obesity is incompletely understood. The observation that weight loss is associated with decreases in TSH and FT3 has highlighted that TSH elevation may be a consequence and not the cause of obesity [11–16, 18, 19]. Even in patients with a clinical diagnosis of hypothyroidism, there are reports of decreased levels of TSH after bariatric surgery [13, 14, 26, 27]. In two studies, all the patients with untreated subclinical hypothyroidism reached normal values of TSH 12 months after bariatric surgery [13, 14].

The increase of TSH may represent a compensatory activation of hypothalamus-pituitary-thyroid axis in response to excessive body weight [28]. This activation appears to be mediated at least in part by hypothalamic or pituitary effects of leptin [29, 30]. In this study, the observation that FT3 is higher in high-normal TSH group further supports an activation of thyroid axis in morbid obesity.

Interestingly, the patients with higher TSH, those with higher baseline BMI and those with higher FT3 were those that presented a greater decrease of TSH 12 months after surgery. The mechanisms of TSH decrease after bariatric surgery remain incompletely understood and remain to be further elucidated. One of the most plausible explanations is the decrease of leptin levels following bariatric surgery [31]. With decreasing amount of body fat, the decreasing leptin circulating levels [32] reduce the central stimulation of the thyroid axis [29, 30] and promote a decrease of TSH. The observation that this decrease is only significant in the subgroup with baseline TSH >2.5 mU/L suggests that this decrease depends on the baseline overactivation of the thyroid axis. Morbid obesity is known to be a disease with a great heterogeneity of pathophysiological mechanisms and of endocrine adaptations [33]

and, probably, only a subgroup of patients present thyroid axis overactivation and consequently TSH decrease after obesity treatment. In summary, bariatric surgery appears to promote a normalization of the thyroid axis function that in the patients with greater baseline dysregulation of the axis will lead to a greater decrease of TSH.

From a clinical point of view, our study highlights that patients with morbid obesity and that present higher TSH levels tend to normalize after bariatric surgery. This points out that caution must be employed when interpreting TSH levels in patients with morbid obesity. Values approaching the upper-limit of the reference interval may not represent a subnormal thyroid function, but a compensatory response of the thyroid axis to morbid obesity. Furthermore, our study emphasizes that the use of narrower reference ranges of serum TSH with a decrease of the upper limit of serum TSH levels, as proposed by several authors [20, 21], is not appropriate to the diagnosis of thyroid diseases in patients with morbid obesity.

We must acknowledge some limitations of our work. First, we only evaluated FT3 and FT4 before surgery, and consequently we cannot evaluate the impact of the variation of these parameters on TSH after bariatric surgery. The absence of FT3 and FT4 levels after weight loss hampers interpretation of our findings. Second, we evaluated the variation of TSH using only two moments (before and 12 months after the surgery). As TSH levels may vary with acute illness or other interfering factors, the observed variation in individual patients may also reflect factors other than the effect of weight loss after bariatric surgery. However, as we used a large group of patients, we believe that this fact does not interfere with the interpretation of our results. And third, we only evaluated the effect on TSH levels 12 months after bariatric surgery and so, our results must not be extrapolated to the long-term effects of bariatric surgery on thyroid function. Given that most previous studies have only evaluated the effect on the first 6–24 months on a small number of patients, we believe that future studies with longer follow-up on a large number of patients are important in this field.

Conclusion

In patients with morbid obesity and normal thyroid function, bariatric surgery promotes a significant decrease of TSH levels 12 months after the procedure. This decrease is significantly greater in patients with baseline high-normal TSH levels and is independently associated with EBWL after bariatric surgery, baseline BMI and baseline FT3 levels.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent For this type of study formal consent is not required.

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Article 4

Thyroid Hormones within the Normal Range and Cardiac Function in the General Population: The EPIPorto Study

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Thyroid Hormones within the Normal Range and Cardiac Function in the General Population: The EPIPorto Study

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Keywords

Thyroid hormones · Cardiac function · Cardiac structure ·
Thyroid stimulating hormone · Free T4 · Free T3

Abstract

Background: Hypothyroidism and hyperthyroidism are associated with marked changes in cardiac structure and function. However, the association of thyroid function within the normal range with cardiac structure and function in the general population remains uncertain. **Methods:** Eight hundred thirty-five subjects aged ≥ 45 years from the EPIPorto cohort (evaluation between 2006 and 2008) were cross-sectionally analyzed. We excluded participants with TSH, free T4 (FT4), or free T3 (FT3) outside of the reference range or with self-reported cardiovascular or thyroid disease. Cardiac structure and function were evaluated by echocardiogra-

phy. We used linear regression models unadjusted and adjusted for sex and age (model 1), and sex, age, BMI, diabetes, hypertension, and smoking (model 2). Nonlinear associations were assessed using restricted cubic splines. **Results:** The mean age was 61.5 years (SD 10.5); 61.1% of the patients were women. In the adjusted model 2, heart rate was positively associated with FT3; diastolic blood pressure was positively associated with TSH; LV end-diastolic and end-systolic volumes were inversely associated with TSH, and ejection fraction was nonlinearly associated with FT3, with higher ejection fractions near the limits of the reference range. Left ventricle (LV) posterior wall thickness was nonlinearly associated with FT4 in the adjusted model 1, with a greater thickness near the limits of the reference range. Regarding diastolic function, no significant associations were observed in adjusted models. **Conclusions:** Thyroid function within the reference range was associated with heart rate, blood pres-

sure, cardiac structure, and function. Increasing thyroid function (lower TSH, higher FT4, or higher FT3) was associated with a higher heart rate, a lower diastolic blood pressure, and larger LV volumes. LV wall thickness and ejection fraction had a U-shaped association with thyroid hormones.

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Introduction

Cardiovascular disease is the leading cause of death worldwide. Traditional risk factors, including diabetes, dyslipidemia, and hypertension, contribute significantly to the development of cardiovascular disease [1]. Intensive treatment of these risk factors is associated with an important reduction of the risk of cardiovascular events [2–4]. However, even after correcting these risk factors, many patients still have a significant residual risk, highlighting the importance of understanding other mechanisms of cardiovascular dysfunction [5, 6].

Thyroid hormones play a critical role in cardiovascular system development and homeostasis [7]. Both overt hypothyroidism and hyperthyroidism contribute to a high risk of atherosclerotic disease and heart failure [8–10]. Even subclinical thyroid dysfunction is known to increase the cardiovascular risk, with both subclinical hypothyroidism and hyperthyroidism being associated with heart failure and atherosclerotic disease [11–15]. Furthermore, subclinical hyperthyroidism has been associated with an increased incidence of cardiac arrhythmias, particularly atrial fibrillation [15, 16], and subclinical hypothyroidism has been linked to an increased risk of hypertension [17, 18].

While the effects of overt and subclinical thyroid dysfunction on cardiac function have been well characterized [19, 20], the association of thyroid hormone levels within the reference range with cardiac function remains uncertain. Therefore, we aimed to evaluate the association between thyroid function within the reference range and cardiac function in the general population.

Materials and Methods

Study Design and Participants

The EPIPorto study is a population-based cohort study [21], ongoing for >20 years, with the main aim of assessing the determinants of health in the adult population of Porto, Portugal. For the baseline evaluation (1999–2003), 2,485 persons were randomly selected and have been repeatedly evaluated over time. Between October 2006 and July 2008, participants aged 45 years or older were eligible to

Table 1. Clinical and echocardiographic characteristics of the study sample

Age, years	61.5±10.5
Females	61.1
BMI	27.4±4.6
Diabetes mellitus	11.3
Dyslipidemia	47.8
Hypertension	54.8
Current smokers	14.7
Treatment with β -blockers	6.6
Heart rate, bpm	63.4±9.7
Blood pressure, mm Hg	
Systolic	132.9±19.6
Diastolic	78.3±11.3
LA volume, mL/m ²	28.5±9.6
LV geometry	
Septum, mm	8.6±1.5
Posterior wall, mm	7.9±1.2
LV mass, g/m ²	79.1±18.3
LVEDV, mL/m ²	66.0±16.0
LVESV, mL/m ²	26.6±8.9
Systolic function	
Ejection fraction, %	60.5±6.2
Diastolic function	
Mitral E/A ratio	0.97±0.32
Deceleration time, ms	235.8±53.6
E' velocity, cm/s	10.6±3.2
E/E' ratio	7.3±2.6

Values are presented as means $\pm$ SD or percents. The total number of patients is 835. LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume.

undergo a systematic evaluation of parameters of cardiac structure and function, which included a cardiovascular clinical history, a physical examination, a detailed anthropometric evaluation, collection of fasting blood samples, and a transthoracic echocardiogram. Among the 2,048 cohort members in the eligible age range at that time, 134 (6.5%) had died, 198 (9.7%) refused reevaluation, and 580 (28.3%) were lost to follow-up. We further excluded 301 patients with a previous myocardial infarction, percutaneous or surgical revascularization, a prior cardiac surgery, significant (moderate to severe) valvular heart disease, a history of thyroid disease, or treatment with drugs that interfere with thyroid function, as well as those without serum available for determination of thyroid function or with TSH, free thyroxine (FT4), or free triiodothyronine (FT3) outside of the reference range (0.35–4.94 μ IU/mL for TSH, 0.70–1.48 ng/dL for FT4, and 1.71–3.71 pg/mL for FT3). Eight hundred thirty-five participants were included in our analysis.

Thyroid Function Measurements

TSH, FT3, and FT4 were measured in serum samples stored at -80°C by an electrochemiluminescence immunoassay using an Abbott Architect i2000 analyzer (Abbott Diagnostics). It has been previously shown that TSH and thyroid hormones can be analyzed reliably in frozen stored samples [22].

Table 2. Linear association of thyroid function with heart rate and blood pressure

	TSH ($\mu\text{IU/mL}$)		FT4 (ng/dL)		FT3 (pg/mL)	
	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value
<i>Heart rate (bpm)</i>						
Unadjusted	-0.35 (-1.79 to 1.09)	0.634	2.98 (-2.32 to 8.28)	0.270	1.52 (-0.26 to 3.30)	0.094
Model 1 ^a	-0.76 (-2.20 to 0.68)	0.303	2.42 (-2.87 to 7.71)	0.370	1.86 (0.08 to 3.64)	0.041
Model 2 ^b	-0.75 (-2.17 to 0.67)	0.299	1.38 (-3.83 to 6.60)	0.603	1.99 (0.23 to 3.75)	0.026
<i>Blood pressure (mm Hg)</i>						
<i>Systolic</i>						
Unadjusted	0.58 (-2.32 to 3.49)	0.693	7.67 (-3.00 to 18.36)	0.159	-0.15 (-3.73 to 3.43)	0.935
Model 1 ^a	0.42 (-2.32 to 3.16)	0.763	1.69 (-8.35 to 11.74)	0.741	2.90 (-0.48 to 6.27)	0.093
Model 2 ^b	0.14 (-2.15 to 2.43)	0.906	-1.99 (-10.38 to 6.40)	0.642	1.99 (-0.84 to 4.81)	0.168
<i>Diastolic</i>						
Unadjusted	1.77 (0.10 to 3.44)	0.038	1.11 (-5.06 to 7.28)	0.724	1.03 (-1.03 to 3.10)	0.328
Model 1 ^a	1.88 (0.19 to 3.57)	0.029	1.62 (-4.59 to 7.83)	0.609	0.81 (-1.29 to 2.90)	0.450
Model 2 ^b	1.54 (0.10 to 2.98)	0.036	0.41 (-4.88 to 5.69)	0.880	-0.06 (-1.84 to 1.72)	0.948

The values shown were estimated by multiple linear regression, with TSH, FT4, or FT3 as independent variable and heart rate, systolic blood pressure, and diastolic blood pressure as dependent variables, adjusting for several variables as stated. TSH was log-transformed. Participants treated with β -blockers were excluded from heart rate and blood pressure analyses. Statistically significant associations are shown in bold. ^a Adjusted for age and sex. ^b Adjusted for age, sex, BMI, hypertension, diabetes, and current smoking.

Echocardiographic Evaluation and Blood Pressure Evaluation

All echocardiography studies were acquired by 1 of 4 cardiologists, using the same equipment (Hewlett-Packard Sonos 5500). Images were stored for later offline analysis by 2 experienced cardiologists who were blinded to clinical data. Cardiac chamber dimensions and volumes and left ventricular (LV) mass were indexed to body surface area. Diastolic function was assessed by measuring mitral inflow velocities (E-wave, A wave, and E/A ratio) and the E-wave deceleration time and the isovolumetric relaxation time using pulsed-wave Doppler in the apical 4-chamber view. Velocities were recorded at end expiration and averaged over 3 consecutive cardiac cycles. Pulsed-wave tissue Doppler velocities were acquired at end expiration, in the apical 4-chamber view, on the lateral side of the mitral annulus, measuring early diastolic (E') and late diastolic (A') velocities and estimating the E/E' ratio accordingly. Systolic function was evaluated by ejection fraction calculation using the modified biplane Simpson rule.

Blood pressure was determined by 2 measurements separated by at least 5 min after a 10-min rest. When the difference between measurements was larger than 5 mm Hg for systolic or diastolic blood pressure, a third measurement was taken and the mean of the 2 closest values was considered.

Comorbidities and Clinical Definitions

BMI was calculated as weight in kilograms divided by the square of height in meters. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg or a diastolic blood pressure ≥ 90 mm Hg at the time of the visit [23] or as use of antihypertensive drugs. Diabetes was defined as a fasting blood glucose level ≥ 126 mg/dL [24], a self-reported history of diabetes, or use of diabetes medications. Dyslipidemia was defined as low-density lipoprotein cholesterol ≥ 160 mg/dL, triglycerides ≥ 200 mg/dL, high-density lipoprotein cholesterol < 40 mg/dL [25], or use of lipid-lowering drugs.

Statistical Analysis

We used linear regression models to evaluate the associations of TSH, FT4, and FT3 (independent variables) with heart rate, blood pressure, cardiac structure, and cardiac function parameters (dependent variables). These associations were evaluated in unadjusted models and adjusted models for sex and age (model 1) and for sex, age, BMI, diabetes, hypertension, and current smoking (model 2). These variables were included as potential confounders due to their known effects on thyroid function [26, 27] and cardiac function [28]. Participants treated with β -blockers were excluded from analyses of heart rate, blood pressure, and cardiac function. As previous studies have shown some similar cardiac manifestations in participants with hypothyroidism and hyperthyroidism [29], we also assessed nonlinear associations using restricted cubic splines with 3 knots at the 10th, 50th, and 90th percentiles [30]. This approach allows the identification of changes in cardiac structure or function that occur with both increasing and decreasing thyroid function within the normal range.

Continuous variables are presented as means ($\pm$ SD). Categorical variables are presented as percentages. Comparisons between groups were made using *t* tests and the χ^2 test. Two-sided *p* < 0.05 was considered statistically significant. Statistical analyses were performed using Stata software, version 14.2 (StataCorp).

Results

Study Population

A total of 835 participants (510 women and 325 men) were included in our analysis after applying the inclusion and exclusion criteria. Participants who were ex-

Table 3. Linear association of thyroid function with cardiac structure

	TSH (μIU/mL)		FT4 (ng/dL)		FT3 (pg/mL)	
	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
<i>LA volume (mL/m²)</i>						
Unadjusted	-0.81 (-2.19 to 0.57)	0.250	2.42 (-2.72 to 7.57)	0.356	-2.44 (-4.16 to -0.72)	0.006
Model 1 ^a	-1.06 (-2.41 to 0.29)	1.123	0.56 (-4.47 to 5.58)	0.828	-1.38 (-3.08 to 0.32)	0.112
Model 2 ^b	-1.16 (-2.52 to 0.20)	0.095	0.71 (-4.35 to 5.76)	0.783	-1.60 (-3.31 to 0.11)	0.066
<i>LV geometry</i>						
<i>Septum (mm)</i>						
Unadjusted	-0.06 (-0.26 to 0.15)	0.593	0.65 (-0.11 to 1.42)	0.095	-0.26 (-0.52 to -0.003)	0.048
Model 1 ^a	0.003 (-0.19 to 0.20)	0.970	0.30 (-0.42 to 1.02)	0.406	-0.07 (-0.31 to 0.18)	0.582
Model 2 ^b	0.01 (-0.18 to 0.19)	0.953	0.17 (-0.51 to 0.84)	0.627	-0.15 (-0.38 to 0.08)	0.203
<i>Posterior wall (mm)</i>						
Unadjusted	-0.01 (-0.18 to 0.16)	0.883	0.40 (-0.22 to 1.02)	0.207	-0.09 (-0.30 to 0.12)	0.389
Model 1 ^a	0.05 (-0.11 to 0.21)	0.528	0.12 (-0.46 to 0.70)	0.678	0.06 (-0.13 to 0.26)	0.525
Model 2 ^b	0.04 (-0.11 to 0.18)	0.630	0.00 (-0.53 to 0.53)	1.000	0.00 (-0.18 to 0.18)	0.999
<i>LV mass (g/m²)</i>						
Unadjusted	-2.00 (-4.61 to 0.60)	0.132	9.48 (-0.17 to 19.13)	0.054	-3.57 (-6.82 to -0.31)	0.032
Model 1 ^a	-1.28 (-3.75 to 1.18)	0.307	5.17 (-3.93 to 14.26)	0.265	-1.16 (-4.27 to 1.94)	0.463
Model 2 ^b	-1.31 (-3.70 to 1.07)	0.281	2.93 (-5.88 to 11.74)	0.514	-1.71 (-4.72 to 1.29)	0.264
<i>LVEDV (mL/m²)</i>						
Unadjusted	-4.38 (-6.67 to -2.09)	<0.001	0.20 (-8.38 to 8.78)	0.963	0.44 (-2.44 to 3.32)	0.766
Model 1 ^a	-3.55 (-5.81 to -1.29)	0.002	0.36 (-8.07 to 8.79)	0.934	0.23 (-2.64 to 3.09)	0.877
Model 2 ^b	-3.70 (-5.98 to -1.42)	0.001	0.34 (-8.13 to 8.82)	0.936	0.03 (-2.85 to 2.91)	0.984
<i>LVESV (mL/m²)</i>						
Unadjusted	-2.46 (-3.74 to -1.18)	<0.001	1.39 (-3.40 to 6.17)	0.570	0.53 (-1.08 to 2.13)	0.521
Model 1 ^a	-2.03 (-3.29 to -0.76)	0.002	1.38 (-3.33 to 6.08)	0.566	0.49 (-1.11 to 2.09)	0.551
Model 2 ^b	-2.03 (-3.30 to -0.75)	0.002	1.36 (-3.40 to 6.11)	0.575	0.43 (-1.18 to 2.05)	0.597

The values shown were estimated by multiple linear regression, with TSH, FT4, or FT3 as the independent variable and cardiac structure parameters as dependent variables, adjusting for several variables as stated. TSH was log-transformed. LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume. Statistically significant associations are shown in bold. ^a Adjusted for age and sex. ^b Adjusted for age, sex, BMI, hypertension, diabetes, and current smoking.

cluded or lost to follow-up did not differ from those included at baseline regarding sex, BMI, or smoking status, but they were older and had a higher prevalence of comorbidities (online suppl. Table 1, see online Supplementary Materials). The general characteristics and echocardiographic findings of the included population are shown in Table 1. The mean age was 61.5 years (SD 10.5) years. Eleven percent of the participants had diabetes mellitus, nearly half had dyslipidemia (47.8%), and more than half had hypertension (54.8%).

Association of Thyroid Function with Heart Rate and Blood Pressure

The associations of thyroid function with heart rate and blood pressure in univariable and multivariable analysis are shown in Table 2. We observed that heart rate

increased significantly with increasing FT3 levels after adjustment for age and sex (model 1) and further adjustment for potential confounding factors of BMI, hypertension, diabetes, and smoking (model 2). Regarding blood pressure, mean diastolic blood pressure was positively associated with TSH levels both in univariate and in multivariate regression models.

Association of Thyroid Function with Cardiac Structure

Concerning cardiac structure (Table 3), both LV end-diastolic and end-systolic volumes were found to be inversely associated with TSH levels in the unadjusted and adjusted regression models. In addition, analysis by restricted cubic splines (Fig. 1) showed U-shaped associations between FT3 levels and LV cavity volumes in unadjusted and adjusted models.

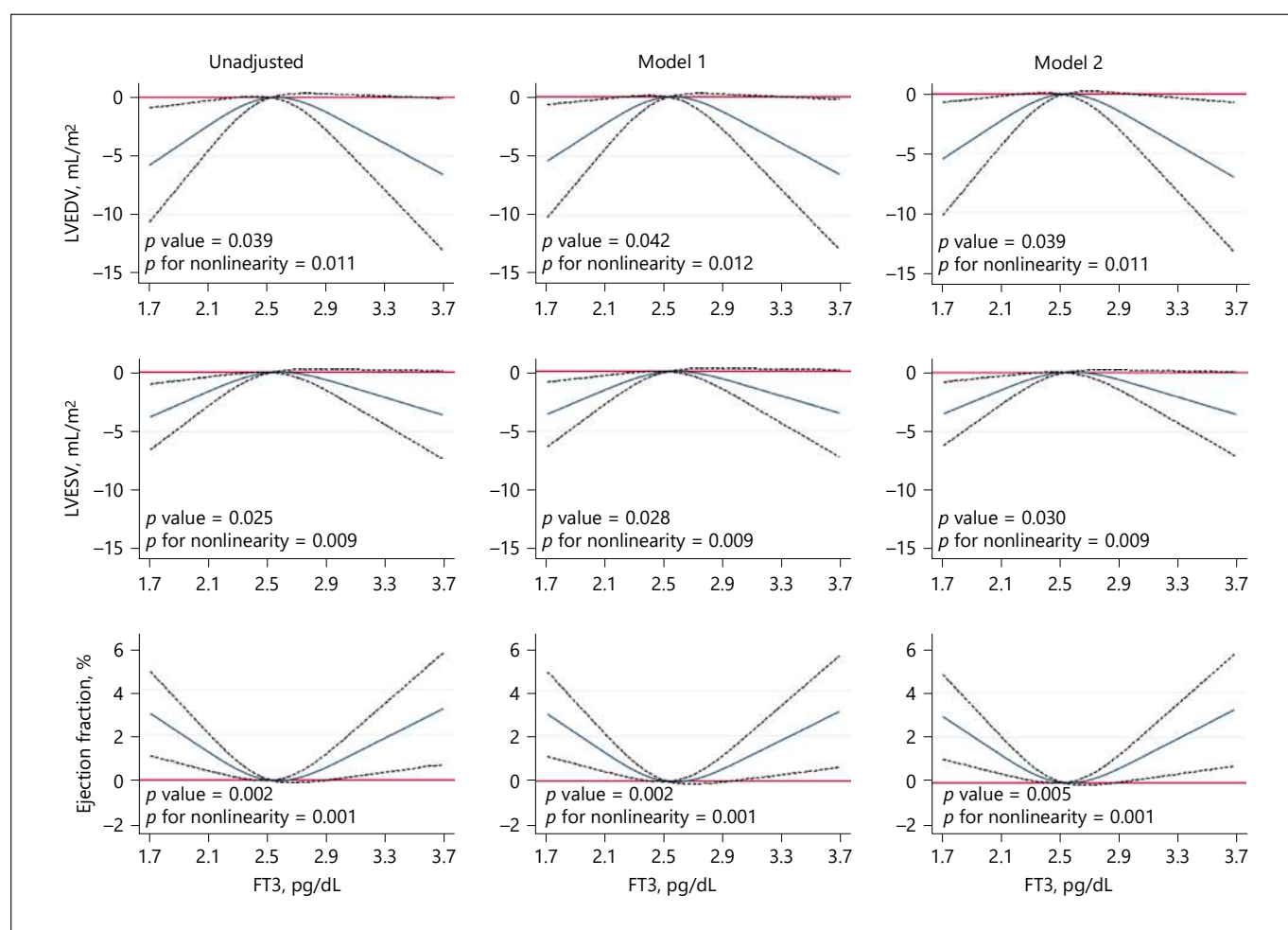


Fig. 1. Restricted cubic splines analysis of the continuous relationship between FT3 and LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), and ejection fraction. Participants treated with β -blockers were excluded from these analyses. Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, BMI, hypertension, diabetes and current smoking.

Negative correlations of FT3 levels with left atrium (LA) volume ($\beta = -2.44$, range -4.16 to -0.72 ; $p = 0.006$) and parameters of LV geometry, specifically septum thickness ($\beta = -0.26$, range -0.52 to -0.003 ; $p = 0.048$) and LV mass ($\beta = -3.57$, range -6.82 to -0.31 ; $p = 0.032$), were also found, but these were not verified after adjustment in models 1 and 2.

Analysis of FT4 levels by restricted cubic splines with septum thickness, posterior wall thickness, and LV mass (Fig. 2) showed only a nonlinear relationship between FT4 and LV posterior wall thickness, with the higher values of posterior wall thickness among participants with higher and lower FT4 levels within the reference range. However, this association was not significant after adjustments in model 2.

Association of Thyroid Function with Systolic and Diastolic Function

Linear regression analysis between thyroid function and systolic and diastolic functions are shown in Table 4. Concerning systolic function, TSH levels were positively associated with ejection fraction ($\beta = 0.96$, range 0.03 – 1.89 ; $p = 0.042$). This association was no longer significant after adjustments in models 1 and 2. On the other hand, analysis between FT3 levels and ejection fraction by restricted cubic splines (Fig. 1) showed a strong nonlinear association between these variables (p for nonlinearity = 0.001), with a higher ejection fraction in extreme FT3 levels within the reference range. These associations remained significant after adjustments in both models.

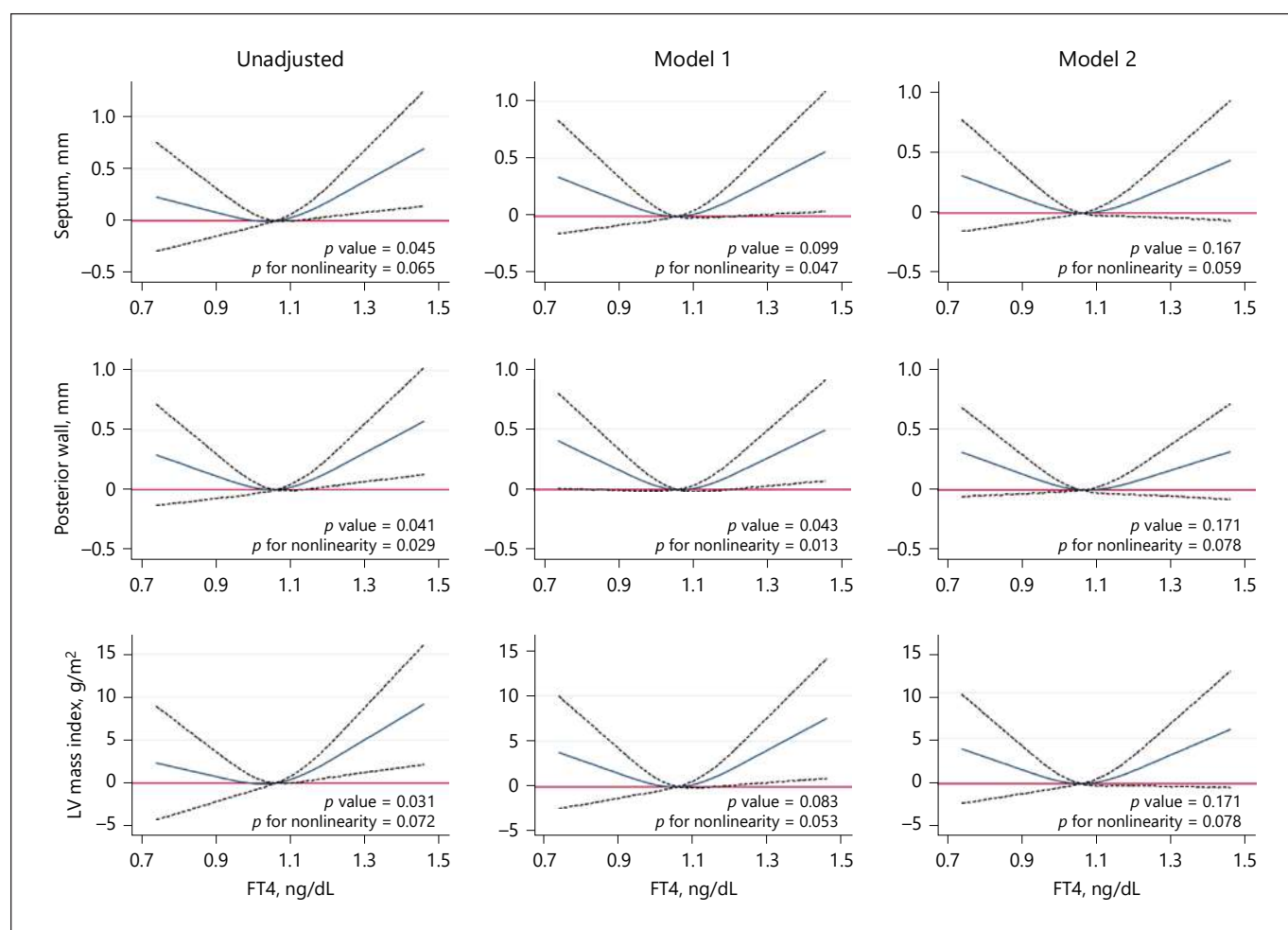


Fig. 2. Restricted cubic splines analysis of the continuous relationship between FT4 and septum thickness, posterior wall thickness, and LV mass. Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, BMI, hypertension, diabetes, and current smoking.

In diastolic function, FT3 levels were positively correlated with E' velocity ($\beta = 0.80$; range 0.21–1.39; $p = 0.008$), unlike FT4 levels, which were negatively correlated with this variable ($\beta = -2.76$, range -4.52 to -0.99; $p = 0.002$) as well as with the mitral E/A ratio ($\beta = -0.22$, range -0.39 to -0.04; $p = 0.015$). Deceleration time increased significantly with increasing FT4 levels ($\beta = 29.58$, range 0.43–58.74; $p = 0.047$). All of these associations were no longer significant after adjustments in models 1 and 2.

Discussion

In a population-based sample, thyroid hormone levels within the reference range were significantly associated with heart rate, blood pressure, and cardiac structure and

function. Our study shows robust positive associations between FT3 levels and heart rate, as well as between TSH levels and diastolic blood pressure, which suggests that increasing thyroid hormone levels are associated with a higher heart rate and a lower diastolic blood pressure. At a structural level, the inverse association found between TSH levels and LV end-systolic and end-diastolic volumes also indicates that an increase in thyroid function is associated with larger LV cavity volumes. On the other hand, nonlinear associations of FT4 and FT3 with LV wall thickness and LV ejection fraction, respectively, suggest that thyroid hormone concentrations near the extremes of the reference range are associated with a greater wall thickness and a higher ejection fraction.

Previous studies evaluating the association of thyroid hormones with cardiac function have shown contradic-

Table 4. Linear association of thyroid function with systolic and diastolic function

	TSH (μ IU/mL)		FT4 (ng/dL)		FT3 (pg/mL)	
	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value
<i>Systolic function</i>						
Ejection fraction (%)						
Unadjusted	0.96 (0.03 to 1.89)	0.042	-1.42 (-4.86 to 2.01)	0.416	-0.44 (-1.59 to 0.71)	0.449
Model 1 ^a	0.78 (-0.16 to 1.71)	0.103	-1.44 (-4.87 to 2.00)	0.412	-0.45 (-1.61 to 0.71)	0.556
Model 2 ^b	0.76 (-0.18 to 1.70)	0.111	-1.47 (-4.92 to 1.98)	0.404	-0.36 (-1.52 to 0.80)	0.543
<i>Diastolic function</i>						
Mitral E/A ratio						
Unadjusted	-0.01 (-0.04 to 0.05)	0.831	-0.22 (-0.39 to -0.04)	0.015	0.03 (-0.02 to 0.09)	0.240
Model 1 ^a	0.01 (-0.03 to 0.05)	0.676	-0.09 (-0.25 to 0.06)	0.247	-0.03 (-0.02 to 0.02)	0.293
Model 2 ^b	0.01 (-0.03 to 0.05)	0.617	-0.08 (-0.23 to 0.07)	0.296	-0.02 (-0.07 to 0.03)	0.393
Deceleration time (ms)						
Unadjusted	1.26 (-6.63 to 9.16)	0.451	29.58 (0.43 to 58.74)	0.047	-2.00 (-11.75 to 7.74)	0.687
Model 1 ^a	1.57 (-5.94 to 9.07)	0.682	15.11 (-12.54 to 42.77)	0.284	5.48 (-3.81 to 14.76)	0.248
Model 2 ^b	2.15 (-5.32 to 9.61)	0.573	15.92 (-11.54 to 43.38)	0.255	3.83 (-5.41 to 13.07)	0.416
E' velocity (cm/s)						
Unadjusted	-0.02 (-0.50 to 0.46)	0.941	-2.76 (-4.52 to -0.99)	0.002	0.80 (0.21 to 1.39)	0.008
Model 1 ^a	0.08 (-0.33 to 0.49)	0.699	-1.29 (-2.79 to 0.21)	0.091	0.07 (-0.44 to 0.58)	0.787
Model 2 ^b	0.09 (-0.32 to 0.49)	0.674	-1.13 (-2.62 to 0.35)	0.134	0.12 (-0.38 to 0.62)	0.634
E/E' ratio						
Unadjusted	0.20 (-0.17 to 0.56)	0.287	1.26 (-0.08 to 2.60)	0.065	-0.41 (-0.85 to 0.04)	0.074
Model 1 ^a	0.04 (-0.29 to 0.37)	0.824	0.45 (-0.77 to 1.66)	0.471	0.01 (-0.40 to 0.42)	0.980
Model 2 ^b	0.04 (-0.28 to 0.37)	0.789	0.23 (-0.96 to 1.42)	0.701	-0.02 (-0.42 to 0.38)	0.911

The values shown were estimated by multiple linear regression, with TSH, FT4, or FT3 as the independent variable and cardiac function parameters as dependent variables, adjusting for several variables as stated. TSH was log-transformed. Participants treated with β -blockers were excluded from systolic function and diastolic function analyses. Statistically significant associations are shown in bold.

^a Adjusted for age and sex. ^b Adjusted for age, sex, BMI, hypertension, diabetes, and current smoking.

tory results. Pearce et al. [31] evaluated the association of TSH with LV structure and function in the Framingham Heart Study. There were no significant associations between TSH and LV mass, LV wall thickness, LA, or LV systolic function. However, TSH <0.5 mU/L was associated with improved LV contractility, as assessed by echocardiographic LV fractional shortening, among women, but this parameter is no longer recommended in clinical practice [31]. In that study, associations of T4 and T3 with cardiac function were not assessed.

In a study of 318 participants with hypertension (mean age in the euthyroid group: 68 years; 10% with heart failure), Iida et al. [32] described that thyroid hormones within the normal reference range were positively associated with LV mass index. On the other hand, in a sample restricted to participants aged between 35 and 55 years without hypertension or cardiovascular disease, Roef et al. [33] showed that higher thyroid hormone levels within the normal range were associated with heart rate and smaller LV cavity sizes but not with wall thickness after

adjustment for blood pressure. In this study, thyroid hormone levels were positively associated with early and late ventricular filling, in contrast to our findings.

The differences between studies are probably related to the populations evaluated. While the study by Roef et al. [33] included only young and healthy participants, the study by Iida et al. [32] was restricted to older hypertensive individuals. On the other hand, our study represents a particularly important segment of the population. As we evaluated participants aged 45 years or older with a proportion of hypertension, diabetes, and dyslipidemia representative of the general population, our study better represents the population usually targeted for cardiovascular preventive interventions.

Thyroid hormones modulate cardiac function through genomic and nongenomic mechanisms [8, 33, 34]. T3 is the active form of thyroid hormones and it is responsible for most cardiovascular effects. In humans, type 2 deiodinase is expressed in the heart and allows local conversion of T4 to T3 [8]. TSH level is as a sensitive marker of

thyroid function as TSH secretion increases with decreasing thyroid hormone levels. However, some studies have suggested that TSH may also have direct effects on heart and vessels [35–37]. In the myocardium, thyroid hormone stimulates channels and transporters involved in calcium fluxes, improving both contraction and relaxation. Thyroid hormone also improves myocardial relaxation through upregulation of sarcoplasmic reticulum calcium-activated ATPase (SERCA2) and Na⁺/K⁺-ATPase, and downregulation of phospholamban [8, 34]. On the other hand, thyroid hormones directly induce myocardial hypertrophy through activation of PI3K/AKT/mTOR and GK3 β signaling pathways [8]. While the direct effects of thyroid hormones on diastolic function may be more marked in younger healthier individuals (like the population studied by Roef et al. [33]), the hypertrophic effects of thyroid hormones may be more marked in the elderly hypertensive population (like the population studied by Iida et al. [32]). In our population, neither diastolic function nor LV mass was linearly associated with thyroid hormone levels. Interestingly, both subclinical hypo- and hyperthyroidism are associated with diastolic dysfunction and myocardial hypertrophy, probably through different mechanisms [38, 39]. In our study, FT4 was nonlinearly associated with LV wall thickness. The fact that associations of FT4 with LV wall thickness were no longer significant after adjustment for model 2 suggests that some of the thyroid hormone effects on the LV mass may be mediated by effects on hypertension or the glycemic profile. These nonlinear associations of FT4 with LV wall thickness highlight that the hypertrophic effects of low and high thyroid hormone levels may extend to the normal reference range of thyroid hormones. The absence of associations with diastolic function suggests that the known direct effects of thyroid hormones on cardiac relaxation may be opposed by detrimental effects on myocardial hypertrophy.

The nonlinear association of FT3 with LV ejection fraction is probably the result of 2 different mechanisms. While the increase in LV ejection fraction with increasing FT3 above the median is probably the consequence of the positive inotropic effects of thyroid hormones, the increase in LV ejection fraction with FT3 below the median may be related to the increase in diastolic filling time with decreasing heart rate [40].

Studies in patients with hypo- and hyperthyroidism have shown that heart rate and blood pressure are strongly associated with thyroid dysfunction [29, 40]. While patients with overt hypothyroidism usually have bradycardia and a high diastolic blood pressure, patients with

overt hyperthyroidism usually have tachycardia and increased systolic blood pressure [40, 41]. Our results suggest that, even within the normal range, variations of thyroid function have a relevant effect on heart rate and blood pressure, which has also been suggested by previous studies [17, 42]. Overt and subclinical hyperthyroidism has also been associated with an increased risk of cardiac arrhythmias [43–45] and with volume overload [46]. In the Rotterdam Study, Chaker et al. [47, 48] found that increasing thyroid function was associated with a higher risk of atrial fibrillation in euthyroid participants and a higher risk of sudden cardiac death. The tachycardic effects appear to be dependent on direct cardiac electrophysiological effects [49–51] and β -adrenergic stimulation [52], while the volume overload is probably mediated by peripheral vasodilation with compensatory activation of the renin-angiotensin-aldosterone axis and increased renal reabsorption of sodium and water [46]. These effects may be observed even within the normal range as suggested by our findings of an increased heart rate with higher FT3 and an association of lower TSH levels with a lower diastolic blood pressure and with higher LV cavity volumes.

Atherosclerotic risk is also thought to be influenced by variations of thyroid hormones within the normal range. Both low and high-normal thyroid functions have been associated with atherosclerotic disease in several population-based cohorts. In the HUNT study, Asvold et al. [53, 54] found that higher TSH levels were associated with a higher risk of coronary heart disease mortality, but only in women. On the other hand, a recent meta-analysis did not find an association between TSH levels and coronary heart disease events and mortality [55]. However, a U-shaped association between FT4 within the normal range and the risk of coronary events was present. A more atherogenic lipid profile with lower FT4 levels may explain the higher cardiovascular risk with a low-normal thyroid status [56]. In another study, Bano et al. [57] showed that a higher FT4 level was associated with a higher risk of subclinical and clinical atherosclerosis. A procoagulant state linked to a high-normal thyroid function may be a possible mechanism to explain this association [58]. Other plausible mechanism would be related to the increasing workload of the heart due to an increased heart rate and myocardial hypertrophy [41].

The results of our analysis are clinically relevant as they highlight that, even within the normal range, thyroid hormones strongly modulate cardiovascular function. The use of detailed echocardiography data in a sample representative of the general population is a strength of our study, as previous studies that evaluated the associa-

tion of thyroid hormones with echocardiographic parameters focused on specific subpopulations [32, 33]. Furthermore, the identification of a U-shaped association of thyroid hormones with wall thickness and ejection fraction is important as it highlights that both normal-low and normal-high thyroid hormones are associated with a higher ejection fraction and a greater wall thickness. In those treated with levothyroxine the risk of adverse cardiovascular outcomes may increase both with undertreatment and with overtreatment [59]. The clinical impact of small changes in thyroid hormones within the normal range concerning cardiovascular outcomes is still not clear. Although some authors have suggested the use of different targets within the normal range according to the clinical context [60], the evidence for such approach is scarce. Furthermore, treatment with levothyroxine is usually associated with higher T4 and lower T3 levels compared with the general population [46], which may not completely restore the euthyroid state [61]. Further studies, ideally randomized clinical trials, are needed to evaluate the potential cardiovascular benefits of individualized targets and the effects of the combination of T4/T3 treatment in participants treated with thyroid hormones.

Regarding the limitations of our study, the cross-sectional design limits our ability to make causal inferences. We cannot exclude that residual confounding (including drugs and unmeasured comorbidities) or loss to follow-up of participants initially included in this cohort may have influenced our results. Furthermore, we must highlight that we evaluated the association of thyroid function within the normal range with cardiac function at a population level. Thyroid function has an important interindividual variation that may have decreased our ability to detect significant associations [62]. Prospective studies are needed to evaluate the effects of intraindividual variations of thyroid hormone levels. As we only evaluated participants who were 45 years or older, our results may not be applicable to younger individuals. Regarding the assessment of echocardiography data, we did not evaluate intraobserver or interobserver variability. However, all of the cardiologists involved in this study had extensive experience in echocardiography and worked at the same institution, and a detailed procedure protocol was discussed between the team, prior to the start of the study, to harmonize the methodology and the measurements. Finally, we only evaluated free thyroid hormone concentrations. Although only the free fraction is considered biologically active [63, 64], we cannot exclude that different associations could have been found if we had evaluated total thyroid hormone concentrations.

In conclusion, variations of thyroid function within the reference range are associated with heart rate, blood pressure, cardiac structure, and cardiac function in the general population. Our results suggest that increasing thyroid function (lower TSH, higher FT4, or higher FT3) is associated with a higher heart rate, a lower diastolic blood pressure, and larger LV cavity volumes. On the other hand, both LV wall thickness and LV ejection fraction have a U-shaped association with thyroid hormones, with a greater wall thickness and a higher ejection fraction near the extremes of the normal range of thyroid hormone concentrations.

Statement of Ethics

Consent was obtained from each subject after a full explanation of the purpose and nature of all of the procedures used. This study was approved by the Ethics Committee of the São João Hospital Center/Faculty of Medicine of University of Porto.

Conflict of Interest Statement

The authors have no conflict of interests to declare.

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Author Contributions

J.S.N., R.F.-C., M.B.-C., A.R.L., and A.A. contributed to the conception and design of this work, the statistical analysis, and interpretation of data. S.M., A.O., J.T.G., D.C., and A.L.-M. contributed to interpretation of data. J.S.N., M.B.-C., and A.R.L. prepared the first draft of this paper. All of the authors critically revised this work and approved the final version of this paper.

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Supplementary Table 1. Comparison at the baseline visit (1999-2003) of included participants vs. those excluded from analysis

	Included (n=835)	Excluded (n=1213)	P value
Age (years), mean (SD)	55.8 ± 10.5	59.4 ± 12.4	< 0.001
Female sex, %	61.1	62.3	0.57
Body mass index (kg/m ²), mean (SD)	27.2 ± 4.6	27.9 ± 5.2	0.13
Diabetes mellitus, %	4.4	9.0	< 0.001
Dyslipidemia, %	41.1	49.4	< 0.001
Hypertension, %	45.7	58.1	< 0.001
Current smoker, %	18.9	21.2	0.20

Supplementary Table 1. Comparison at the baseline visit (1999-2003) of included participants vs. those excluded from analysis. Among 2048 participants with 45 years or more in visit between October 2006 and July 2008, 134 (6.5%) had died, 198 (9.7%) refused re-evaluation and 580 (28.3%) were lost to follow-up. We further excluded 301 patients with previous myocardial infarction, percutaneous or surgical revascularization, prior cardiac surgery, significant (moderate to severe) valvular heart disease, history of thyroid disease, treatment with drugs that interfere with thyroid function; without serum available for determination of thyroid function; or with TSH, free thyroxine (FT4) or free triiodothyronine (FT3) outside of the reference range. SD: standard deviation.



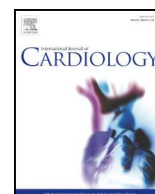
Article 5

Lower free triiodothyronine levels within the reference range are associated with higher cardiovascular mortality: An analysis of the NHANES

Neves JS, Leitão L, Baeta Baptista R, Bigotte Vieira M, Magriço R, Viegas Dias C, Oliveira A, Falcão-Pires I, Lourenço A, Carvalho D, Leite-Moreira A.

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Lower free triiodothyronine levels within the reference range are associated with higher cardiovascular mortality: An analysis of the NHANES☆

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ABSTRACT

Background: Thyroid hormones play a central role in cardiovascular homeostasis. Lower free triiodothyronine (FT3) levels have been associated with worse prognosis in several conditions. However, contrary to thyrotropin (TSH) and free thyroxine (FT4), the role of FT3 in morbidity and mortality in the general population remains uncertain. Our objective was to evaluate the association between within the normal range FT3 levels and mortality in the general population.

Methods: We evaluated 7116 adults in the National Health and Nutrition Examination Survey (NHANES) 2001–2002, 2007–2008, and 2009–2010 cycles with mortality evaluated as of December 2011. Exclusion criteria were: pregnancy; history of thyroid disease; use of thyroid-related drugs; and TSH, FT4, or FT3 level outside the reference range.

Results: During a median follow-up of 45 months, 357 participants died. In unadjusted analysis, lower FT3 levels were associated with higher all-cause (HR per 0.1 pg/mL increase in FT3: 0.82 [95% confidence interval, 0.78–0.87]), cardiovascular (HR 0.74 [0.66–0.83]), cancer-related (HR 0.88 [0.80–0.97]) and other cause-related mortality (HR 0.83 [0.77–0.90]). After adjustment with Cox proportional hazard models, lower FT3 levels remained significantly associated with higher cardiovascular mortality (HR 0.83 [0.75–0.93]), but not with all-cause (HR 0.97 [0.92–1.02]), cancer-related (HR 1.02 [0.89–1.17]), or other cause-related mortality (HR 1.00 [0.92–1.10]).

Conclusions: Lower levels of FT3 within the reference range may independently predict higher cardiovascular mortality in the general population.

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1. Introduction

Thyroid hormones control metabolism of every tissue and play a central role in cardiovascular homeostasis [1,2]. Both hypothyroidism and hyperthyroidism increase risk of all-cause and cardiovascular

mortality [2–4], but even variations within the normal range of TSH and free thyroxine (FT4) are associated with increased morbidity and mortality in the general population. High-normal thyrotropin (TSH) levels increase all-cause and cardiovascular mortality [5,6]. Conversely, low-normal TSH levels increase risk of atrial fibrillation [7,8] and all-cause mortality [9]. Regarding FT4, low FT4 levels have been associated with increased all-cause mortality [5,10,11], and high FT4 levels have been related to sudden death [12], atrial fibrillation [5,8], and atherosclerotic cardiovascular morbidity and mortality [13].

Lower free triiodothyronine (FT3) levels have been associated with worse prognosis in chronic heart failure [14], acute myocardial infarction [15], cerebrovascular disease [16], chronic kidney disease [17],

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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and in critically illness [18]. However, the role of variations within the normal range in the risk of mortality in the general population remains uncertain.

Therefore, we aimed to evaluate the association between FT3 levels and all-cause, cardiovascular, cancer and other cause-related mortality in the general population.

2. Methods

2.1. Study design and participants

We analyzed data from the National Health and Nutrition Examination Survey (NHANES). The NHANES is a nationally representative survey of the civilian noninstitutionalized U.S. population conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). The NCHS Research Ethics Review Board reviewed and approved the study protocol.

We included adults aged 18 years or older who participated in the continuous NHANES survey cycles of 2001–2002, 2007–2008, and 2009–2010 in which FT4 and FT3 levels were assessed at baseline. We excluded pregnant women, participants with a prior history of thyroid disease, participants receiving thyroid-related drugs (levothyroxine, liothyronine, thyroid desiccated, methimazole, or propylthiouracil), and those with TSH, FT4 or FT3 outside the reference range.

2.2. Thyroid function evaluation

In NHANES blood samples were processed, stored and shipped to Collaborative Laboratory Services, Ottumwa, Iowa. TSH level was measured with a third generation two-site immunoassay, FT4 levels were measured using a two-step enzyme immunoassay, FT3 levels were measured with a competitive binding immunoassay. Reference ranges in NHANES were defined as serum TSH level of 0.39–4.60 mIU/L; serum FT4 level of 0.6–1.6 ng/dL; and serum FT3 level of 2.5–3.9 pg/mL. Antithyroperoxidase and antithyroglobulin antibody titers were measured with two-step immunoassays. Detailed laboratory methods for NHANES are described elsewhere [19].

2.3. Outcomes

The main outcomes of our study were all-cause mortality, cardiovascular mortality, cancer mortality and other cause-related mortality. Mortality status and cause of death were determined by NHANES linked National Death Index public-access files through December 31, 2011.

2.4. Statistical analysis

Our study population was categorized by tertiles according to FT3 levels and by age- and sex-specific FT3 tertiles. The association of FT3 levels with all-cause mortality, cardiovascular mortality, cancer mortality and mortality from other causes was assessed with unadjusted, age and sex adjusted, and fully adjusted Cox proportional hazard models. Confounders were classified as: age (18–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, 70–79 years, ≥80 years); sex; race (Non-Hispanic White, Non-Hispanic Black, Mexican American, Other Hispanic, Other Race); body mass index (BMI; <20, 20 to <25, 25 to <30, 30 to <35, 35 to <40, ≥40 kg/m²); smoking (non-smoker, former smoker, current smoker); education (less than 9th grade, 9–11th grade, high-school grade, some college or associate's (AA) degree, college graduate and above); annual family income

(<\$25,000, \$25,000 to \$75,000, >\$75,000); diabetes mellitus (self-reported diabetes, hemoglobin A1c ≥ 6.5%, fasting plasma glucose level ≥126 mg/dL or use of glucose lowering drugs); hypertension (self-reported hypertension, systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg or use of medication to lower blood pressure); dyslipidemia (self-reported dyslipidemia, total cholesterol ≥240 mg/dL, low-density cholesterol ≥160 mg/dL, high-density cholesterol <40 mg/dL, triglycerides ≥200 mg/dL or use of lipid lowering drugs); chronic kidney disease (glomerular filtration rate <60 mL/min/1.73 m²); previous cardiovascular disease (self-reported ischemic heart disease, heart failure or previous stroke); self-reported history of cancer; FT4 and TSH levels. Age- and sex-specific FT3 tertile survival was evaluated by Kaplan-Meier curves and log-rank tests. Unadjusted and adjusted models using restricted cubic spline analyses with three-knots were also performed to flexibly display the relationship between FT3 and mortality. Subgroup analyses were conducted for sex, age (<65 years or ≥65 years), baseline cardiovascular diseases and tertiles of FT4 or TSH using the likelihood ratio test for interactions.

As complementary analyses, we performed five additional Cox proportional hazards models. In the first model, we excluded participants with positive thyroid antibodies. In the second model, we also included participants with FT3 between 2.0 pg/mL and the lower limit of the reference range. In the third model, we analyzed participants with normal TSH and normal FT4, including in the analysis patients with FT3 outside the reference range (participants with FT3 below the reference range and participants with FT3 above the reference range). In the fourth model, we analyzed all participants without overt thyroid dysfunction, which was defined as high TSH with low FT4 (overt hypothyroidism) or low TSH and high FT4 and/or FT3 (overt hyperthyroidism). In the fifth model, we repeated the main analysis excluding participants treated with amiodarone.

Continuous variables are presented as mean ± standard deviation and categorical variables are presented as percentages. A two-sided p-value of <0.05 was considered statistically significant. Analyses were performed using Stata (version 14.2).

3. Results

3.1. Clinical characteristics

We evaluated data from 7116 participants in the NHANES. Fifty-two percent were male and mean age was 45 ± 14 years. Higher FT3 levels were associated with younger age and male sex (Supplemental Table 1). In the characterization according to the age- and sex-specific FT3 tertiles, subjects with higher FT3 were more likely to be non-white, current smokers, to have a lower level of education, lower annual family income, higher BMI, hypertension, and higher levels of FT4, compared with those with lower FT3. They were also less likely to have CKD (Table 1).

3.2. Free triiodothyronine levels and all-cause mortality

Lower normal FT3 levels were associated with an increase in all-cause mortality [hazard ratio (HR) per 0.1 pg/mL increase in FT3: 0.82, 95% CI 0.78–0.87, p < 0.001] in the continuous unadjusted analysis and in the restricted cubic splines unadjusted analysis. This association was no longer significant after adjustment for age and sex (HR 0.96,

Table 1
Baseline characteristics of the population and according to age- and sex-specific tertiles of FT3.

	Total population (n = 7116)	Age- and sex-specific tertiles of FT3			p value
		Lower tertile	Middle tertile	Upper tertile	
Male sex, %	51.9	–	–	–	
Age, years	45.0 ± 14.3	–	–	–	
White race, %	69.5	70.9	70.4	66.5	0.035
Income < \$25,000, %	26.8	25.3	26.2	29.4	0.024
Education < 9th grade, %	6.6	5.0	6.8	8.5	<0.001
BMI, kg/m ²	28.2 ± 5.5	27.4 ± 5.3	28.2 ± 5.2	29.0 ± 6.0	<0.001
Diabetes, %	10.5	11.4	10.1	9.6	0.081
Dyslipidemia, %	53.6	50.2	54.7	57.0	0.003
Hypertension, %	36.6	35.3	35.6	39.3	0.013
Chronic kidney disease, %	4.9	6.6	4.7	2.8	<0.001
Smoking, %	19.6	17.7	20.1	21.7	0.019
History of CV disease, %	7.3	7.8	6.6	7.5	0.663
History of cancer, %	8.7	8.3	9.3	8.5	0.746
TSH, mIU/L	1.79 ± 0.83	1.82 ± 0.82	1.78 ± 0.83	1.76 ± 0.84	0.047
FT4, ng/dL	0.78 ± 0.10	0.77 ± 0.09	0.78 ± 0.09	0.80 ± 0.11	<0.001
FT3, pg/mL	3.17 ± 0.26	2.90 ± 0.16	3.20 ± 0.13	3.49 ± 0.16	<0.001

Abbreviations: BMI, body mass index; CV, cardiovascular.

P-values <0.05 are presented in bold.

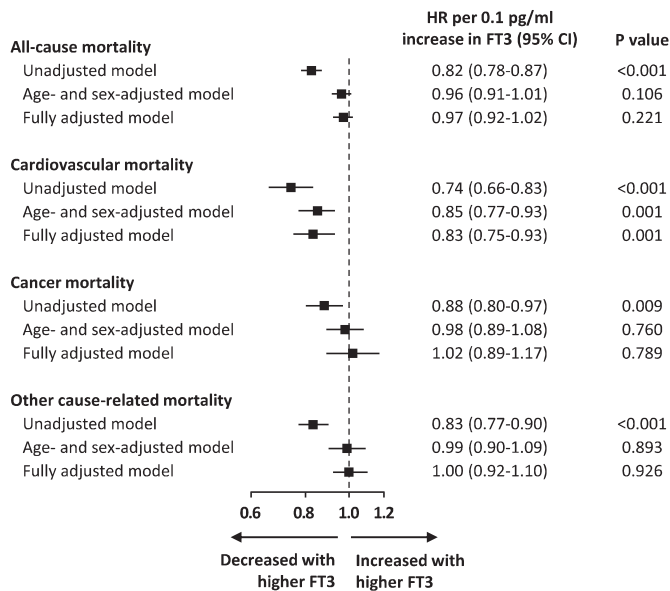


Fig. 1. Association of FT3 with all-cause, cardiovascular, cancer, and other cause-related mortality. Fully adjusted model: age, gender, race, BMI, smoking, education, annual family income, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, previous cardiovascular disease, history of cancer, FT4 and TSH levels. CI: confidence interval; HR: hazard ratio.

95% CI 0.91–1.01, $p = 0.106$) in the fully adjusted model (HR 0.97, 95% CI 0.92–1.02, $p = 0.221$) (Fig. 1), in the analysis according to age- and sex-specific tertiles of FT3 (Fig. 2), and in the adjusted restricted cubic splines analysis (Supplemental Fig. 2).

3.3. Free triiodothyronine levels and cardiovascular mortality

During follow-up, 82 deaths occurred from cardiovascular disease. In the unadjusted and adjusted models, higher FT3 levels were associated with lower cardiovascular mortality (Fig. 1). In the fully adjusted model, the HR for cardiovascular mortality was 0.83 (95% CI 0.75–0.93, $p = 0.001$) per 0.1 pg/mL increase in FT3. Comparing with participants in the upper age- and sex-specific tertile of FT3 (Fig. 2), participants in the middle and lower tertile had higher cardiovascular mortality [HR 1.61 (95% CI 0.91–2.84) and HR 2.41 (95% CI 1.32–4.41), respectively; p for trend = 0.008]. The analysis of FT3 as a continuous variable using restricted cubic spline models also showed an association

between lower levels of FT3 and higher risk of cardiovascular mortality both in unadjusted and in adjusted models (Supplemental Fig. 2).

3.4. Free triiodothyronine levels, cancer mortality and other cause-related mortality

During follow-up, 98 participants died due to cancer and 177 due to other causes. After multivariate adjustment, there was no significant association between FT3 levels and mortality from cancer or other causes (Fig. 1 and Supplemental Fig. 2). We also found no significant differences in cancer and other cause-related mortality according to age- and sex-specific FT3 tertiles (Supplemental Fig. 1).

3.5. Subgroup analyses and complementary analyses

There was no significant interaction between FT3 levels and age groups, sex, baseline cardiovascular disease, TSH tertile, or FT4 tertile regarding all-cause or cardiovascular mortality (Fig. 3). There were also no significant interactions between FT3 levels and these subgroups concerning cancer and other cause-related mortality (Supplemental Fig. 3).

The complementary analyses presented results that were consistent with the main analyses, either excluding the 743 patients with positive thyroid antibodies (Supplemental Table 2), including the 115 participants with FT3 between 2.0 pg/mL and the lower limit of the reference range for FT3 (Supplemental Table 3), including the 470 participants with FT3 levels outside the reference range (Supplemental Table 4), including the 832 participants without overt thyroid dysfunction (Supplemental Table 5), or excluding the 63 participants who were treated with amiodarone (Supplemental Table 6).

4. Discussion

Our study showed an inverse association between FT3 levels within the reference range and cardiovascular mortality. The inverse association of FT3 with cardiovascular mortality remained statistically significant after adjustment for demographic variables, relevant comorbidities, FT4 and TSH levels. No interactions regarding cardiovascular mortality were found between FT3 and age, sex, baseline cardiovascular disease and tertiles of FT4 or TSH. Consequently, our study suggests that a lower FT3 level, even within the reference range, is an independent predictor of higher cardiovascular mortality.

Most studies that have evaluated the effects of thyroid hormones levels within the reference range on mortality have focused mainly on TSH and FT4 [5,6,9–11]. Although lower levels of FT3 have

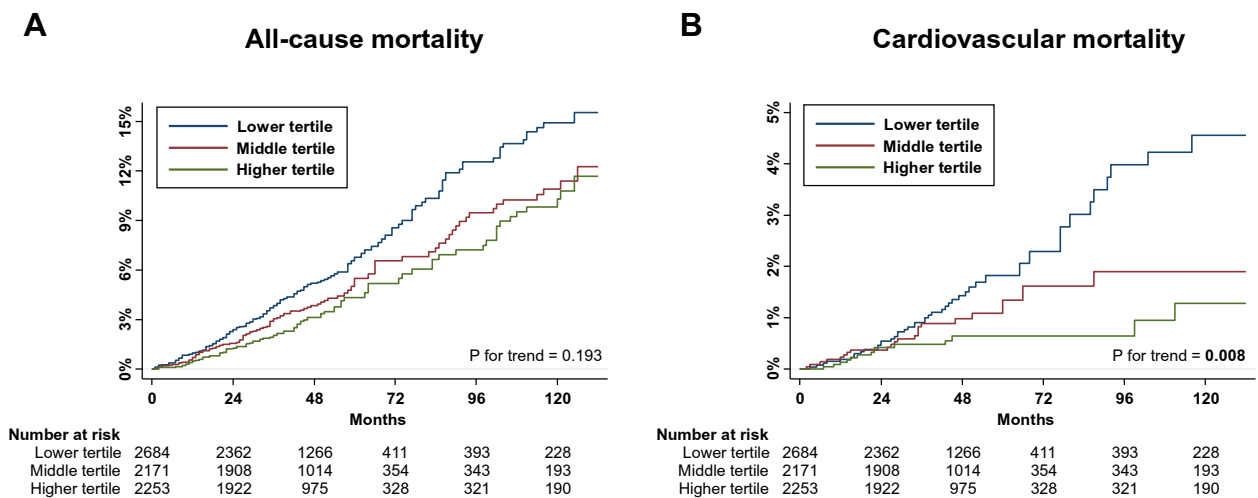


Fig. 2. Kaplan-Meier curves for all-cause (A) and cardiovascular mortality (B) by age- and sex-specific FT3 tertiles.

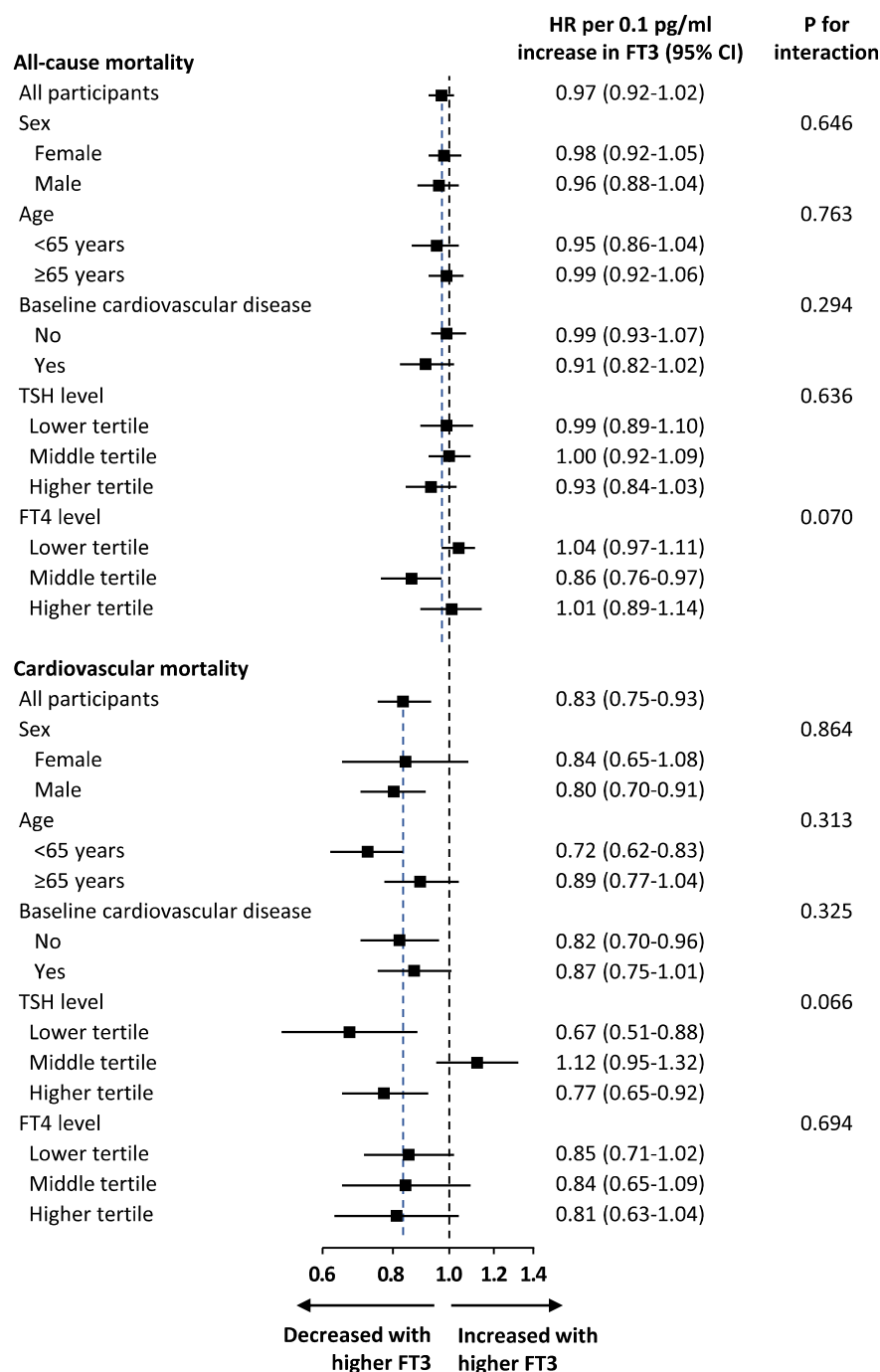


Fig. 3. Subgroup analysis of the association between FT3 levels, all-cause mortality, and cardiovascular mortality. Analysis adjusted for age, sex, race, BMI, smoking, education, annual family income, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, previous cardiovascular disease, history of cancer, FT4 and TSH levels. HR: hazard ratio.

been consistently associated with worse prognosis in several diseases [14–16], to our knowledge this is the first study showing an association between lower levels of FT3 and higher cardiovascular mortality in the general population. In a prospective cohort study of ambulatory patients with chronic heart failure, not only low T3 levels, but also T3 evaluated as a continuous variable was associated with a composite end point of adverse cardiovascular outcomes (ventricular assist device placement, heart transplantation, or death) [20]. Among patients hospitalized with previous cardiovascular disease, Pfister et al. [21] also shown that lower FT3 levels within the reference range were associated with higher mortality. Meuwese et al. [22], also showed in patients with chronic kidney disease in maintenance hemodialysis

that relatively lower basal triiodothyronine concentrations were associated with higher mortality comparing with patients with higher levels.

Several studies have previously assessed the association of FT3 levels with all-cause mortality among elderly patients. In the Milan Geriatrics 75+ Cohort Study, lower FT3 was associated with increased all-cause mortality among older outpatients with normal TSH [23]. Also, in the Leiden 85-Plus Study, including 599 participants followed from 85 years through 89 years of age, lower FT3 was associated with all-cause mortality [24]. In the Chianti Area Study, including 951 subjects with 65 years or more, there was also a trend for participants with lower FT3 levels to present higher all-cause mortality [25].

Regarding cardiovascular mortality, no association was found in the Chianti Area Study [25] or in the Kangbuk Samsung Health Study including Korean healthy participants, with a mean age of 40 years and only 5% above 60 years of age [10]. Differences among the populations studied, particularly regarding age, probably explain much of the heterogeneity in the results found across studies.

Multiple pathways have been implicated in cardiovascular protection by thyroid hormones [2]. Triiodothyronine modulates lipid profile, endothelial function, blood pressure, and directly improves cardiac function [2]. Thyroid hormones have also been proposed to be cardioprotective through modulation of mitochondrial function and reduction of cardiomyocytes apoptosis [26,27]. Triiodothyronine is the biologically active thyroid hormone and therefore an association between free triiodothyronine levels and cardiovascular mortality is not surprising. However, it is also possible that lower FT3 levels may only constitute a marker rather than a direct mediator of increased cardiovascular risk. Low triiodothyronine levels are commonly found in patients with acute critical illness and severe chronic diseases [28]. The occurrence of low triiodothyronine syndrome, one of the most common forms of non-thyroidal illness syndrome (alterations in thyroid hormones without any underlying intrinsic thyroid disorder), can be explained by changes in deiodinase activity, thyroid hormone binding, peripheral metabolism, or changes in the hypothalamic-pituitary-thyroid axis [28]. The severity of disease has been correlated with the decrease of thyroid hormone levels. Whether these changes are detrimental or adaptive during acute or chronic disease remains controversial. In a recent meta-analysis of studies including patients with cardiovascular disease, low triiodothyronine syndrome was independently associated with increased all-cause and cardiovascular mortality [29]. Our study reinforces results from this meta-analysis regarding cardiovascular mortality but not all-cause mortality in the general population.

We analyzed data from the NHANES which includes a large number of participants and is designed to be representative of the non-institutionalized US population, spanning a wide age distribution. The availability of comprehensive information about the participants allowed for adjustment for the main biologically plausible confounders. Our results were consistent across main and complementary analyses.

Nevertheless, we must highlight that thyroid function was only evaluated at baseline. We cannot exclude that the associations with mortality would differ if FT3 was also evaluated during follow-up. Furthermore, in NHANES the evaluation of reverse triiodothyronine levels was not performed. It is possible that the effects of FT3 in mortality differ according to the levels of reverse triiodothyronine. We must also highlight that previous cardiovascular disease was self-reported by the participants. We cannot exclude the presence of bias on the classification of baseline cardiovascular disease. Moreover, as all observational studies, our analysis must be interpreted as exploratory. It is possible that residual confounding exists due to unmeasured variables.

Our results suggest that FT3 levels may contribute to the stratification of cardiovascular risk in the general population. Even after adjustment for TSH, FT4, and traditional cardiovascular risk factors, lower FT3 levels within the reference range independently predicted cardiovascular mortality. If these findings are confirmed in other cohorts, the evaluation of FT3 levels might be included in cardiovascular risk scores allowing a finer hierarchization of the patient's cardiovascular risk profile.

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Contributions: Research idea: JSN. Literature search, study design, data analysis and first manuscript draft preparation: JSN, LL, RBB, MBV, RM, CVD. Interpretation of the results and critical revision of the manuscript: AO, IFP, AL, DC, ALM. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijcard.2019.03.009>.

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SUPPLEMENTAL MATERIAL

Lower FT3 within the reference range is associated with higher cardiovascular mortality: an analysis of the NHANES

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Supplemental table 1. Baseline characteristics of the population according to FT3 tertile

	Lower tertile (3.3-3.9 pg/mL)	Middle tertile (3.0-3.3 pg/mL)	Upper tertile (2.5-3.0 pg/mL)	P value
Male sex, %	36.5	53.9	67.6	<0.001
Age, years	51.3 ± 15.0	44.6 ± 13.2	38.0 ± 12.1	<0.001
White race, %	73.3	70.4	70.4	<0.001
Income < \$25000, %	25.7	26.0	29.1	0.038
Education < 9 th grade, %	6.6	6.1	7.2	0.558
BMI, kg/m ²	27.6 ± 5.5	28.2 ± 5.3	28.8 ± 5.7	<0.001
Diabetes, %	13.7	9.8	7.5	<0.001
Dyslipidemia, %	54.3	51.8	54.9	0.810
Hypertension, %	43.8	35.0	29.9	<0.001
Chronic kidney disease, %	10.0	2.9	1.2	<0.001
Smoking, %	15.9	20.0	23.7	<0.001
History of CV disease, %	10.2	6.7	4.4	<0.001
History of cancer, %	11.3	8.9	5.3	<0.001
Free T4, ng/dL	0.77 ± 0.10	0.78 ± 0.10	0.80 ± 0.11	<0.001
TSH, mIU/L	1.88 ± 0.86	1.78 ± 0.83	1.69 ± 0.78	<0.001

Abbreviations: BMI, body mass index; CV, cardiovascular.

Supplemental table 2. Association of FT3 with all-cause, cardiovascular, cancer, and other cause-related mortality excluding participants with positive thyroid antibodies

	Hazard ratio per each 0.1 pg/mL increase in FT3	P value
All-cause mortality		
Unadjusted model	0.83 (0.78-0.88)	<0.001
Age and sex adjusted model	0.96 (0.90-1.01)	0.177
Fully adjusted model	0.98 (0.92-1.04)	0.433
CV mortality		
Unadjusted model	0.72 (0.62-0.83)	<0.001
Age and sex adjusted model	0.82 (0.73-0.93)	0.002
Fully adjusted model	0.81 (0.70-0.94)	0.005
Cancer mortality		
Unadjusted model	0.88 (0.79-0.98)	0.023
Age and sex adjusted model	0.99 (0.89-1.10)	0.860
Fully adjusted model	1.03 (0.89-1.19)	0.710
Other cause-related mortality		
Unadjusted model	0.85 (0.78-0.93)	<0.001
Age and sex adjusted model	1.01 (0.91-1.11)	0.913
Fully adjusted model	1.03 (0.94-1.12)	0.576

Abbreviations: CV, cardiovascular.

Fully adjusted model: age, sex, race, BMI, smoking, education, annual salary, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, previous cardiovascular disease, history of cancer, FT4 and TSH levels.

Supplemental table 3. Association of FT3 with all-cause, cardiovascular, cancer, and other cause-related mortality including participants with FT3 above 2.0 pg/mL

	Hazard ratio per each 0.1 pg/mL increase in FT3	P value
All-cause mortality		
Unadjusted model	0.81 (0.77-0.85)	<0.001
Age and sex adjusted model	0.93 (0.88-0.99)	0.020
Fully adjusted model	0.97 (0.92-1.01)	0.124
CV mortality		
Unadjusted model	0.74 (0.67-0.81)	<0.001
Age and sex adjusted model	0.85 (0.79-0.92)	<0.001
Fully adjusted model	0.84 (0.78-0.92)	<0.001
Cancer mortality		
Unadjusted model	0.87 (0.80-0.95)	0.003
Age and sex adjusted model	0.97 (0.89-1.07)	0.579
Fully adjusted model	1.01 (0.90-1.14)	0.816
Other cause-related mortality		
Unadjusted model	0.81 (0.74-0.88)	<0.001
Age and sex adjusted model	0.95 (0.85-1.06)	0.359
Fully adjusted model	1.00 (0.92-1.09)	0.999

Abbreviations: CV, cardiovascular.

Fully adjusted model: age, sex, race, BMI, smoking, education, annual salary, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, previous cardiovascular disease, history of cancer, FT4 and TSH levels.

Supplemental table 4. Association of FT3 with all-cause, cardiovascular, cancer, and other cause-related mortality including participants with FT3 outside the reference range (restricting the analysis to participants with normal TSH and normal FT4)

	Hazard ratio per each 0.1 pg/mL increase in FT3	P value
All-cause mortality		
Unadjusted model	0.81 (0.78-0.85)	<0.001
Age and sex adjusted model	0.94 (0.89-0.99)	0.025
Fully adjusted model	0.97 (0.92-1.01)	0.110
CV mortality		
Unadjusted model	0.74 (0.68-0.81)	<0.001
Age and sex adjusted model	0.86 (0.80-0.92)	<0.001
Fully adjusted model	0.84 (0.78-0.91)	<0.001
Cancer mortality		
Unadjusted model	0.88 (0.81-0.95)	0.002
Age and sex adjusted model	0.98 (0.91-1.07)	0.688
Fully adjusted model	1.00 (0.90-1.10)	0.973
Other cause-related mortality		
Unadjusted model	0.81 (0.75-0.88)	<0.001
Age and sex adjusted model	0.96 (0.87-1.06)	0.369
Fully adjusted model	1.00 (0.93-1.08)	0.922

Abbreviations: CV, cardiovascular.

Fully adjusted model: age, sex, race, BMI, smoking, education, annual salary, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, previous cardiovascular disease, history of cancer, FT4 and TSH levels.

Supplemental table 5. Association of FT3 with all-cause, cardiovascular, cancer, and other cause-related mortality including participants with FT3 outside the reference range (restricting the analysis to participants without overt thyroid dysfunction)

	Hazard ratio per each 0.1 pg/mL increase in FT3	P value
All-cause mortality		
Unadjusted model	0.82 (0.78-0.86)	<0.001
Age and sex adjusted model	0.95 (0.90-1.00)	0.036
Fully adjusted model	0.97 (0.93-1.01)	0.107
CV mortality		
Unadjusted model	0.75 (0.69-0.82)	<0.001
Age and sex adjusted model	0.87 (0.81-0.94)	<0.001
Fully adjusted model	0.86 (0.80-0.93)	<0.001
Cancer mortality		
Unadjusted model	0.87 (0.81-0.94)	0.001
Age and sex adjusted model	0.98 (0.91-1.06)	0.632
Fully adjusted model	0.99 (0.91-1.09)	0.894
Other cause-related mortality		
Unadjusted model	0.82 (0.76-0.89)	<0.001
Age and sex adjusted model	0.96 (0.88-1.05)	0.383
Fully adjusted model	0.99 (0.92-1.07)	0.921

Abbreviations: CV, cardiovascular.

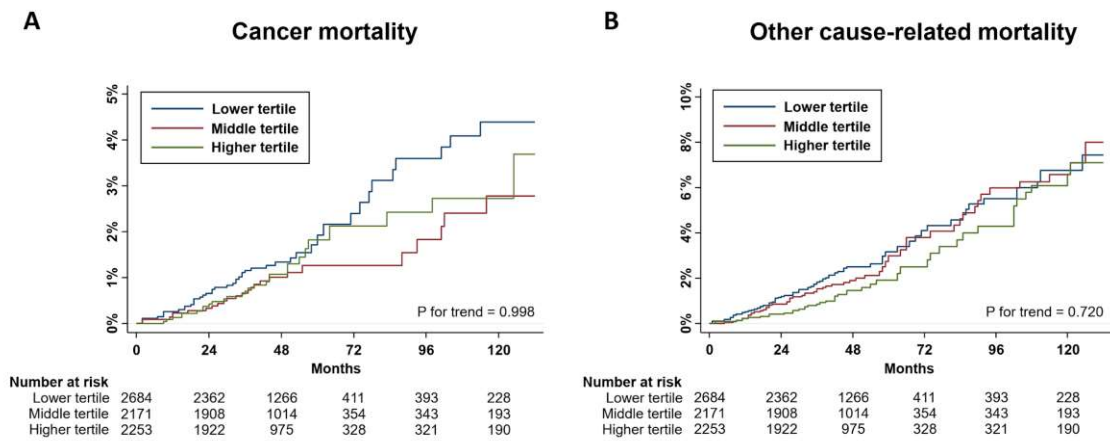
Fully adjusted model: age, sex, race, BMI, smoking, education, annual salary, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, previous cardiovascular disease, history of cancer, FT4 and TSH levels.

Supplemental table 6. Association of FT3 with all-cause, cardiovascular, cancer, and other cause-related mortality excluding participants treated with amiodarone

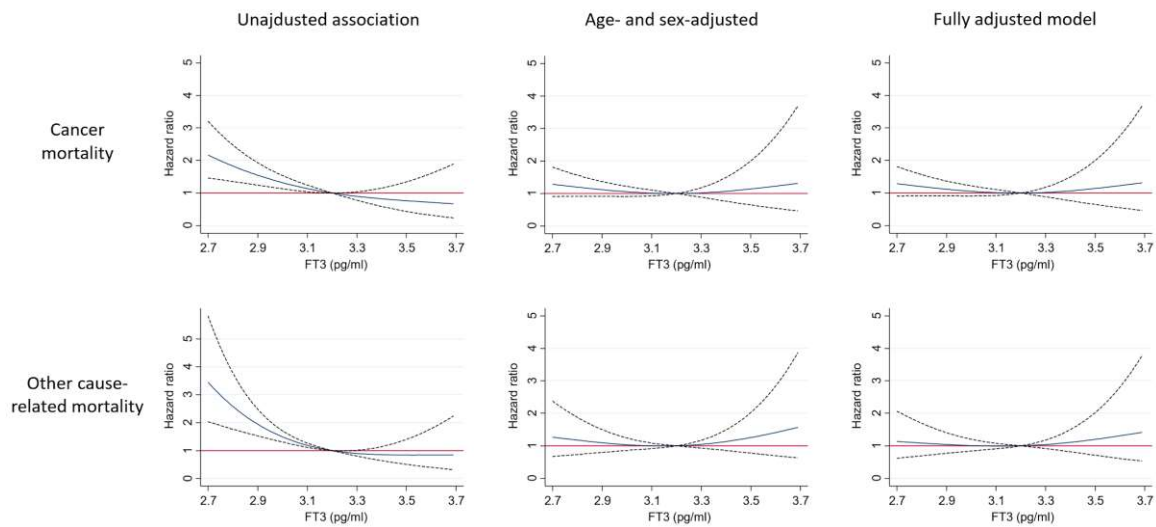
	Hazard ratio per each 0.1 pg/mL increase in FT3	P value
All-cause mortality		
Unadjusted model	0.82 (0.78-0.87)	<0.001
Age and sex adjusted model	0.96 (0.91-1.01)	0.104
Fully adjusted model	0.97 (0.92-1.02)	0.217
CV mortality		
Unadjusted model	0.74 (0.65-0.83)	<0.001
Age and sex adjusted model	0.85 (0.77-0.93)	0.001
Fully adjusted model	0.83 (0.75-0.93)	0.001
Cancer mortality		
Unadjusted model	0.88 (0.80-0.97)	0.009
Age and sex adjusted model	0.98 (0.89-1.09)	0.741
Fully adjusted model	1.02 (0.89-1.17)	0.798
Other cause-related mortality		
Unadjusted model	0.83 (0.77-0.90)	<0.001
Age and sex adjusted model	0.99 (0.90-1.09)	0.879
Fully adjusted model	1.00 (0.92-1.10)	0.931

Abbreviations: CV, cardiovascular.

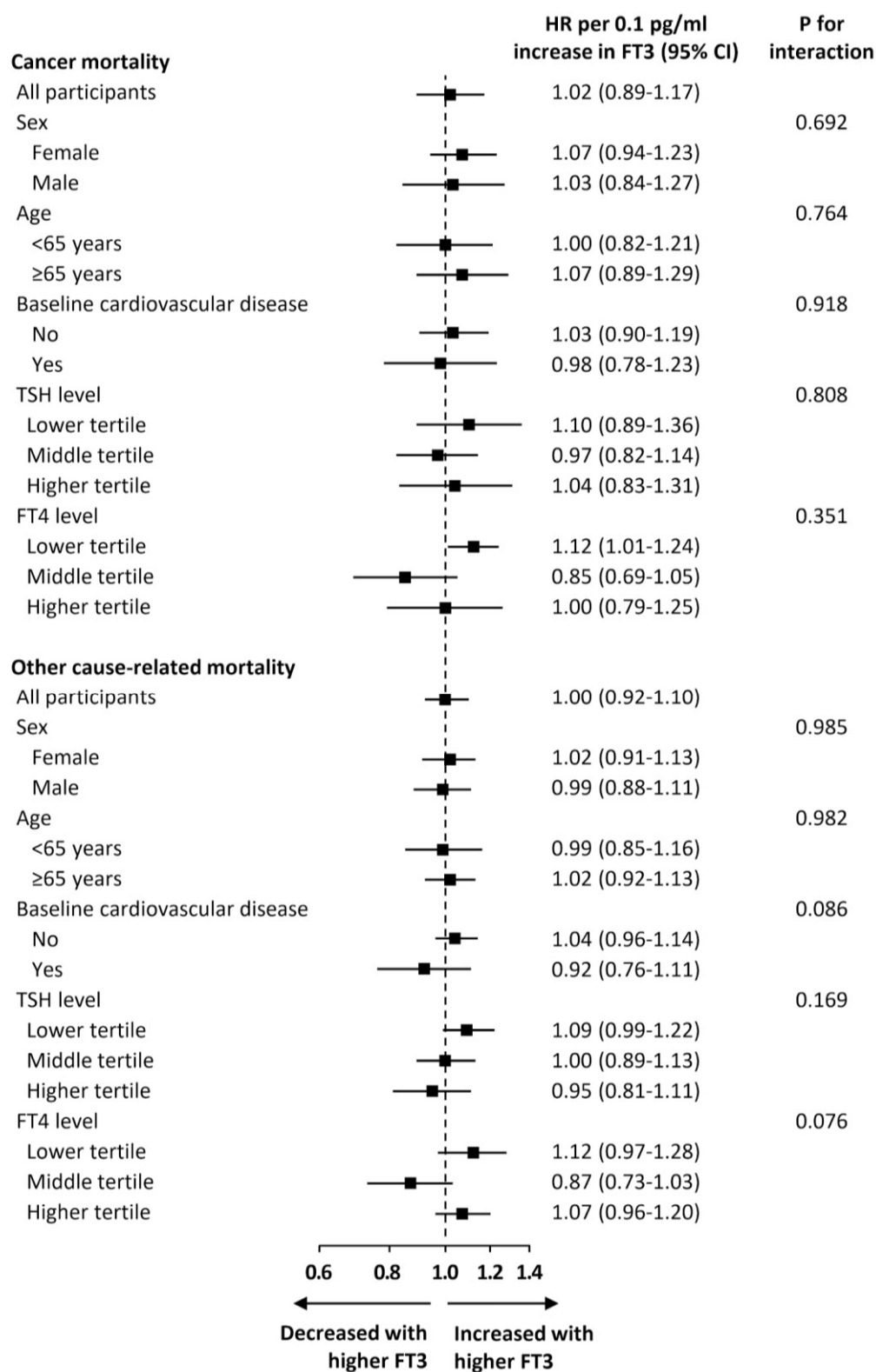
Fully adjusted model: age, sex, race, BMI, smoking, education, annual salary, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, previous cardiovascular disease, history of cancer, FT4 and TSH levels.



Supplemental figure 1 - Kaplan-Meier curves for cancer (A) and other cause-related mortality (B) by age- and sex-specific FT3 tertiles.



Supplemental figure 2 - Relationship between FT3 levels, cancer mortality, and other cause-related mortality using restricted cubic spline models. Fully adjusted model: age, sex, race, BMI, smoking, education, annual salary, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, previous cardiovascular disease, history of cancer, FT4 and TSH levels.



Supplemental figure 3 - Subgroup analysis of the association between FT3 levels, cancer mortality, and other cause-related mortality. Analysis adjusted for age, sex, race, BMI, smoking, education, annual family income, diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, previous cardiovascular disease, history of cancer, FT4 and TSH levels. HR: Hazard ratio.

Article 6

Effects of Triiodothyronine Treatment in an Animal Model of Heart Failure with Preserved Ejection Fraction

Neves JS, Leite AR, Conceição G, Gonçalves A, Borges-Canha M, Vale C, Von-Hafe M, Martins D, Miranda-Silva D, Leite S, Oliveira E, Sousa-Mendes C, Chaves J, Lourenço I, Grijota-Martínez C, Báñez-López S, Miranda IM, Almeida-Coelho J, Vasques-Nóvoa F, Carvalho D, Lourenço A, Falcão-Pires I, Leite-Moreira A.

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Effects of Triiodothyronine Treatment in an Animal Model of Heart Failure with Preserved Ejection Fraction

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Background: Low levels of triiodothyronine (T3) are common in patients with heart failure (HF). Our aim was to evaluate the effects of supplementation with low and replacement doses of T3 in an animal model of HF with preserved ejection fraction (HFpEF).

Methods: We evaluated four groups: ZSF1 Lean ($n=8$, Lean-Ctrl), ZSF1 Obese (rat model of metabolic-induced HFpEF, $n=13$, HFpEF), ZSF1 Obese treated with a replacement dose of T3 ($n=8$, HFpEF-T3high), and ZSF1 Obese treated with a low-dose of T3 ($n=8$, HFpEF-T3low). T3 was administered in drinking water from weeks 13 to 24. The animals underwent anthropometric and metabolic assessments, echocardiography, and peak effort testing with maximum O₂ consumption (VO₂max) determination at 22 weeks, and a terminal hemodynamic evaluation at 24 weeks. Afterwhile myocardial samples were collected for single cardiomyocyte evaluation and molecular studies.

Results: HFpEF animals showed lower serum and myocardial thyroid hormone levels than Lean-Ctrl. Treatment with T3 did not normalize serum T3 levels, but increased myocardial T3 levels to normal levels in the HFpEF-T3high group. Body weight was significantly decreased in both the T3-treated groups, comparing with HFpEF. An improvement in glucose metabolism was observed only in HFpEF-T3high. Both the treated groups had improved diastolic and systolic function *in vivo*, as well as improved Ca²⁺ transients and sarcomere shortening and relaxation *in vitro*. Comparing with HFpEF animals, HFpEF-T3high had increased heart rate and a higher rate of premature ventricular contractions. Animals treated with T3 had higher myocardial expression of calcium transporter ryanodine receptor 2 (RYR2) and α -myosin heavy chain (MHC), with a lower expression of β -MHC. VO₂max was not influenced by treatment with T3. Cardiomyocytes size and myocardial fibrosis were reduced in both the treated groups. Three animals died in the HFpEF-T3high group.

Conclusions: Treatment with T3 was shown to improve metabolic profile, myocardial calcium handling, and cardiac function. While the low dose was well-tolerated and safe, the replacement dose was associated with

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increased heart rate, and increased risk of arrhythmias and sudden death. Modulation of thyroid hormones may be a potential therapeutic target in HFpEF; however, it is important to take into account the narrow therapeutic window of T3 in this condition.

Keywords: heart failure, heart failure with preserved ejection fraction, triiodothyronine, thyroid hormones, metabolic syndrome, diastolic function

Introduction

THYROID HORMONES ARE important modulators of cardiovascular function.^{1,2} Hypothyroidism has been associated with an increased risk of myocardial dysfunction and heart failure (HF).^{3,4} Several guidelines propose the screening of thyroid dysfunction in HF and the correction of thyroid function to improve prognosis in HF.^{5,6}

Patients with HF frequently have low triiodothyronine (T3) levels related to a high prevalence of nonthyroidal illness syndrome.^{4,7–10} In these patients, the cardiovascular dysfunction and the proinflammatory systemic status change deiodinase activity, leading to lower conversion of thyroxine (T4) into T3 and higher thyroid hormone breakdown.¹¹ Even in patients with normal plasma thyroid hormone levels, local imbalances in deiodinase activity may lead to local myocardial hypothyroidism.¹² Previous studies have evaluated the effects of thyroid hormone supplementation in animal models of HF with reduced ejection fraction (HFrEF),¹³ postmyocardial infarction,¹⁴ experimental *diabetes mellitus*,¹⁵ and hypertensive heart disease,^{16,17} in which most studies suggested benefits, but some showed evidence for potential harm with thyroid hormone supplementation, particularly at higher doses.^{16,18,19}

Randomized clinical trials (RCTs) in humans have also suggested a potential benefit of supplementation of T3 in HFrEF,²⁰ after acute myocardial infarction²¹ and in patients undergoing cardiac surgery.²² However, these studies had limited follow-up and were underpowered to detect potential adverse effects.

Few studies have assessed the effects of thyroid hormone supplementation in HF with preserved ejection fraction (HFpEF). The evaluation of thyroid hormone supplementation in HFpEF is relevant because (1) HFpEF is responsible for at least half of the cases of HF²³; (2) diastolic dysfunction and systemic metabolic dysfunction are central elements of the pathophysiology of HFpEF²⁴; and (3) thyroid hormones improve diastolic function and systemic metabolic function.¹²

Therefore, the aim of this study was to evaluate the effects of treatment with low dose and replacement dose of T3 in an animal model of HFpEF.

Materials and Methods

Experimental model

Two independent cohorts of male ZSF1 Lean (Lean-Ctrl, $n=8$) and ZSF1 Obese (HFpEF groups, $n=29$) rats were purchased from Charles River Laboratories (Barcelona, Spain) at the age of 12 weeks. The sample size was decided according to previous studies and preliminary data.^{15,25} The animals were housed in groups of two per cage in an isolated ventilated cage system (IVC), and maintained on a 12-hour

light–dark cycle, with full access to the recommended diet (LabDiet® 5008; International Product Supplies Ltd.) and water. ZSF1 Obese Rat is an experimental rat model of HFpEF associated with metabolic syndrome. The detailed characterization of this animal model was previously described.^{26,27}

All procedures were reviewed and approved by the Ethics Committee of the Faculty of Medicine of University of Porto (FMUP), FMUP Animal Welfare and Ethics Review Body (Orgão de Bem-Estar e Ética Animal [ORBEA]), and the Portuguese competent authority (Direção Geral de Alimentação e Veterinária [DGAV], process No. 010334) and were done according to the Guide for the Care by the National Institutes of Health (NIH Publication No. 85–23, revised 2011), EU Directive 2010/63/EU and Decreto-lei 113/2013 national legislation. All procedures were carried out by properly trained and licensed researchers.

Experimental procedure

At 13 weeks of age, ZSF1 Obese rats were randomly allocated to receive T3 (T6397; Sigma-Aldrich) supplementation in drinking water in replacement dose (HFpEF-T3high, $n=8$), low dose (HFpEF-T3low, $n=8$), or solo drinking water (HFpEF, $n=13$), as described in the Supplementary Data. Replacement dose T3 was calculated to normalize myocardial T3 levels according to the literature and preliminary data (data not shown).²⁸ In the HFpEF-T3high group, animals received an initial T3 concentration in the water of 0.04 $\mu\text{g/mL}$ (40 $\mu\text{g/mL}$ T3, 50% ethanol, 48.5% glycerol) from 13 to 16 weeks of life, increasing after that to 0.06 $\mu\text{g/mL}$ (60 $\mu\text{g/mL}$ T3, 50% ethanol, 48.5% glycerol). HFpEF-T3low received half of the maximum dose used for HFpEF-T3high groups (0.03 $\mu\text{g/mL}$ of T3 concentration in the drinking water—30 $\mu\text{g/mL}$ T3, 50% ethanol, 48.5% glycerol, starting at 13 weeks until the end of the protocol).

Oral glucose tolerance testing, insulin resistance, metabolic cage studies, and peak effort testing

At 22 weeks, animals underwent oral glucose and insulin resistance (IR) testing as previously described.²⁵ On the following day, animals underwent a 24-hour acclimatization period in metabolic cages. Water and food intake and urine output were registered for 24 hours (Techniplast, Buguggiate, Italy). The maximum oxygen consumption (VO_2max) by maximum effort test was evaluated at 22 weeks of age as described in the Supplementary Data.

Echocardiographic evaluation

Echocardiography evaluation was performed at 22 weeks of age using a 15-MHz linear probe and an echocardiography system (Acuson Sequoia C512; Siemens). Structural and

functional evaluation was performed as previously described.²⁹ At least three stable cardiac cycles were averaged for all measurements.

Hemodynamic and electrocardiographic evaluation

At 24 weeks of age, animals were sedated, anesthetized, and mechanically ventilated as previously described.²⁹ The surgical preparation consisted of a left thoracotomy, and placement of a pressure-volume catheter in the left ventricle (LV) through the apex (SPR-847; Millar Instruments) and a flow probe (2.5PS; Transonic) in the ascending aorta. Hemodynamic recordings were made after a stabilization period of 15 minutes, as previously described.²⁵ Electrocardiographic (ECG) recordings were also obtained. Detection of premature ventricular contractions (PVCs) was based on the RR-interval signal during stable periods. The RR interval features were normalized by the mean value of all RR intervals within the same ECG recording.³⁰ Classification of PVCs was performed as previously described.³¹

At the end, the animals were euthanized under anesthesia by exsanguination. Once concluded, the heart was removed, and the apex was cut for molecular studies. The remaining heart was quickly used for cardiomyocyte isolation. Blood samples were collected, and the remaining organs were excised, weighed, and stored at -80°C . The weights were normalized to tibia length.

Serum and tissue thyroid hormone evaluation

At 24 weeks, circulating thyrotropin (TSH) concentration was evaluated by enzyme-linked immunosorbent assay (ELISA; E-EL-R0976). T4 and T3 were quantified by specific and highly sensitive radioimmunoassays in the serum, LV, adipose tissue, and skeletal muscle (*gastrocnemius*), after appropriate extraction and purification of tissue extracts as described.³² High specific activity T4 and T3 labeled with ^{125}I were synthesized as described.³³

Histological evaluation of myocardium

We evaluated LV myocardial fibrosis using Sirius red staining as described in the Supplementary Data.

Single cardiomyocyte evaluation

The hearts were enzymatically perfused to isolate cardiomyocytes, as described in the Supplementary Data. Cardiomyocytes were placed in temperature-controlled MatTek dishes at 37°C , containing 1.8 mM Ca^{2+} Tyrode's buffer. Platinum electrodes were immersed in the solution for stimulation, coupled to a MyoPacer. Ca^{2+} transients and sarcomere shortening were simultaneously recorded in an inverted microscope by an IonOptix system (IonOptix, Milton, MA) similar to what has been previously described.^{34,35} Briefly, cardiomyocytes were placed in a heated chamber mounted on the stage of an Olympus AE31 inverted microscope. After selection of an isolated, rod-shaped cardiomyocyte with clear striation and that did not spontaneously contract, the analysis was initiated.

Before measuring, the solution containing the intact isolated cardiomyocytes was incubated for 20 minutes with a Ca^{2+} -sensitive dye, 1 mM FURA-2AM (F1221; Thermo Fisher Scientific®, Waltham, MA). Loaded cells were

electrically stimulated at different frequencies with 14 ms bipolar pulse waves. The fluorescence emitted at 340 and 380 nm was alternately recorded and a ratiometric fluorescence/fluorescence ratio was the indicator of cytosolic Ca^{2+} concentration.

Real-time quantitative polymerase chain reaction

RNA was extracted from the LV after tissue disruption with zirconium beads and TRIzol. Following centrifugation, RNA in the aqueous phase was precipitated with isopropanol and washed with 70% ethanol. After resuspension, quantification of RNA concentration was performed in the NanoDrop (Thermo Fisher Scientific) and integrity was evaluated by electrophoresis. Synthesis of complementary DNA (cDNA; $100\text{ ng}/\mu\text{L}$) was executed using the *SensiFAST™ cDNA Synthesis Kit* (Meridian Bioscience, Inc.®, Cincinnati, OH), with reactions conducted in a *T100 thermal cycler* (Bio-Rad®, Hercules, CA). Quantitative polymerase chain reactions (qPCRs) were run in triplicate following the protocol of *SENSiFAST SYBR Hi-ROX Mix* (Biolone) using the *PikoReal™ Real-Time PCR System* (Thermo Fisher Scientific), as previously described.^{36,37} Sequences of the specific PCR primer pairs used are in Supplementary Table S2.

The β -actin gene was used to normalize target gene expression. Initial mRNA expression data were log-transformed using the $2^{-\Delta\Delta\text{CT}}$ method and expressed as relative to the mean obtained for the Lean-Ctrl group.

Immunoblotting

Immunoblotting was performed as previously described.³⁸ Briefly, protein extracts from the heart were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 4 – 20% gradient polyacrylamide gels (Criterion™ TGX™ Precast Gels, No. 5671095; Bio-Rad) and then electroblotted into nitrocellulose membranes (Trans-Blot® Turbo; Bio-Rad). Blots were blocked and incubated with primary antibodies to phospholamban (MA3-922; Thermo Fisher Scientific), ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2 (SERCA2a; PA1-211A/IRDye 800; Thermo Fisher Scientific), and thyroid hormone receptors α (PA1-211A; Thermo Fisher Scientific) and β (ab180612; Abcam) overnight at 4°C . The immunoblots were subsequently washed and incubated with 700 or 800 nm infrared dye-conjugated antibodies (Nos. 926-68020 and 926-32211; LI-COR Biosciences). The membrane was imaged by scanning at 800 and 700 nm with an Odyssey Infrared Imaging System (LI-COR Biosciences). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (ab9485; Abcam) and the control group was set as reference.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 9.4.1; Dotmatics) and Stata (version 17.0; Stata-Corp). For normally distributed variables, we performed a one-way analysis of variance test, followed by Tukey's multiple comparisons test to assess differences between groups. For non-normally distributed variables, we performed the Kruskal–Wallis test, followed by Dunn's multiple

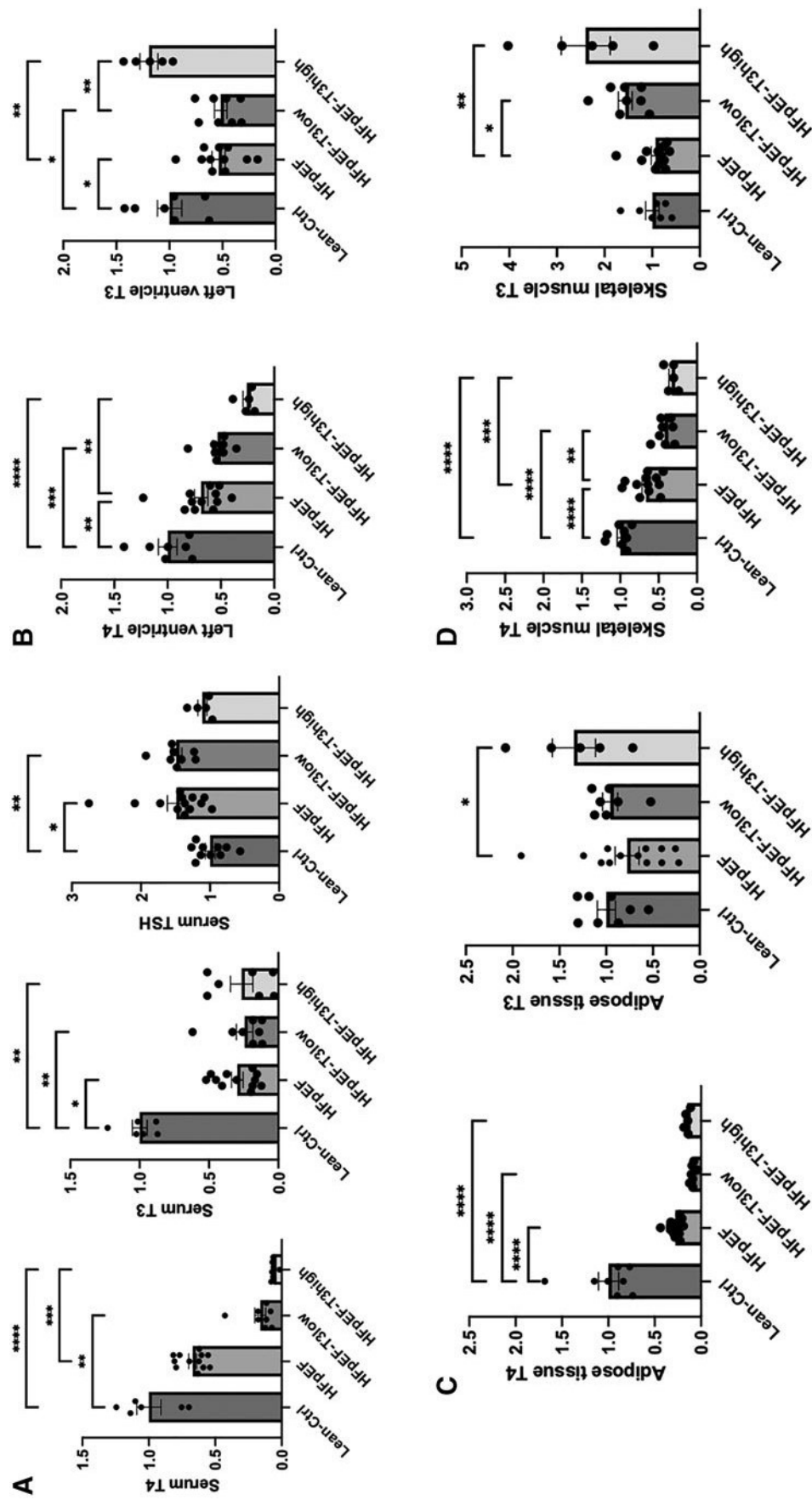


FIG. 1. Serum and tissue thyroid hormones at 24 weeks from Lean-Ctrl ($n=13$), HFpEF ($n=8$), HFpEF-T3low ($n=8$), and HFpEF-T3high ($n=5$). TSH was evaluated by ELISA and T4 and T3 were quantified by specific and highly sensitive radioimmunoassay. Results are expressed as relative fold increase above the mean value of thyroid hormone levels from the Lean-Ctrl group arbitrarily fixed at 1. (A) Serum thyroid hormone levels. (B) Left ventricle thyroid hormone levels. (C) Subcutaneous adipose tissue thyroid hormone levels. (D) Skeletal muscle thyroid hormone levels. The number of filled circles represent the number of samples for each group. Data are represented by mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. ELISA, enzyme-linked immunosorbent assay; HFpEF, heart failure with preserved ejection fraction; SEM, standard error mean; T3, triiodothyronine; T4, thyroxine; TSH, thyrotropin.

comparison test. Group data are presented as mean $\pm$ standard error mean or as median (interquartile range). Statistical significance was set at a p -value <0.05 .

Results

Serum and tissue thyroid hormones

HFpEF animals were shown to have lower serum and myocardial thyroid hormone levels than Lean-Ctrl (Fig. 1A, B). Treatment with both low and replacement dose of T3 did not normalize serum T3 levels, but increased myocardial T3 levels, with correction of myocardial T3 levels in the HFpEF-T3high group. TSH levels were higher in HFpEF than Lean-Ctrl and were normalized by replacement-dose but not low-dose T3 (Fig. 1A). Serum and tissue T4 levels were decreased in HFpEF-T3low and HFpEF-T3high (Fig. 1A, B). Levels of T3 at adipose level were higher in the HFpEF-T3high than in the HFpEF (Fig. 1C), and at skeletal muscle level were higher in HFpEF-T3high and HFpEF-T3low groups, compared with Lean-Ctrl and HFpEF (Fig. 1D).

Anthropometric parameters, metabolic effects, and peak effort testing

HFpEF had higher body weight, perigonadal fat, perirenal fat, and liver weight than Lean-Ctrl. In comparison with HFpEF, body weight was significantly decreased in HFpEF-T3low and HFpEF-T3high groups (Table 1).

Area under the curve in the oral glucose tolerance test (OGTT AUC) and IR (IR AUC) were significantly higher in the HFpEF compared with Lean-Ctrl. An improvement in both OGTT AUC and IR AUC was observed in the HFpEF-T3high group but not in the HFpEF-T3low group.

Compared with Lean-Ctrl, HFpEF animals had lower VO_2 max, and lower distance run. Treatment with T3 did not significantly change VO_2 max or distance run.

Echocardiography

In the echocardiographic evaluation at 22 weeks of age, HFpEF had a higher LV mass, isovolumetric relaxation time

(IVRT), E/e' (ratio between peak E wave velocity and peak E' wave velocity), left atrial (LA) area, and lower S' (peak systolic velocity of tissue Doppler at the lateral mitral annulus) than the Lean-Ctrl (Table 2). HFpEF-T3high and HFpEF-T3low groups had echocardiographic evidence of improved diastolic and systolic function. Both the treated groups had improved IVRT, ejection time, and S' compared with HFpEF. HFpEF-T3high showed higher heart rate (315.5 ± 9.9 vs. 297.8 ± 9.1 bpm, $p < 0.05$) and lower E/e' than HFpEF. The LV mass and LA area were not significantly different between the HFpEF and treated groups.

Hemodynamic and ECG evaluations

In the hemodynamic evaluation at 24 weeks of age, HFpEF had an increased LV stiffness as assessed by the β (chamber stiffness constant derived from exponential), LV end-diastolic pressure, and arterial elastance (Table 3). A trend for a prolonged time constant of isovolumetric relaxation (Tau) in HFpEF was also present ($p = 0.061$). LV systolic function was confirmed to be normal in HFpEF rats, evident from LV ejection fraction, LV end-systolic pressure, dP/dt_{max} , and end-systolic pressure–volume relationship E_{es} (slope of indexed linear end-systolic pressure–volume relationship). Treatment with T3 decreased Tau and arterial elastance in both groups compared with HFpEF, and normalized the LV stiffness to the levels of Lean-Ctrl group.

HFpEF-T3high group also had higher cardiac index (158.0 ± 9.5 vs. 121.3 ± 4.9 $\mu\text{L}/\text{min}/\text{cm}^2$, $p < 0.05$) and ejection fraction ($76.7\% \pm 5.9\%$ vs. $63.2\% \pm 2.3\%$, $p < 0.05$) than the HFpEF group, as well as a lower diastolic blood pressure than the Lean-Ctrl ($p < 0.05$) and HFpEF ($p = 0.070$) groups. HFpEF-T3 low group showed a trend for a lower β compared with the HFpEF group ($p = 0.066$), and a lower end-diastolic pressure (9.2 ± 1.3 vs. 12.0 ± 0.9 mmHg, $p < 0.05$) and dP/dt_{min} ($-14,289 \pm 1395$ vs. -9947 ± 525 mmHg/s; $p < 0.05$) than the HFpEF group.

Concerning ECG recording during the hemodynamics (Fig. 2), a higher rate of PVCs per minute was found in the HFpEF-T3high group in comparison with the Lean-Ctrl and HFpEF groups.

TABLE 1. ANTHROPOMETRIC, METABOLIC PARAMETERS AND PEAK EFFORT TESTING

	Lean-Ctrl (n=8)	HFpEF (n=13)	HFpEF-T3low (n=8)	HFpEF-T3high (n=5)
Body weight, g	449 $\pm$ 7	616 $\pm$ 11 ^a	572 $\pm$ 8^{a,b}	534 $\pm$ 8^{a,b}
Perigonadal fat, mg/cm	699 $\pm$ 54	1504 $\pm$ 71 ^a	1374 $\pm$ 59 ^a	1331 $\pm$ 29 ^a
Perirenal fat, mg/cm	769 $\pm$ 46	3910 $\pm$ 187 ^a	3819 $\pm$ 277 ^a	2785 $\pm$ 114^{a-c}
Liver weight, mg/cm	3109 $\pm$ 95	10,079 $\pm$ 162 ^a	9303 $\pm$ 335 ^a	7035 $\pm$ 360^{a-c}
Right kidney, mg/cm	366 $\pm$ 7	585 $\pm$ 19 ^a	614 $\pm$ 22 ^a	582 $\pm$ 20 ^a
OGTT AUC, mg/min/dL	12,493 $\pm$ 277	39,182 $\pm$ 2111 ^a	36,381 $\pm$ 3045 ^a	24,056 $\pm$ 1561^{a-c}
IR AUC, mg/min/dL	6706 $\pm$ 339	20,060 $\pm$ 1815 ^a	21,686 $\pm$ 1436 ^a	11,385 $\pm$ 221^{b,c}
VO_2 max, mL/min/kg ^{0.75}	32.9 $\pm$ 0.9	21.2 $\pm$ 1.0 ^a	24.2 $\pm$ 1.6 ^a	23.6 $\pm$ 1.0 ^a
Running distance, cm	45,862 $\pm$ 2812	14,009 $\pm$ 1065 ^a	13,945 $\pm$ 1092 ^a	16,090 $\pm$ 531 ^a

Anthropometric parameters at week 24. IR test, glucose tolerance test and peak effort testing were conducted at week 22. Organs weight were normalized for tibial length. Significant differences between HFpEF-T3low or HFpEF-T3high groups vs. HFpEF are highlighted in bold.

^a $p < 0.05$ vs. Lean-Ctrl.

^b $p < 0.05$ vs. HFpEF.

^c $p < 0.05$ vs. HFpEF-T3low.

AUC, area under the curve; HFpEF, heart failure with preserved ejection fraction; IR, insulin resistance; OGTT, oral glucose tolerance test; T3, triiodothyronine; VO_2 max, maximum oxygen consumption.

TABLE 2. ECHOCARDIOGRAPHIC EVALUATION AT 22 WEEKS OF AGE

	<i>Lean-Ctrl</i> (n = 8)	<i>HFpEF</i> (n = 13)	<i>HFpEF-T3low</i> (n = 8)	<i>HFpEF-T3high</i> (n = 5)
LV mass, g	1.18 ± 0.11	1.47 ± 0.06 ^a	1.64 ± 0.04 ^a	1.50 ± 0.08 ^a
HR, bpm	297.8 ± 9.1	272.7 ± 7.0	298.7 ± 9.1	315.5 ± 9.9^b
IVCT, ms	16.4 ± 1.2	16.1 ± 0.6	13.8 ± 1.0	14.0 ± 0.7
IVRT, ms	20.3 ± 1.2	25.8 ± 0.7 ^a	20.4 ± 0.7^b	20.5 ± 1.2^b
ET, ms	74.9 ± 2.2	81.9 ± 3.9	65.0 ± 4.3^{a,b}	65.1 ± 2.1^{a,b}
S', m/s	0.066 ± 0.003	0.049 ± 0.001 ^a	0.063 ± 0.005^b	0.060 ± 0.002^b
E/e'	15.0 ± 1.7	21.5 ± 0.8 ^a	20.0 ± 0.9 ^a	16.2 ± 1.1^b
E/A	1.95 ± 0.26	1.66 ± 0.09	1.74 ± 0.13	1.80 ± 0.17
LAA (cm ²)	0.28 ± 0.01	0.35 ± 0.01 ^a	0.36 ± 0.01 ^a	0.32 ± 0.01 ^c

Significant differences between HFpEF-T3low or HFpEF-T3high groups vs. HFpEF are highlighted in bold. E/A: ratio between peak E and A waves of pulsed-wave Doppler mitral flow velocity; E/e': ratio between peak E wave velocity of pulsed-wave Doppler mitral flow and peak E' wave velocity of tissue Doppler at the lateral mitral annulus; S': peak systolic velocity of tissue Doppler at the lateral mitral annulus.

^a*p* < 0.05 vs. Lean-Ctrl.

^b*p* < 0.05 vs. HFpEF.

^c*p* < 0.05 vs. HFpEF-T3low.

ET, ejection time; HR, heart rate; IVCT, isovolumetric contraction time; IVRT, isovolumetric relaxation time; LAA, left atrial area; LV, left ventricle.

Single cardiomyocyte sarcomere shortening and cytosolic Ca²⁺ transients

Comparing with Lean-Ctrl, Ca²⁺ transients were delayed in HFpEF group both in contraction, as assessed by time to peak Ca²⁺, and in relaxation, as measured in half-time of Ca²⁺ decay (Fig. 3A–C). HFpEF also had a shorter sarcomere length at baseline, a longer time to full contraction, and time to half-relaxation (Fig. 3E–G). Treatment with T3 improved Ca²⁺ transients and sarcomere shortening and relaxation. Both the treated groups had improved half-time of peak Ca²⁺ decay compared with HFpEF (Fig. 3C), but only HFpEF-

T3high had improved time to peak Ca²⁺ (Fig. 3B). Both HFpEF-T3high and HFpEF-T3low had improved sarcomere length, time to full contraction, and time to half-relaxation compared with HFpEF (Fig. 3D–G).

LV fibrosis

Fibrosis was increased in HFpEF in comparison with Lean-Ctrl. Compared with HFpEF, both low and replacement dose of T3 decreased myocardial fibrosis (Fig. 4). HFpEF-T3low group showed less fibrosis than HFpEF-T3high group (*p* < 0.01).

TABLE 3. HEMODYNAMIC EVALUATION AT 24 WEEKS OF AGE

	<i>Lean-Ctrl</i> (n = 8)	<i>HFpEF</i> (n = 13)	<i>HFpEF-T3low</i> (n = 8)	<i>HFpEF-T3high</i> (n = 5)
CI, $\mu\text{L}/\text{min}/\text{cm}^2$	134.8 ± 6.2	121.3 ± 4.9	126.1 ± 11.0	158.0 ± 9.5^a
HR, bpm	333.2 ± 9.5	277.9 ± 8.4 ^b	299.8 ± 10.9	313.5 ± 16.0
SBP, mmHg	147.3 ± 3.8	150.6 ± 2.8	155.1 ± 2.8	148.2 ± 4.9
MAP, mmHg	126.7 ± 3.8	128.7 ± 3.6	127.7 ± 3.4	119.0 ± 5.3
DBP, mmHg	105.3 ± 3.7	99.6 ± 3.3	98.0 ± 4.3	84.6 ± 5.4 ^b
EF, %	58.2 ± 1.2	63.2 ± 2.3	64.5 ± 3.0	76.7 ± 5.9^{a,b}
Tau, ms	11.0 ± 0.4	12.6 ± 0.3	9.5 ± 0.6^a	8.6 ± 0.7^{a,b}
LVESP, mmHg	146.8 ± 3.7	145.2 ± 2.9	146.9 ± 4.4	132.9 ± 9.2
LVEDP, mmHg	7.0 ± 0.9	12.0 ± 0.9 ^b	9.2 ± 1.3^a	9.7 ± 1.0
dP/dt _{max} , mmHg/s	10,300 ± 687	9765 ± 378	11,815 ± 444^a	11,801 ± 745
dP/dt _{min} , mmHg/s	−10,203 ± 415	−9947 ± 525	−14,289 ± 1395^{a,b}	−10,586 ± 867
EDV _i , $\mu\text{L}/\text{cm}^2$	0.74 ± 0.02	0.72 ± 0.03	0.67 ± 0.06	0.68 ± 0.05
Ea _i , mmHg/ $\mu\text{L}/\text{cm}^2$	533.8 ± 5.8	658.8 ± 7.8 ^b	626.4 ± 6.1^{a,b}	629.3 ± 7.8^{a,b}
β , $\mu\text{L}/\text{cm}^2$	4.34 ± 0.58	7.66 ± 0.69 ^b	5.07 ± 0.93	5.93 ± 0.53
Ees _i , mmHg/ $\mu\text{L}/\text{cm}^2$	163.5 ± 13.3	203.2 ± 19.1	225.5 ± 31.8	241.8 ± 36.3

Significant differences between HFpEF-T3low or HFpEF-T3high groups vs. HFpEF are highlighted in bold.

β : β chamber stiffness constant derived from exponential end-diastolic pressure–volume relationship; dP/dt_{max}: maximum rate of pressure rise; dP/dt_{min}: maximum rate of pressure fall; Ees_i: slope of indexed linear end-systolic pressure–volume relationship; Tau: exponential decay of the ventricular pressure during isovolumic relaxation.

^a*p* < 0.05 vs. HFpEF.

^b*p* < 0.05 vs. Lean-Ctrl.

^c*p* < 0.05 vs. HFpEF-T3low.

CI, cardiac index; DBP, diastolic blood pressure; Ea_i, indexed arterial elastance; EDV_i, end-diastolic volume index; EF, ejection fraction; LVEDP, left ventricle end-diastolic pressure; LVESP, left ventricle end-systolic pressure; MAP, mean arterial pressure; SBP, systolic blood pressure.

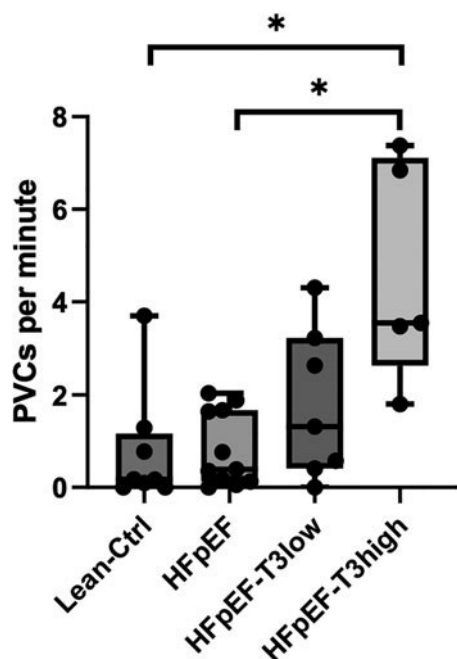


FIG. 2. Rate of PVCs during electrocardiographic monitoring performed in hemodynamic evaluation for the different experimental groups: Lean-Ctrl (ZSF1 Lean, $n=8$), HFpEF (placebo-treated ZSF1 Obese, $n=11$), HFpEF-T3low (ZSF1 Obese+low T3, $n=7$), and HFpEF-T3high (ZSF1 Obese+replacement T3 dose, $n=5$). Extreme outliers were removed ($n=2$ for HFpEF; $n=1$ for HFpEF-T3low). Error bars express the maximum and minimum value. The box plot bold lines represent the median and the first and third quartiles. Statistical analysis was performed using Kruskal–Wallis with *post hoc* comparisons by Dunn's multiple comparisons test. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$. PVCs, premature ventricular contractions.

Gene expression and immunoblotting

Treatment with replacement dose of T3 decreased the expression of β -myosin heavy chain (β -MHC) and increased the expression of α -MHC (Fig. 5B); increased the expression of the calcium handling proteins (Fig. 5C); of several antioxidant proteins (Fig. 5D); and of proinflammatory genes interleukin (IL)-1 α , IL-6 ($p=0.053$), and tumor necrosis factor (TNF)- α ($p=0.061$) (Fig. 5F). Treatment with low-dose T3 also decreased the expression of β -MHC ($p=0.094$), and increased the expression of ryanodine receptor 2; of antioxidant proteins methylmalonate semialdehyde dehy-

drogenase (ALDH6A1; $p=0.082$) and aldehyde dehydrogenase 2 (ALDH2; $p=0.084$); and of the extracellular matrix regulators matrix metalloproteinase (MMP) 2 and tissue inhibitor of metalloproteinases 2 (TIMP2) (Fig. 5E). No significant changes were found on the LV expression of brain natriuretic peptide (BNP) with T3 supplementation (Fig. 5A).

Western immunoblotting showed that SERCA2a protein content was similar between groups (Fig. 6B), but an increase in the phosphorylation of phospholamban in HFpEF-T3high group compared with HFpEF (Fig. 6A), and a decrease in the levels of thyroid hormone receptor β in both the treated groups (Fig. 6C).

Mortality

Mortality was increased in the HFpEF-T3high. No animals died in the Lean-Ctrl, HFpEF, and HFpEF-T3low groups. In the HFpEF-T3high group, three animals had sudden death at week 18 (one animal) and week 21 (two animals). Autopsy of the three animals with sudden death did not reveal myocardial infarction or other evident cause for death; an arrhythmic event was assumed as the probable cause of death.

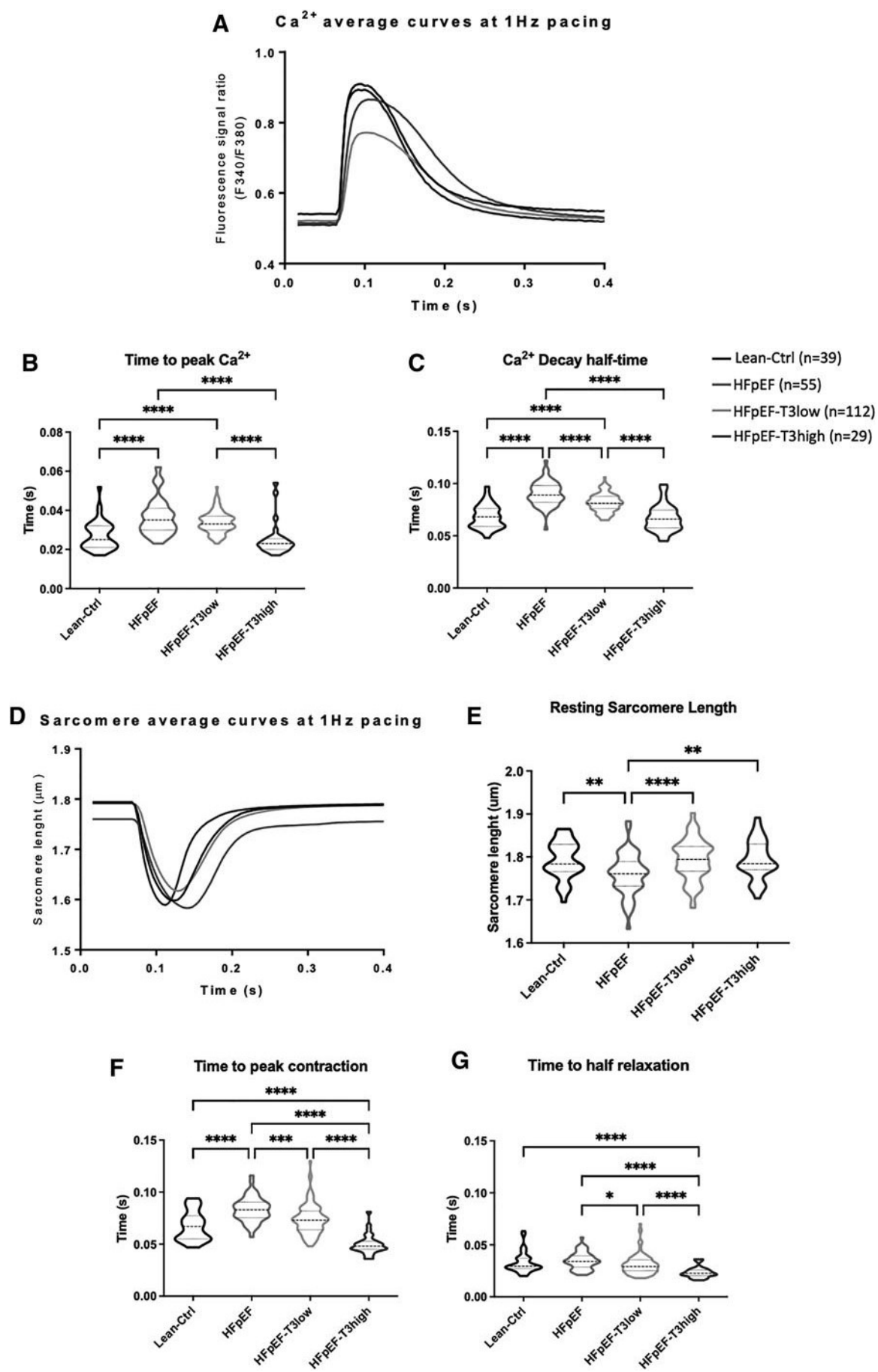
Discussion

In an animal model of HFpEF with metabolic syndrome, treatment with T3 improved metabolic and cardiac functions. While low-dose treatment was well tolerated, treatment with replacement dose was associated with increased risk of sudden cardiac death.

Previous studies had already showed that HF is associated with relevant changes in thyroid hormone level.^{1,7–10,39} Myocardial hypertrophy and HF have been shown to reduce myocardial T3 levels.^{40,41} Furthermore, lower levels of serum T3 are associated with worse prognosis both in patients with HFrEF and HFpEF.^{8–10,42,43} Our study is unique in that an animal model of HFpEF with metabolic syndrome was used. In accordance with the previous studies,^{15,17} HFpEF group also showed decreased serum and myocardial T3 levels, in comparison with the control group.

In our study, treatment with T3 was able to reduce body weight and improve insulin sensitivity, as well as the response to oral glucose test. At the myocardium level, the significant increase in the expression of calcium handling proteins and of MHC- β translated into improved calcium handling and improved sarcomere contraction and relaxation. While the replacement dose was able to improve the contraction and relaxation of the sarcomere to a greater extent than the low T3 dose, the lower dose reduced more the cardiomyocyte hypertrophy and the myocardial fibrosis. This

FIG. 3. Single cardiomyocyte evaluation. (A) Average Ca^{2+} transient fluorescence ratio plotting recording with 1 Hz electrical stimulation frequency for the different experimental groups: Lean-Ctrl (ZSF1 Lean, $n=39$), HFpEF (placebo-treated ZSF1 Obese, $n=55$), HFpEF-T3low (ZSF1 Obese+low T3, $n=112$), and HFpEF-T3high (ZSF1 Obese+replacement T3 dose, $n=29$). (B) Time elapsed from electric stimulus until peak cytoplasmic Ca^{2+} level. (C) Time interval from peak Ca^{2+} to its half decay (50% of peak Ca^{2+}). (D) Average sarcomere length recording with 1 Hz electrical stimulation frequency for the different experimental groups: Lean-Ctrl (ZSF1 Lean, $n=40$), HFpEF (placebo-treated ZSF1 Obese, $n=53$), HFpEF-T3low (ZSF1 Obese+low T3, $n=108$), and HFpEF-T3high (ZSF1 Obese+replacement T3 dose, $n=32$). (E) Time elapsed from electric stimulus to peak sarcomere shortening. (F) Time interval from peak sarcomere shortening to a half-relaxed state. Error bars express the maximum and minimum value of the parameter assumed. The middle bold dashed line represents the median, while the dotted lines illustrate the first and third quartiles. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$.



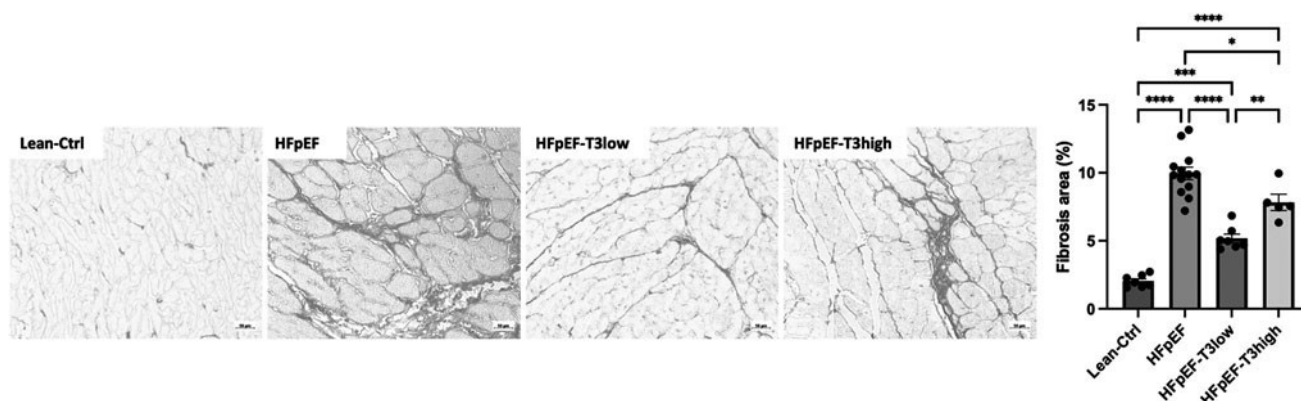


FIG. 4. Sirius red-stained histological sections ($\times 100$ magnification) of myocardium from Lean-Ctrl (ZSF1 Lean, $n=6$), HFpEF (placebo-treated ZSF1 Obese, $n=13$), HFpEF-T3low (ZSF1 Obese+low T3 dose, $n=8$), and HFpEF-T3high (ZSF1 Obese+replacement T3 dose, $n=5$), and the corresponding statistical analysis. Fibrosis was increased in HFpEF in comparison with Lean-Ctrl and the treatment of ZSF1 Obese with T3 (low and replacement doses) attenuated fibrosis. Data are represented by mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Statistical analysis was performed using one-way ANOVA with *post hoc* comparisons by Tukey's multiple comparisons test. ANOVA, analysis of variance.

suggests that, while some benefits of thyroid hormones at cardiac level are more pronounced with increasing doses, other effects may attenuate with higher doses. This is in line with the known risk of adverse cardiovascular effects of both low and high thyroid hormone levels.^{1,2,4}

The improvement of cardiac function with T3 supplementation is in accordance with most previous studies performed in different animal models of cardiovascular disease. In an animal model of myocardial-infarction-induced HFrEF, treatment with T3 improved systolic and diastolic function.¹³ In animal models of hypertensive heart disease, treatment with T3 improved diastolic function, attenuated cardiac dysfunction, and reduced adverse ventricular remodeling.^{16,17} There is also limited evidence of potential benefits of treatment with T3 in humans. In a randomized double-blind study of 50 patients with HFrEF and low T3 syndrome, supplementation with low-dose T3 for 6 weeks increased the LV ejection fraction and improved the 6-minute walk distance.²⁰

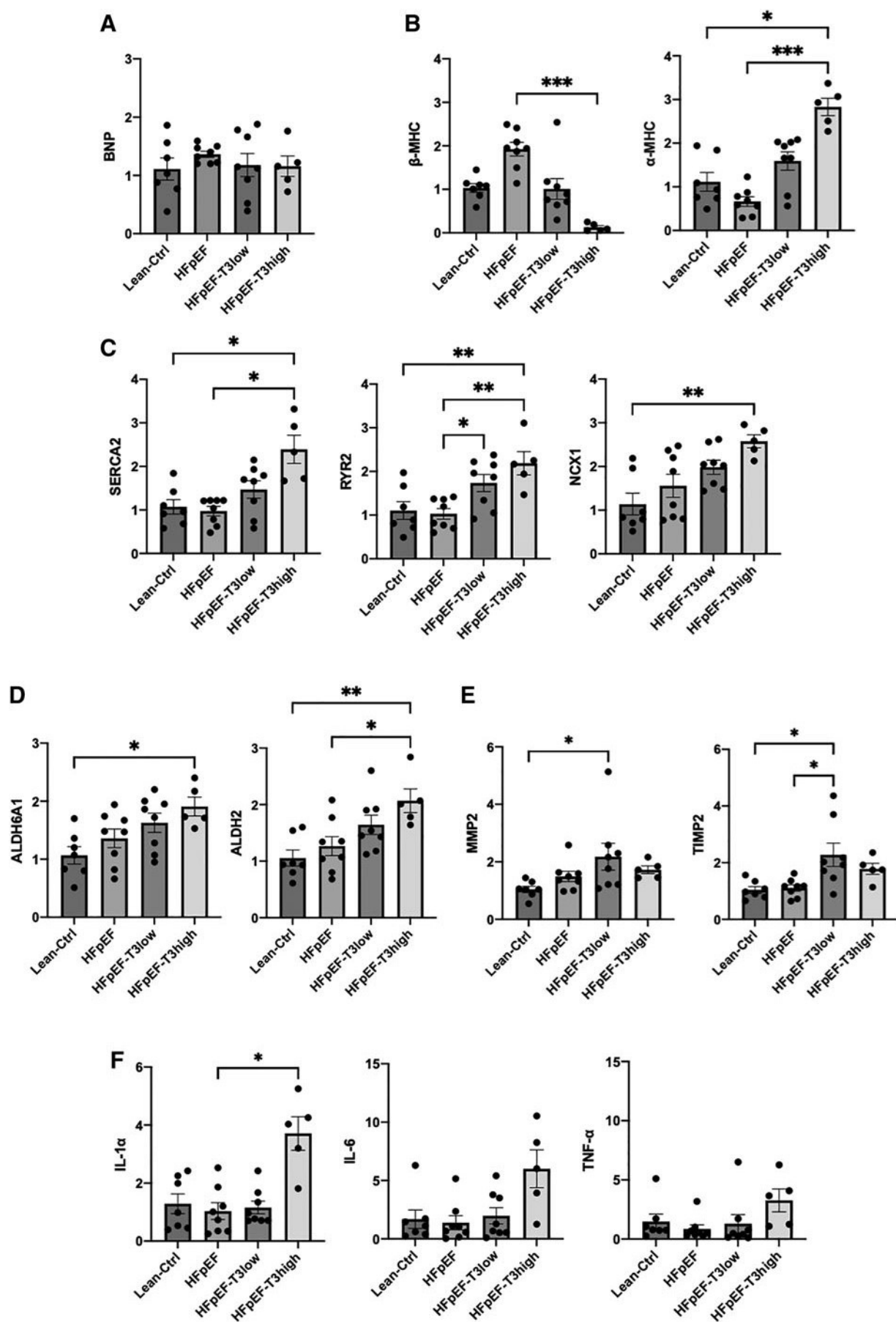
One of the main findings of our study was the increased risk of sudden death with replacement doses of T3. HF is a condition with high risk of sudden death, mostly due to arrhythmic events.^{44,45} Thyroid hormones increase the sensitivity to catecholamines and modulate the activity of multiple cardiac ion transporters, thus increasing the heart rate and the risk of both atrial and ventricle arrhythmic events.^{46,47} While

correction of hypothyroidism is not associated with an increased risk of arrhythmic events,⁴⁸ in a susceptible heart, normalization of T3 levels may increase the susceptibility to arrhythmic events. Although we cannot definitely ascertain that the cause of death in the group treated with replacement dose of T3 was arrhythmic, this is plausible from a pathological perspective and supported by our results.⁴⁹

In fact, we show that the high-dose treated group had an increased heart rate during *in vivo* echocardiographic evaluation and a higher rate of PVCs during hemodynamics. Also, the increased phosphorylation of phospholamban in this group enhances beta-adrenergic response.⁵⁰ The increased risk of arrhythmic events is well established for overt and subclinical hyperthyroidism.⁵¹ Even within the euthyroid range, higher levels of T4 have also been associated with an increased risk of arrhythmia and sudden cardiac death.^{52–54} In an RCT of patients with acute myocardial infarction, treatment with intravenous T3 reduced infarct size at 6 months, but was associated with a tendency for increased risk of atrial fibrillation (19% vs. 5%).²¹

In our study, the low dose was not associated with increased heart rate or sudden death. Low dose of T3 promoted greater decreases in myocardial fibrosis and was not associated with increased expression of proinflammatory interleukins. In agreement with our results for the low-dose treatment, in an animal model of myocardial infarction,

FIG. 5. LV myocardial mRNA expression from Lean-Ctrl (ZSF1 Lean, $n=5$), HFpEF (placebo-treated ZSF1 Obese, $n=5$), HFpEF-T3low (ZSF1 Obese+low T3 dose, $n=5$), and HFpEF-T3high (ZSF1 Obese+replacement T3 dose, $n=5$). (A) LV mRNA expression of BNP. (B) LV mRNA expression of α -MHC and β -MHC. (C) LV mRNA expression of several molecules implicated in calcium handling (SERCA2, RYR2, and sodium–calcium exchanger 1 (NCX1)). (D) LV mRNA expression of antioxidant molecules (ALDH6A1 and ALDH2). (E) LV mRNA expression of MMP2 and TIMP2. (F) LV mRNA expression of several inflammatory molecules (IL-1 α , IL-6, and TNF- α). Relative quantification was achieved using the comparative $2^{-\Delta\Delta C_t}$ method by normalization with the housekeeping gene (beta-actin). The number of filled circles represent the number of samples for each group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. ALDH2, aldehyde dehydrogenase 2; ALDH6A1, methylmalonate semialdehyde dehydrogenase; BNP, brain natriuretic peptide; IL, interleukin; LV, left ventricular; MHC, myosin heavy chain; MMP, matrix metalloproteinase; NCX1, sodium–calcium exchanger 1; RYR2, ryanodine receptor 2; SERCA2, ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2; TIMP2, tissue inhibitor of metalloproteinases 2; TNF, tumor necrosis factor.



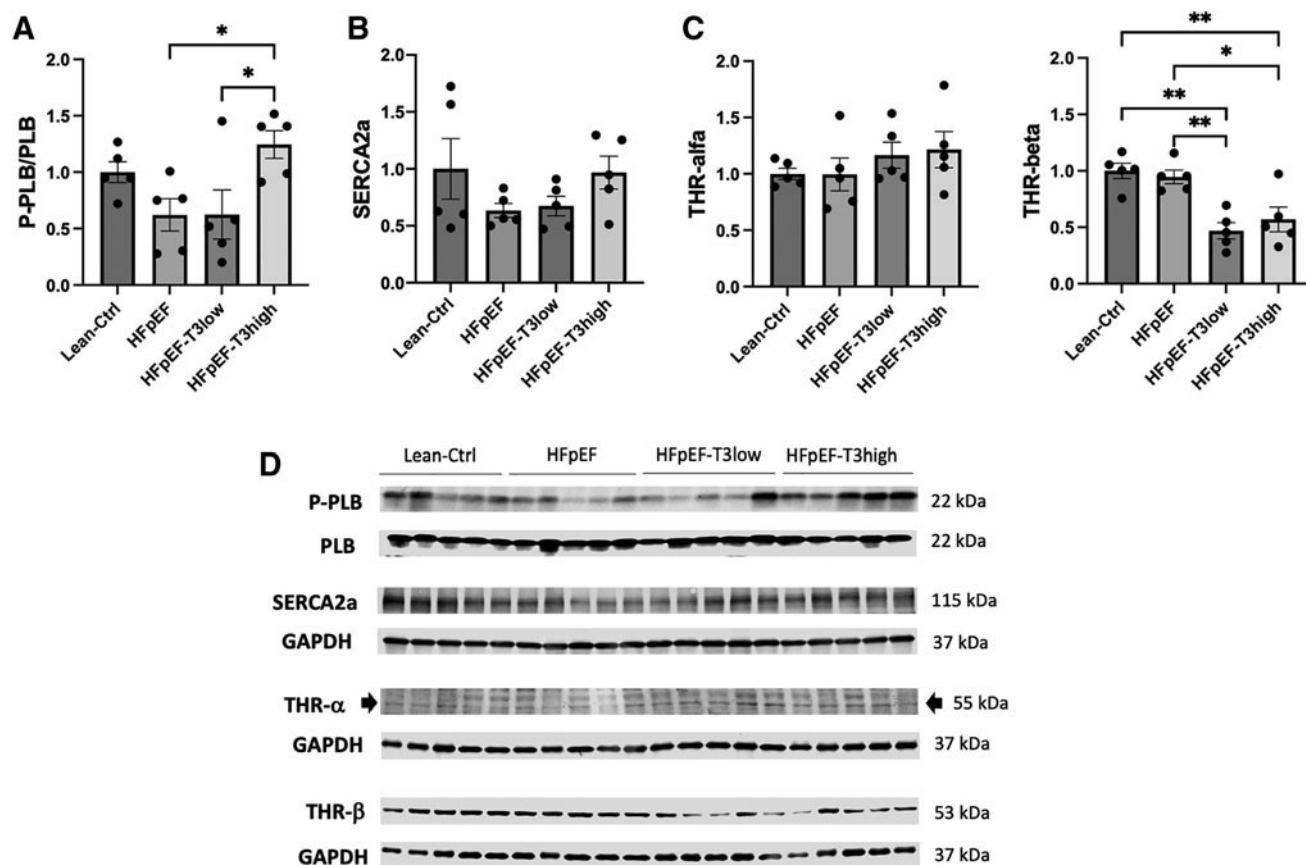


FIG. 6. Protein expressions by immunoblotting of LV samples from Lean-Ctrl (ZSF1 Lean, $n=5$), HFpEF (placebo-treated ZSF1 Obese, $n=5$), HFpEF-T3low (ZSF1 Obese+low T3 dose, $n=5$), and HFpEF-T3high (ZSF1 Obese+replacement T3 dose, $n=5$). (A) PLB and P-PLB protein expressions. Values are expressed as mean $\pm$ SEM. $*p<0.05$; $**p<0.01$; $***p<0.001$; $****p<0.0001$. (B) SERCA2a protein expression. Values are expressed as mean $\pm$ SEM. $*p<0.05$; $**p<0.01$; $***p<0.001$; $****p<0.0001$. (C) THR-alpha and THR-beta protein expressions. Values are expressed as mean $\pm$ SEM. $*p<0.05$; $**p<0.01$; $***p<0.001$; $****p<0.0001$. (D) Representative gel scans of PLB, P-PLB, THR-alpha, and THR-beta, and the loading control GAPDH. Uncropped gels are available in Supplementary Figure S1. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PLB, phospholamban; P-PLB, phospho-phospholamban; THR, thyroid hormone receptor.

treatment with physiological doses of T3 enhanced LV contractility, reduced fibrosis, and reduced the incidence of atrial tachyarrhythmias.¹⁴

From a clinical perspective, our results do not support the use of T3 in replacement doses for HFpEF, given the potential risk of sudden cardiac death. On the contrary, supplementation with low doses of T3 may be a potential therapeutic approach in HFpEF. Future RCTs with thyroid hormones in cardiovascular disease should include a detailed assessment of arrhythmic events.

This study has limitations that we must acknowledge. Despite the well-established role of the ZSF1 Obese Rat as an animal model of HFpEF, this model may not fully replicate the pathophysiology of human HFpEF. Moreover, the complexity and multiple phenotypes of HFpEF in humans may further limit the translatability of our results to the entire spectrum of HFpEF presentations in humans. We have only used male ZSF1 rats, even though HFpEF is more prevalent in women. Nevertheless this HFpEF animal model was developed in males and studies showed similar functional cardiac outcomes in both sexes.^{55–57} T3 was administered in drinking water, which may contribute to variations of expo-

sure to T3. While subcutaneous pellets for continuous release of T3 could have increased the stability of exposure to T3, the oral administration of T3 is more translatable to the expected effects of treatment with oral T3 in humans.⁵⁸

We did not monitor arrhythmic events during the study protocol, which precludes us from definitely stating the exact cause of the cases of sudden death. We only tested the effects of T3 supplementation; we cannot exclude that treatment with T4 could have been associated with greater benefits and/or lower risks.

Conclusions

In conclusion, treatment with T3 was shown to improve metabolic profile, myocardial calcium handling, as well as cardiac function in an animal model of HFpEF. While the low dose was well-tolerated and safe, the replacement dose was associated with increased heart rate and a higher risk of arrhythmias and sudden death. While modulation of thyroid hormones may be a potential therapeutic target in HFpEF, it is important to take into consideration the small therapeutic window of T3 in these patients.

Data Availability Statement

The data sets used during the current study are available from the corresponding author on reasonable request.

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Authors' Contributions

J.S.N.: conceptualization (lead), investigation (equal), formal analysis (equal), visualization (supporting), and writing—original article (lead); A.R.L.: investigation (equal), formal analysis (equal), visualization (lead), and writing—original article (supporting); G.C.: investigation (equal), formal analysis (equal), and writing—review and editing (equal); A.G.: investigation (equal), formal analysis (equal), and writing—review and editing (equal); M.B.-C.: investigation (equal), formal analysis (supporting), and writing—review and editing (equal); C.V.: investigation (equal), formal analysis (supporting), and writing—review and editing (equal); M.V.-H.: investigation (equal), formal analysis (supporting), and writing—review and editing (equal); D.M.: investigation (supporting), formal analysis (supporting), and writing—review and editing (equal); D.M.-S.: investigation (supporting), formal analysis (supporting), and writing—review and editing (equal); S.L.: investigation (supporting), formal analysis (supporting), and writing—review and editing (equal); E.R.-O.: investigation (supporting), formal analysis (supporting), writing—review and editing (equal), and visualization (supporting); C.S.-M.: investigation (supporting) and writing—review and editing (equal).

J.C.: investigation (supporting) and writing—review and editing (equal); I.M.L.: investigation (supporting) and writing—review and editing (equal); C.G.-M.: investigation (supporting), formal analysis (supporting), and writing—review and editing (equal); S.B.-L.: investigation (supporting), formal analysis (supporting), and writing—review and editing (equal); I.M.M.: investigation (supporting) and writing—review and editing (equal); J.A.-C.: investigation (supporting) and writing—review and editing (equal); F.V.-N.: investigation (supporting), formal analysis (supporting), writing—review and editing (equal), and visualization (supporting); D.C.: supervision (supporting) and writing—review and editing (equal); A.L.: conceptualization (supporting), investigation (equal), formal analysis (equal), supervision (supporting), and writing—review and editing (equal); I.F.-P.: conceptualization (supporting), investigation (equal), formal analysis (equal), supervision (supporting), and writing—review and editing (equal); A.L.-M.: conceptualization (supporting), supervision (lead), and writing—review and editing (equal).

Author Disclosure Statement

No conflicts of interest are declared by the authors.

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Supplementary Material

Supplementary Data

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Supplemental Material

Materials and Methods

Experimental Model and Procedure

Twenty-nine ZSF1 Obese rats at 13 weeks of age were submitted to a process of simple randomization using an ordered sequence to receive T3 (T6397, Sigma-Aldrich) supplementation in drinking water in replacement-dose (HFpEF-T3high, n=8), low-dose (HFpEF-T3low, n=8) or solo drinking water (HFpEF, n=13). The experimenters were blinded to study allocation.

Peak effort testing

To determine the maximum oxygen consumption ($\text{VO}_{2\text{max}}$) by maximum effort test, animals at 22 weeks of age were evaluated on a closed chamber treadmill (inclination of 10°) coupled to a gas analyzer (LE8700C and LE405, Panlab Harvard Apparatus®), as previously published.^{1,2} The adaptation period was carried out at a speed of 15 cm.s^{-1} for 3 minutes. The protocol was performed at a speed of 30 cm.s^{-1} , with increments of 5 cm.s^{-1} every minute until the animals reached their maximal aerobic capacity. Oxygen consumption and respiratory exchange ratio were measured with paramagnetic oxygen (75-PSO2, Accuscan Instruments, Inc.) and carbon dioxide analyzers (75-PSCO2, Accuscan Instruments, Inc.).

Histological evaluation of myocardium

All left ventricular (LV) samples were processed using Leica® *Histocore Pearl* automatic processor (Wetzlar, Germany). Paraffin inclusion was performed in

Leica Histocore Arcardia Embedding System. Fibrosis was assessed using 3 μm LV sections stained with Red Sirius and quantification was performed with Image-Pro Plus in 8 randomly selected fields per sample using the 100 $\times$ amplification.

Isolation of single cardiomyocytes

The heart was cannulated via the aorta and perfused at 6mL/min with cold Ca^{2+} -free digestion buffer (DB) (in mM: 130.00 NaCl, 5.40 KCl, 3.00 Pyruvate, 25.00 HEPES, 0.50 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.33 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 22.00 Glucose) supplemented with 0.05mM HEPES and 0.2mM EGTA as quickly as possible. Care was given throughout the whole protocol to avoid any bubble from entering the perfusion system as it drastically affects the isolation.

Once the drips were clear (no visible blood denoting that coronary arteries are cleaned), perfusate was changed to solution 1 (Table S1) at 37°C. Enzyme digestion was performed for 35 to 55 minutes.

After transferring the heart to solution 2 (Table S1) at 37°C, the tissue was minced and mechanical trituration for 3 minutes, the solution was then strained through a 200 μm mesh and centrifuged at 500 rpm for 1 min, at room temperature. The pelleted cells were resuspended in solution 3 (Table S1) and left to precipitate for 10 minutes at room temperature. The solution cells were then resuspended in solution 4 (Table S1) for another 10 minutes.

Finally, the supernatant was removed and exchanged by Tyrode's buffer (in mM: 133.5 NaCl, 5.0 KCl, 1.2 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 1.2 MgSO_4 , 10.0 HEPES and 22.0 Glucose) with 1.8mM Ca^{2+} .

Table S1 Digestion solutions used in the isolation procedure

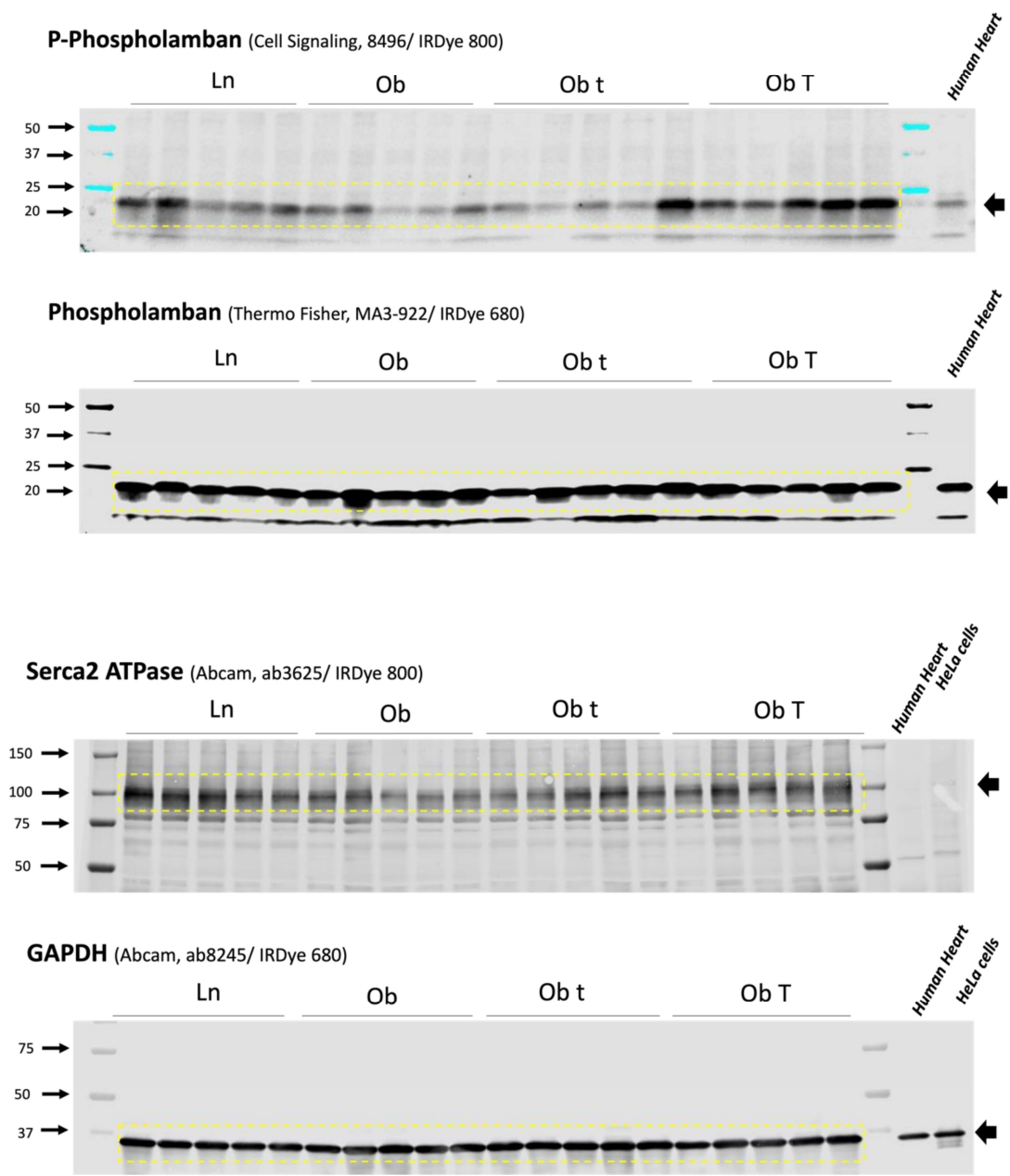
Solution	1	2	3	4
Collagenase type 2 (17101, Gibco), mg/ml	1.2	0.3	-	-
CaCl ₂ , mM	0.05	0.1	0.25	0.5
Bovine Serum Albumin (MB04602), mg/ml	-	10	10	10

*Real-time quantitative Polymerase Chain Reaction (RT-qPCR)***Table S2** Primer pairs used in real-time quantitative polymerase chain reaction (RT-qPCR) in myocardial tissue.

Genes	Primer sequences (5' to 3')
Brain natriuretic peptide (BNP) RnNPPB_F RnNPPB_R	GGATTGGCGCAGTCAGTCGTT CCGCAGGCAGAGTCAGAAG
Myosin heavy chain α (α-MHC) RnMHC α _F RnMHC α _R	TCGGGTGAAGGTGGGTAA AGGGTTGCGTTGATGCGT
Myosin heavy chain β (β-MHC) RnMHC β _F RnMHC β _R	CTTCCCCAACCGCATTCTTTAT CTTCTCAGCCCCTTTCCG
Methylmalonate semialdehyde dehydrogenase (ALDH6A1) RnALDH6A1_F RnALDH6A1_R	TCCAGCATCTTCCTTCTCTTCA AACCCGACCAACAACCTCA
Aldehyde Dehydrogenase 2 (ALDH2) RnALDH2_F RnALDH2_R	TTACTTCATCCAGCCCACC CCCCACAACCTCCTCAAT
Tumor necrosis factor α (TNF-α) RnTNF α _F RnTNF α _R	CCACCACGCTCTTCTGTCT CTACGGGCTTGTCACCTCG

Interleukin-6 (IL-6) RnIL6_F RnIL6_R	ATCTGCTCTGGTCTTCTGGA TTGCTCTGAATGACTCTGGCT
Interleukin-1α (IL-1α) RnIL1 α _F RnIL1 α _R	GGTGGTGGTGTCTCAGCAAC TGGGTTGGATGGTCTCTTCT
Matrix metalloproteinase-2 (MMP2) RnMMP2_F RnMMP2_R	GGCACCACCGAGGATTATG CCAGAAAAGTGAAGGGAAGAC
Tissue inhibitor of metalloproteinases 2 (TIMP2) RnTIMP2_F RnTIMP2_R	GAAGGCGGAAGGAGATGG GTTTAGGCTCTTCTTCTGGGT
Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1A) RnPGC1A_F RnPGC1A_R	CAGCCACTCCACCAAGAAAG TCACCAAACAGCCGTAGACT
ATPase Sarcoplasmic/Endoplasmic Reticulum Ca²⁺ Transporting 2 (SERCA2A) RnSERCA2A_F RnSERCA2A_R	CACTGCGCTGGGGTTCAATCCTC GCAGCGCCAACATAACAGCCAATA
Ryanodine receptor II (RYR2) RnRYR2_F RnRYR2_R	GAAAAGGTGCGGGTTGGAG CTGGAAGGCTGCGTCTAC
Na⁺/Ca²⁺ exchanger 1 (NCX1) RnNCX1_F RnNCX1_R	AGACCCAGAAGGAAATCAGAGT AGACAAGCAATCGCAGACACG
β-actin RnACTB_F RnACTB_R	5'-ATGGTGGGTATGGGTCAGA-3' 5'-CTCATTGTAGAAAGTGTGGTGCC-3'

Results



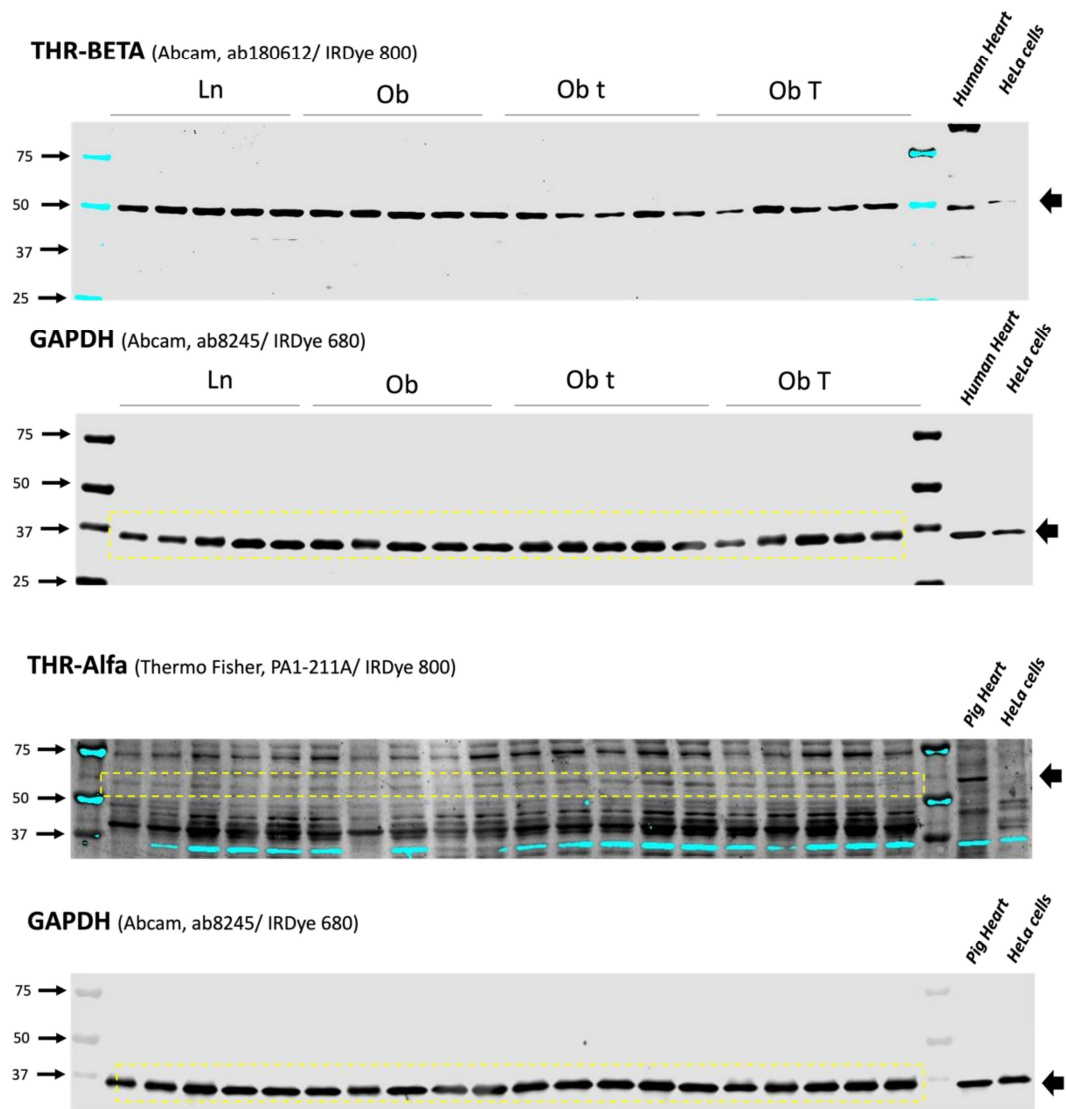


Figure S1. Uncropped gels of left ventricular phospholambam, phospho-phospholambam, SERCA2 ATPase, THR-Beta, GAPDH, THR-Beta and THR-Alpha. SERCA2, sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2; THR, thyroid hormone receptors; GAPDH, glyceraldehyde-3-phosphate dehydrogenase (loading control).

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Chapter 3 | Part B

Prediabetes and cardiovascular risk



Article 7

Impaired Fasting Glucose and Chronic Kidney Disease, Albuminuria, or Worsening Kidney Function: a Secondary Analysis of the SPRINT

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Impaired Fasting Glucose and Chronic Kidney Disease, Albuminuria, or Worsening Kidney Function: A Secondary Analysis of SPRINT

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Purpose: Diabetes mellitus is a risk factor for the development and progression of chronic kidney disease (CKD). However, the association of prediabetes with adverse kidney outcomes is uncertain.

Methods: We performed a secondary analysis of the Systolic Blood Pressure Intervention Trial (SPRINT), including 9361 participants without diabetes at baseline. We categorized participants according to fasting glucose level as having impaired fasting glucose [≥ 100 mg/dL (≥ 5.6 mmol/L)] or normoglycemia [< 100 mg/dL (< 5.6 mmol/L)]. Unadjusted and adjusted proportional hazards models were fitted to estimate the association of impaired fasting glucose (vs normoglycemia) with a composite outcome of worsening kidney function [$\geq 30\%$ decrease in estimated glomerular filtration rate (eGFR) to < 60 mL/min/1.73 m² in participants without baseline CKD; $\geq 50\%$ decrease in eGFR or need for long-term dialysis/kidney transplantation in participants with CKD] or incident albuminuria (doubling of urinary albumin/creatinine ratio from < 10 mg/g to > 10 mg/g). These outcomes were also evaluated separately and according to CKD status at baseline.

Results: Participants' mean age was 67.9 ± 9.4 years, 35.5% were female, and 31.4% were black. The median follow-up was 3.3 years, and 41.8% had impaired fasting glucose. Impaired fasting glucose was not associated with higher rates of the composite outcome [hazard ratio (HR): 0.97; 95% CI: 0.8 to 1.16], worsening kidney function (HR: 1.02; 95% CI: 0.75 to 1.37), or albuminuria (HR: 0.98; 95% CI: 0.78 to 1.23). Similarly, there was no association of impaired fasting glucose with outcomes according to baseline CKD status.

Conclusions: Impaired fasting glucose at baseline was not associated with the development of worsening kidney function or albuminuria in participants of SPRINT. (*J Clin Endocrinol Metab* 104: 4024–4032, 2019)

Diabetes mellitus (DM) is a major global health problem, affecting 14.3% of adults in the United States (1) with type 2 DM accounting for the majority of adult cases (2). DM in general is known to be a major risk factor for the development and progression of chronic kidney disease (CKD) and albuminuria (3), and it remains the predominant cause of end-stage renal disease (ESRD) in the United States (4). Both CKD and DM are associated with an increased risk for cardiovascular (CV) events and death (5), with their coexistence associated with a multiplicative risk (6).

Diabetes is generally thought to develop along a spectrum from normoglycemia to abnormal glycemia, with an intervening period of “prediabetes” (*i.e.*, abnormal glucose concentrations that do not meet the diagnostic criteria for diabetes). According to the recommendations of the American Diabetes Association (7), prediabetes is defined by impaired fasting glucose [fasting plasma glucose level of 100 to 125 mg/dL (5.6 to 6.9 mmol/L)], impaired glucose tolerance [2-hour plasma glucose value between 140 and 199 mg/dL (between 7.8 and 11.0 mmol/L) after a 75-g oral glucose tolerance test], or HbA1c value of 5.7% to 6.4%. As with DM in general, the worldwide prevalence of prediabetes is increasing, with more than 470 million people expected to have this condition by 2030 (8). Although the presence of prediabetes is associated with higher CV risk, the association of prediabetes with development of CKD is less clear (9–12). The large number of nondiabetic participants, baseline determination of fasting plasma glucose level, detailed documentation, and follow-up of the Systolic Blood Pressure Intervention Trial (SPRINT) provided a unique opportunity to assess the association of impaired fasting glucose with adverse renal outcomes. We hypothesized that the presence of baseline impaired fasting glucose would be associated with a higher risk of worsening kidney function, CKD, and incident albuminuria.

Research Design and Methods

Study design and population

We performed a secondary analysis of SPRINT data to evaluate the association of impaired fasting glucose with adverse renal outcomes. SPRINT was a multicenter, randomized, controlled trial including 9361 patients aged ≥ 50 years with hypertension and an increased risk of CV events. Increased CV risk was defined by the presence of at least one of the following: clinical or subclinical CV disease; CKD, defined by an estimated glomerular filtration rate (eGFR) of 20 to 59 mL/min/1.73 m²; a 10-year risk of CV disease of 15% or greater on the basis of the Framingham risk score; or age ≥ 75

years. Patients with DM or prior stroke were excluded. Detailed inclusion and exclusion criteria have been published (13). Eligible participants were randomly assigned to a systolic blood pressure target of <140 mm Hg (standard treatment) or <120 mm Hg (intensive treatment). The study was approved by the institutional review board at each participating study site, and written informed consent was obtained from all participants. The intervention was stopped early after a median follow-up of 3.26 years owing to a significantly lower rate of the primary composite outcome in the intensive-treatment group (13). The data for this analysis were obtained as a part of the SPRINT Data Analysis Challenge (14).

Exposures

Participants were categorized into two groups according to fasting plasma glucose level at randomization, using the American Diabetes Association cutoff of 100 mg/dL (5.6 mmol/L) (15). Participants with a fasting plasma glucose level ≥ 100 mg/dL (≥ 5.6 mmol/L) were classified as having impaired fasting glucose; those with fasting plasma glucose level <100 mg/dL (<5.6 mmol/L) were classified as being normoglycemic. We excluded participants with missing data on fasting plasma glucose level at the randomization visit and those with missing data on all the renal outcomes evaluated ($n = 40$).

Study outcomes

The composite renal outcome was defined by worsening kidney function or incident albuminuria, both as prespecified in SPRINT. In participants without CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of $\geq 30\%$ to a value of <60 mL/min/1.73 m² on two consecutive laboratory determinations collected at 3-month intervals. In participants with CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of $\geq 50\%$ or the development of ESRD requiring long-term dialysis or kidney transplantation. Incident albuminuria was defined for all study participants by a doubling of the urinary albumin/creatinine ratio from <10 mg/g at baseline to >10 mg/g during follow-up. The increase in urinary albumin/creatinine ratio had to be observed at two visits at least 3 months apart. As a secondary analysis, we evaluated the association of impaired fasting glucose with all-cause mortality. Medical records were obtained for documentation of clinical events and were reviewed by investigators masked to treatment assignment. At-risk time was considered to begin at the time of randomization. Participants were followed up until the occurrence of the outcomes of interest, censoring (date of last event ascertainment), or study completion.

Study data

Detailed definitions of the variables used in SPRINT were previously reported (16). Briefly, trained study personnel collected sociodemographic characteristics at baseline using structured interviews. Clinical and laboratory evaluations were performed every 3 months thereafter. The blood samples were centrifuged and shipped on ice to the central laboratory. Fasting plasma glucose level was measured in serum using the hexokinase method on a Roche analyzer. Four racial/ethnic groups were considered: non-Hispanic white, non-Hispanic black, Hispanic, and other race or ethnicity. Blood pressure was measured with an automated measurement system (Model 907; Omron Healthcare). A mean of three blood pressure measurements at an office visit while the patient was seated and after 5 minutes of quiet rest was considered. Body mass index (BMI) was calculated as the body weight in kilograms divided by the square of height in meters.

Statistical analysis

Continuous variables were examined graphically and recorded as means ($\pm$ SDs) for normally distributed data or as medians (with interquartile ranges) for non-normally distributed data. Comparisons were made using *t* tests or Wilcoxon rank sum tests as appropriate. Categorical variables were examined by frequency distribution and recorded as proportions. Comparisons were made using the χ^2 test.

Log-rank tests were used to compare Kaplan-Meier estimates for survival curves in the two treatment groups in a time-to-first-event analysis. Hazard ratios (HRs) and 95% CIs were estimated with a Cox proportional-hazards model, with stratification according to center. Initially, unadjusted models were fitted. Subsequently, multivariable models that adjusted for baseline age, sex, race (black, Hispanic, white, or other), smoking status (current, former/never), systolic blood pressure, prior CV disease, BMI, statin use, aspirin use, and trial treatment arm were fitted. The presence of effect modification for prespecified variables (age, sex, and treatment group) was assessed by inclusion of the relevant cross-product terms in adjusted models, with performance of the likelihood ratio test. We also analyzed the outcomes of interest in the prespecified subgroups of baseline CKD status (with or without CKD at baseline).

We performed two sensitivity analyses. First, we performed all the previously described analyses after excluding SPRINT participants who had a baseline fasting plasma glucose level ≥ 126 mg/dL (≥ 7.0 mmol/L), the cutoff for a diagnosis of diabetes ($n = 295$). Second, we performed all the previously described analyses, using

a more stringent cutoff of ≥ 110 mg/dL (≥ 6.1 mmol/L) to define impaired fasting glucose as recommended by some organizations, including the World Health Organization (17). Two-sided *P* values < 0.05 were considered statistically significant. All statistical analyses were performed using Stata software, version 14.2 (StataCorp).

Results

Baseline characteristics

A total of 9321 participants were analyzed, of whom 3897 (41.8%) had impaired fasting glucose and 5424 (58.2%) had normoglycemia. The mean age of the cohort was 67.9 ± 9.4 years; 35.5% were female, and 31.4% were black. At baseline, participants with impaired fasting glucose tended to be younger; were more likely to be male, white, and current smokers; were more likely to have prior CV disease, higher BMI, higher fasting triglyceride levels, and higher eGFR; and were more likely to be using a statin, aspirin, and a higher number of antihypertensive agents. Participants with impaired fasting glucose were also more likely to have lower systolic and diastolic blood pressure, lower total cholesterol, and lower high-density lipoprotein levels. There were no significant differences between groups according to baseline fasting glucose status (Table 1).

Association of impaired fasting glucose with renal outcomes

The median (25th to 75th percentile) follow-up was 3.3 (2.8 to 3.8) years. The composite renal outcome occurred in 221 individuals (5.7%) with impaired fasting glucose and 314 (5.8%) with normoglycemia at baseline. Worsening kidney function occurred in 79 individuals (2.0%) with impaired fasting glucose and 114 (2.1%) with normoglycemia at baseline. Among the 4619 participants at risk of developing albuminuria as prespecified in SPRINT, this outcome occurred in 147 individuals with impaired fasting glucose (7.6% of those at risk and 3.8% of all participants) and 203 individuals with normoglycemia at baseline (7.5% of those at risk and 3.7% of all participants).

Impaired fasting glucose was not associated with higher rates of the composite renal outcome, worsening kidney function, or albuminuria in either unadjusted or adjusted analyses (Fig. 1; Table 2). There was no evidence for effect modification of the composite renal outcome according to randomized treatment assignment ($P_{\text{interaction}} = 0.98$), sex ($P_{\text{interaction}} = 0.17$), or age ($P_{\text{interaction}} = 0.15$). Similarly, there was no evidence for effect modification of worsening kidney function ($P_{\text{interaction}} = 0.38$) or albuminuria ($P_{\text{interaction}} = 0.74$).

Table 1. Baseline Characteristics of Study Participants According to Fasting Glucose Status

	Normoglycemia (n = 5424)	Impaired Fasting Glucose (n = 3897)	P Value ^a
Age, y	68.1 ± 9.6	67.6 ± 9.2	0.02
Female sex, n (%)	2155 (39.7)	1152 (29.6)	<0.001
Race or ethnic group, n (%)			<0.001
Non-Hispanic white	2997 (55.3)	2387 (61.3)	
Non-Hispanic black	1764 (32.5)	1021 (26.2)	
Hispanic	565 (10.4)	413 (10.6)	
Other	98 (1.8)	76 (2.0)	
Baseline blood pressure, mm Hg			
Systolic	140.2 ± 15.7	138.9 ± 15.4	<0.001
Diastolic	78.5 ± 11.9	77.7 ± 12.0	0.001
Antihypertensive agents, (n)	2 (1–2)	2 (1–3)	<0.001
Cardiovascular disease, n (%)	1046 (19.3)	823 (21.1)	0.03
Body mass index, kg/m ²	29.1 ± 5.7	30.9 ± 5.6	<0.001
Fasting plasma glucose level			<0.001
mg/dL	91 ± 6	110 ± 13	
mmol/L	5.1 ± 0.3	6.1 ± 0.7	
Fasting total cholesterol level			<0.001
mg/dL	191 ± 41	188 ± 41	
mmol/L	4.9 ± 1.1	4.9 ± 1.1	
Fasting HDL cholesterol level			<0.001
mg/dL	55 ± 15	50 ± 13	
mmol/L	1.4 ± 0.4	1.3 ± 0.3	
Fasting total triglyceride level			<0.001
mg/dL	100 (73–137)	116 (84–166)	
mmol/L	1.1 (0.8–1.5)	1.3 (0.9–1.9)	
Statin use, n (%)	2213 (41.1)	1832 (47.3)	<0.001
Aspirin use, n (%)	2682 (49.6)	2064 (53.0)	0.001
Current smoker, n (%)	2177 (40.1)	1784 (45.8)	<0.001
eGFR, mL/min/1.73 m ²	71.3 ± 20.9	72.4 ± 20.2	0.01
Chronic kidney disease, n (%)	1576 (29.1)	1069 (27.4)	0.09
Albumin/creatinine ratio, mg/g	9.4 (5.7–21.3)	9.5 (5.6–21.5)	0.67

Values for continuous variables are given as mean ± SD or median (25th–75th percentile).

Abbreviation: HDL, high-density lipoprotein.

^aP values refer to a test for difference (t test for normally distributed continuous variables; Wilcoxon rank sum for nonnormally distributed continuous variables; and χ^2 test for categorical variables) according to impaired fasting glucose vs normoglycemia.

according to randomized treatment assignment. The results of the analysis according to subgroup of randomized treatment assignment are presented in an online repository (18).

There was no association of impaired fasting glucose with adverse renal outcomes in the sensitivity analyses that excluded participants with baseline fasting plasma glucose level ≥ 126 mg/dL, consistent with the main

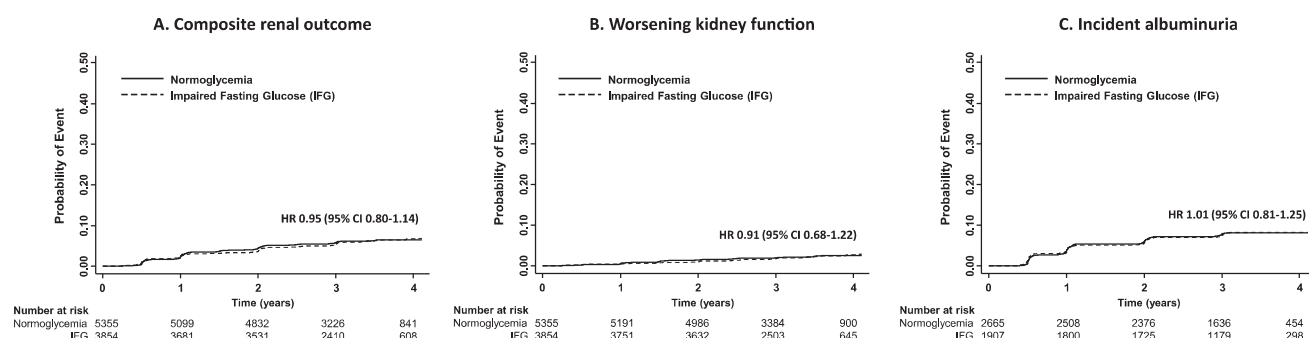


Figure 1. (A) Composite renal outcome, (B) worsening kidney function, and (C) incident albuminuria by fasting glucose status. The composite renal outcome was defined by worsening kidney function or incident albuminuria. In participants without CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 30% or more to a value of <60 mL/min/1.73 m². In participants with CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 50% or more or the development of ESRD requiring long-term dialysis or kidney transplantation. Incident albuminuria was defined for all study participants by a doubling of the ratio of urinary albumin to creatinine from <10 mg/g at baseline to >10 mg/g during follow-up.

Table 2. Association of Impaired Fasting Glucose vs Normoglycemia With Outcomes

Outcome	Normoglycemia	Impaired Fasting Glucose	Hazard Ratio (95% CI)
	No. of Events/No. at Risk (%)	No. of Events/No. at Risk (%)	
Composite adverse renal outcome ^a	314/5424 (5.8)	221/3897 (5.7)	
Unadjusted model			0.95 (0.80–1.14)
Adjusted model			0.97 (0.81–1.16)
Worsening kidney function ^b	114/5424 (2.1)	79/3897 (2.0)	
Unadjusted model			0.91 (0.68–1.22)
Adjusted model			1.02 (0.75–1.37)
Incident albuminuria ^c	203/2691 (7.5)	147/1928 (7.6)	
Unadjusted model			1.01 (0.81–1.25)
Adjusted model			0.98 (0.78–1.23)

Adjusted model: age, sex, race, smoking status, systolic blood pressure, prior cardiovascular disease, body mass index, statin use, aspirin use, and trial treatment arm.

^aDefined by worsening kidney function or incident albuminuria.

^bIn participants without CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 30% or more to a value of <60 mL/min/1.73 m². In participants with CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 50% or more or the development of ESRD requiring long-term dialysis or kidney transplantation.

^cDefined for all study participants by a doubling of the ratio of urinary albumin/creatinine from <10 mg/g at baseline to >10 mg/g during follow-up.

analyses (Table 3). Similarly, when the World Health Organization definition of impaired fasting glucose was assessed, no significant association of impaired fasting glucose with any adverse renal outcomes was found (Table 4).

Association of impaired fasting glucose with renal outcomes according to CKD status at baseline

Among participants without CKD at baseline (n = 6676), 164 (2.5%) developed a ≥30% decrease in eGFR to <60 mL/min/1.73 m², and 242 (of 3593; 6.7%) developed incident albuminuria. In participants with CKD at baseline (n = 2645), 29 (1.1%) presented with a ≥50% decrease in eGFR, long-term dialysis, or kidney transplantation, and 108 (of 1026; 10.5%)

developed incident albuminuria. There were no significant differences in the unadjusted or adjusted effect estimates according to the presence or absence of CKD at baseline (Table 5).

Association of impaired fasting glucose with all-cause mortality

Impaired fasting glucose was not associated with an increased risk for mortality in either unadjusted analysis (HR: 0.93; 95% CI: 0.75 to 1.16) or adjusted analysis (HR: 0.96; 95% CI: 0.77 to 1.20). Furthermore, there was no evidence for effect modification of mortality according to the presence or absence of CKD at baseline ($P_{\text{interaction}} = 0.40$) or randomized treatment assignment ($P_{\text{interaction}} = 0.70$).

Table 3. Association of Impaired Fasting Glucose vs Normoglycemia With Outcomes [Excluding Patients With Fasting Plasma Glucose Level ≥126 mg/dL (≥7.0 mmol/L) at Baseline]

Outcome	Normoglycemia	Impaired Fasting Glucose	Unadjusted Hazard Ratio	Adjusted Hazard Ratio ^a
	No. of Events/No. at Risk (%)	No. of Events/No. at Risk (%)		
Composite adverse renal outcome ^b	314/5424 (5.8)	198/3602 (5.5)	0.93 (0.78–1.12)	0.94 (0.78–1.14)
Worsening kidney function ^c	114/5424 (2.1)	70/3602 (1.9)	0.88 (0.65–1.19)	0.98 (0.72–1.34)
Incident albuminuria ^d	203/2691 (7.5)	133/1796 (7.4)	0.98 (0.78–1.23)	0.96 (0.76–1.21)

^aAdjusted model: age, sex, race, smoking status, systolic blood pressure, prior cardiovascular disease, body mass index, statin use, aspirin use, and trial treatment arm.

^bDefined by worsening kidney function or incident albuminuria.

^cIn participants without CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 30% or more to a value of <60 mL/min/1.73 m². In participants with CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 50% or more or the development of ESRD requiring long-term dialysis or kidney transplantation.

^dDefined for all study participants by a doubling of the ratio of urinary albumin/creatinine from <10 mg/g at baseline to >10 mg/g during follow-up.

Table 4. Association of Impaired Fasting Glucose vs Normoglycemia With Outcomes Using the WHO Definition [Fasting Plasma Glucose ≥ 110 mg/dL (≥ 6.1 mmol/L)]

Outcome	Normoglycemia	Impaired Fasting Glucose	Unadjusted Hazard Ratio	Adjusted Hazard Ratio ^a
	No. of Events/No. at Risk (%)	No. of Events/No. at Risk (%)		
Composite adverse renal outcome ^b	439/7847 (5.6)	96/1474 (6.5)	1.14 (0.92–1.43)	1.17 (0.93–1.47)
Worsening kidney function ^c	157/7847 (2.0)	36/1474 (2.4)	1.18 (0.82–1.70)	1.28 (0.88–1.86)
Incident albuminuria ^d	288/3903 (7.4)	62/716 (8.7)	1.19 (0.90–1.57)	1.21 (0.90–1.61)

^aAdjusted model: age, sex, race, smoking status, systolic blood pressure, prior cardiovascular disease, body mass index, statin use, aspirin use, and trial treatment arm.

^bDefined by worsening kidney function or incident albuminuria.

^cIn participants without CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 30% or more to a value of <60 mL/min/1.73 m². In participants with CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 50% or more or the development of ESRD requiring long-term dialysis or kidney transplantation.

^dDefined for all study participants by a doubling of the ratio of urinary albumin/creatinine from <10 mg/g at baseline to >10 mg/g during follow-up.

Conclusions

In our *post hoc* analysis of participants of the SPRINT trial, impaired fasting glucose at baseline was not associated with a higher incidence of CKD, worsening kidney function, or incident albuminuria in comparison with normoglycemia.

Previous studies evaluating the association of prediabetes with kidney disease have shown contradictory results (8–11, 19–26). Most studies suggesting a higher risk of kidney disease in patients with prediabetes are cross-sectional (21, 23–26), with obvious limitations in estimating temporal associations. One of the largest studies reporting a positive association of prediabetes

Table 5. Association of Impaired Fasting Glucose vs Normoglycemia With Outcomes According to Chronic Kidney Disease Status at Baseline

	Normoglycemia	Impaired Fasting Glucose	Hazard Ratio (95% CI)
	No. of Events/No. at Risk (%)	No. of Events/No. at Risk (%)	
Participants without CKD at baseline			
Composite adverse renal outcome ^a	229/3848 (6.0)	169/2828 (6.0)	
Unadjusted model			0.98 (0.80–1.21)
Adjusted model			1.00 (0.81–1.23)
$\geq 30\%$ reduction in eGFR to <60 mL/min/1.73 m ²	96/3848 (2.5)	68/2828 (2.4)	
Unadjusted model			0.90 (0.65–1.23)
Adjusted model			1.02 (0.73–1.41)
Incident albuminuria ^b	136/2071 (6.7)	106/1522 (7.0)	
Unadjusted model			1.09 (0.83–1.42)
Adjusted model			1.05 (0.80–1.39)
Participants with CKD at baseline	(n = 1576)	(n = 1069)	
Composite adverse renal outcome ^a	85/1576 (5.4)	52/1069 (4.9)	
Unadjusted model			0.85 (0.59–1.23)
Adjusted model			0.86 (0.59–1.26)
$\geq 50\%$ reduction in eGFR, long-term dialysis, or kidney transplantation	10/1576 (0.6)	11/1069 (1.0)	
Unadjusted model			0.80 (0.37–1.74)
Adjusted model			0.92 (0.41–2.06)
Incident albuminuria ^b	67/620 (10.8)	41/406 (10.1)	
Unadjusted model			0.91 (0.59–1.39)
Adjusted model			0.90 (0.57–1.40)

Adjusted model: age, sex, race, smoking status, systolic blood pressure, prior cardiovascular disease, body mass index, statin use, aspirin use, and trial treatment arm.

^aDefined by worsening kidney function or incident albuminuria.

^bDefined for all study participants by a doubling of the ratio of urinary albumin/creatinine from <10 mg/g at baseline to >10 mg/g during follow-up.

with greater risk of CKD was a meta-analysis of 185,452 individuals (mainly Asian and white). Over 835,146 person-years of follow-up, the authors reported a modest increased risk of CKD development (HR: 1.12; 95% CI: 1.02 to 1.21) (11). However, the analyses were limited by significant heterogeneity across studies and limited ability to adjust for potentially confounding variables (11). Our finding of no association of impaired fasting glucose with renal outcomes is consistent with a study of 12,808 participants aged ≥ 20 years. Over a median follow-up of >10 years, both diabetes and hypertension were associated with an increased risk of CKD, whereas neither prediabetes nor prehypertension (individually or combined) was associated with an increased risk of CKD (12). Furthermore, in an analysis of 2398 participants in the Framingham Heart Study offspring cohort, which had a median follow-up of 7.0 years, neither impaired fasting glucose nor impaired glucose tolerance was associated with CKD development after adjustment for potential confounders (10). Of note, these studies did not include time-updated analyses, and most patients in the Framingham cohort had low to moderate CV risk, whereas patients included in the SPRINT trial were at higher risk for CV events.

In this study, impaired fasting glucose was not associated with all-cause mortality. Previous studies have had contradictory results regarding this association. Although most found increased mortality among patients with prediabetes (27–30), others found no association with mortality (31, 32). It should be noted that most previous studies that found an association between prediabetes and all-cause mortality included participants at low or moderate CV risk. The fact that all participants included in SPRINT had a higher baseline CV risk raises the possibility that impaired fasting glucose may not be associated with additional risk for mortality among those who are already at higher risk for CV events.

Prediabetes may be considered part of the continuum from normoglycemia to hyperglycemia with a greater associated risk of complications. Prediabetes raises short-term absolute risk of type 2 DM by threefold to 10-fold (33), and people with diabetes are vulnerable to multiple and complex medical complications, including CKD development and progression to ESRD (15, 34). In addition, prediabetes is frequently associated with comorbidities, particularly hypertension, that are also important risk factors for CKD (35). However, DM is diagnosed on the basis of glucose levels that have been associated with higher risk of development of end-organ complications (34). Thus, it is possible that the pathophysiological mechanisms involved in the renal adverse outcomes observed in DM may not be present at the

glycemic levels in prediabetes. In this regard, it is interesting to note that plasma glucose levels in prediabetes are similar to the actual therapeutic targets for patients with DM, which have been associated reduced risk of renal outcomes (35, 36). It is also possible that lifestyle interventions are more frequently recommended for patients with prediabetes than for normoglycemic participants, eventually minimizing the risk of adverse kidney outcomes. In fact, early identification and treatment of prediabetes has been shown to potentially reduce or delay the progression to DM and related CV and microvascular disease (34).

Regarding the strengths of our study, we performed a secondary analysis of a large multicenter, randomized, controlled trial with rigorous data collection. The presence of detailed information about the participants allowed adjustment for several biologically plausible confounders in a group of individuals with high CV risk. There are, however, limitations of this analysis, which include the observational nature of a *post hoc* study, the potential for residual confounding despite performance of adjusted models, and the potential lack of power to detect significant associations in subgroups. Impaired fasting glucose was defined on the basis of a single measure of fasting glucose level at baseline, and time-updated measures of glycemic control and comorbid disease were not available. Although sensitivity analyses using a different fasting plasma glucose cutoff were consistent with our primary results, we cannot exclude the possibility of differing risks if prediabetes status had been based on HbA1c levels and/or oral glucose tolerance tests and if time-updated glycemic measurements had been available. Furthermore, the risk for adverse renal outcomes with impaired fasting glucose may have been higher if the follow-up had been longer. The limitations of the current data set include the absence of insulin levels, markers of insulin resistance, and detailed information on time-updated eGFR and albuminuria, which precluded the performance of more granular analyses.

In summary, in this *post hoc* analysis of the SPRINT trial, impaired fasting glucose was not associated with a higher incidence of CKD, incident albuminuria, or worsening kidney function. Future studies examining time-updated glycemic status with longer duration of follow-up are needed to examine the longitudinal renal risks of prediabetes.

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Impaired Fasting Glucose and Chronic Kidney Disease, Albuminuria, or Worsening Kidney Function: a Secondary Analysis of the SPRINT

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Supplementary Table 1 - Association of Impaired Fasting Glucose versus Normoglycemia with Outcomes, by SPRINT randomized treatment assignment

	Normoglycemia	Impaired Fasting Glucose	Unadjusted Hazard Ratio	Adjusted Hazard Ratio*
	No. events/No. at risk (%)			
Standard treatment (SBP target of <140 mmHg)				
Composite adverse renal outcome ^a	139/2,703 (5.1%)	100/1,956 (5.1%)	0.96 (0.73 - 1.25)	0.93 (0.71 - 1.22)
Worsening kidney function ^b	33/2,703 (1.2%)	19/1,956 (1.0%)	0.73 (0.41 - 1.31)	0.95 (0.51 - 1.74)
Incident albuminuria ^c	107/1,335 (8.0%)	85/992 (8.6%)	1.03 (0.77 - 1.39)	0.95 (0.70 - 1.29)
Intensive treatment (SBP target of <120 mmHg)				
Composite adverse renal outcome ^a	175/2,721 (6.4%)	121/1,941 (6.2%)	0.94 (0.74 - 1.20)	1.00 (0.78 - 1.28)
Worsening kidney function ^b	81/2,721 (3.0%)	60/1,941 (3.1%)	1.00 (0.71 - 1.41)	1.06 (0.75-1.51)
Incident albuminuria ^c	96/1,356 (7.1%)	62/936 (6.6%)	0.96 (0.68 - 1.35)	1.06 (0.74 - 1.50)

Adjusted model: age, sex, race, smoking status, systolic blood pressure, prior cardiovascular disease, body mass index, statin use, aspirin use, and trial treatment arm.

^a defined by worsening kidney function or incident albuminuria.

^b in participants without CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 30% or more to a value of less than 60 ml per minute per 1.73 m². In participants with CKD at baseline, worsening kidney function was defined by a decrease in the eGFR of 50% or more or the development of ESRD requiring long-term dialysis or kidney transplantation.

^c defined for all study participants by a doubling of the ratio of urinary albumin to creatinine from less than 10 mg/g at baseline to greater than 10 mg/g during follow-up.



Article 8

Association of Prediabetes With CKD Progression and Adverse Cardiovascular Outcomes: An Analysis of the CRIC Study

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Association of Prediabetes With CKD Progression and Adverse Cardiovascular Outcomes: An Analysis of the CRIC Study

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Purpose: Despite our understanding of diabetes as an established risk factor for progressive kidney disease and cardiac complications, the prognostic significance of prediabetes in patients with chronic kidney disease (CKD) remains largely unknown.

Methods: Participants of the Chronic Renal Insufficiency Cohort (CRIC) were categorized as having normoglycemia, prediabetes, or diabetes according to fasting plasma glucose, glycated hemoglobin A1c (HbA1c), and treatment with antidiabetic drugs at baseline. Unadjusted and adjusted proportional hazards models were fit to estimate the association of prediabetes and diabetes (versus normoglycemia) with: (1) composite renal outcome (end-stage renal disease, 50% decline in estimated glomerular filtration rate to ≤ 15 mL/min/1.73 m², or doubling of urine protein-to-creatinine ratio to ≥ 0.22 g/g creatinine); (2) composite cardiovascular (CV) outcome (congestive heart failure, myocardial infarction or stroke); and (3) all-cause mortality.

Results: Of the 3701 individuals analyzed, 945 were normoglycemic, 847 had prediabetes and 1909 had diabetes. The median follow-up was 7.5 years. Prediabetes was not associated with the composite renal outcome (adjusted hazard ratio [aHR] 1.13; 95% confidence interval [CI], 0.96–1.32; $P = 0.14$), but was associated with proteinuria progression (aHR 1.23; 95% CI, 1.03–1.47; $P = 0.02$). Prediabetes was associated with a higher risk of the composite CV outcome (aHR 1.38; 95% CI, 1.05–1.82; $P = 0.02$) and a trend towards all-cause mortality (aHR 1.28; 95% CI, 0.99–1.66; $P = 0.07$). Participants with diabetes had an increased risk of the composite renal outcome, the composite CV outcome, and all-cause mortality.

Conclusions: In individuals with CKD, prediabetes was not associated with composite renal outcome, but was associated with an increased risk of proteinuria progression and adverse CV outcomes. (*J Clin Endocrinol Metab* 105: e1772–e1780, 2020)

Key Words: prediabetes, diabetes, chronic kidney disease, cardiovascular outcomes, renal outcomes, all-cause mortality

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Abbreviations: aHR, adjusted hazard ratio; BMI, body mass index; BNP, brain natriuretic peptide; CHF, congestive heart failure; CI, confidence interval; CKD, chronic kidney disease; CRIC, Chronic Renal Insufficiency Cohort; CV, cardiovascular; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HbA1c, glycated hemoglobin A1c; HF, heart failure; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity troponin T; MI, myocardial infarction; SGLT2, sodium glucose co-transporter 2.

Prediabetes is a highly prevalent condition, affecting about one-third of adults in the United States (1). Patients with prediabetes have an increased risk for diabetes, with 2% to 10% progressing to diabetes each year (2, 3). Although prediabetes is frequently considered an intermediary stage in the progression between normoglycemia and diabetes, many individuals may have prediabetes for several years, while some may never progress.

Chronic kidney disease (CKD) is a heterogeneous group of disorders characterized by alterations in kidney structure and function (4), and is associated with an increased risk of end-stage renal disease (ESRD) and adverse cardiovascular (CV) outcomes (4). In the United States, the prevalence of CKD is estimated to be approximately 14% (5). In patients with CKD, diabetes has been clearly associated with an increased risk of progression to ESRD and adverse CV outcomes (6); however, the prognostic significance of prediabetes remains uncertain.

The Chronic Renal Insufficiency Cohort (CRIC) includes participants with CKD followed up to 10 years. This cohort includes a high proportion of participants with prediabetes and includes adjudicated renal and CV outcomes, providing a unique opportunity to evaluate the associations of prediabetes with these outcomes among patients with CKD. We hypothesized that prediabetes would be associated with an increased risk of CKD progression and adverse CV outcomes in patients with CKD.

Methods

Study participants

The Chronic Renal Insufficiency Cohort (CRIC) Study is a multicenter prospective cohort that recruited an ethnically and racially diverse group of subjects with prevalent CKD across 7 clinical centers in the United States from 2003 to 2008. CRIC was designed to elucidate risk factors for progression of CKD, development of ESRD, and development of CV disease among patients with varying stages of CKD, half of whom had diabetes. Entry criteria included estimated glomerular filtration rate (eGFR) from 20 to 70 mL/min/1.73 m² and age of 21 to 74 years. Individuals with polycystic kidney disease, New York Heart Association class (NYHA) III or IV heart failure (HF), known cirrhosis, HIV/AIDS, multiple myeloma or renal cancer were excluded. Further exclusions included active immunosuppression, recent chemotherapy or immunosuppressive therapy; institutionalization; organ transplantation; pregnancy; or dialysis for a month prior to screening. For this secondary analysis, we excluded participants with missing fasting glucose or glycated hemoglobin (HbA1c) measurements at baseline (n = 238) (Supplementary Figure 1) (7). The study design, baseline characteristics and main results of CRIC have been published previously (8). The CRIC study was approved by the institutional review board

at each participating study site, and written informed consent was obtained from all participants.

Exposure

The main exposure of the current analysis was prediabetes at baseline, defined as either HbA1c of 5.7% to 6.4% or fasting plasma glucose of 100 to 125 mg/dL and no treatment with antidiabetic drugs. Diabetes was defined as one of the following: HbA1c $\geq$ 6.5%, fasting plasma glucose $\geq$ 126 mg/dL, or treatment with antidiabetic drugs at baseline.

In sensitivity analyses, the definitions of prediabetes and diabetes according to HbA1c alone or fasting plasma glucose alone were also evaluated. Previous studies have shown that the association of prediabetes with renal and CV outcomes may differ according to the definition used for classifying prediabetes (9, 10). In this classification, patients treated with antidiabetic drugs were classified as having diabetes and the remaining patients were defined as having normoglycemia, prediabetes, or diabetes, according to fasting plasma glucose or HbA1c cutoffs.

Study outcomes

The prespecified primary endpoints of the present analysis were: (1) composite renal outcome defined as either the development of ESRD (renal transplantation or dialysis initiation), a 50% decline in baseline eGFR (CKD-EPI equation) to $\leq$ 15 mL/min/1.73 m², or doubling of urine protein to creatinine ratio to $\geq$ 0.22 g/g creatinine (this cutoff corresponds to an urinary protein excretion of 300 mg/day and has been used in previous studies assessing progression of CKD) (11); (2) a composite CV outcome of congestive heart failure (CHF), myocardial infarction (MI), or stroke; and (3) all-cause mortality. Individual components of the composite outcomes were also assessed. As exploratory outcomes we also evaluated (1) the composite endpoint of a 50% decline in baseline eGFR (with or without decrease to $\leq$ 15 mL/min/1.73 m²) or development of ESRD; and (2) peripheral artery disease events (amputation due to vascular disease, or peripheral surgical or percutaneous revascularization). For the primary renal endpoint, the development of ESRD and eGFR decline were prespecified endpoints in CRIC. For the purpose of the present analyses, we also included proteinuria in order to more comprehensively capture CKD progression. The CV composite of CHF, MI, or stroke was also prespecified in CRIC. The inclusion of CHF is highly relevant, given the body of literature stressing the increased risk of HF among patients with diabetes (12–14), and the growing evidence favoring new therapies (e.g., sodium glucose co-transporter 2 [SGLT2] inhibitors) to prevent HF events in those with and without diabetes (15–17). Clinical endpoints were adjudicated by an independent clinical events committee and outcomes definitions are presented in Supplementary Table 1 (7). Participants were followed until March 2013, withdrawal of consent, loss to follow-up, or death. Outcomes for both prediabetes and diabetes at baseline (compared with normoglycemic individuals), and for alternative definitions of prediabetes and diabetes by HbA1c criteria alone, and fasting plasma glucose alone, are presented.

Statistical analyses

Continuous variables are described as mean $\pm$ standard deviation or median (25th–75th percentiles) and categorical variables as proportions (percentages). Baseline characteristics

of the study population were compared with ANOVA or the Kruskal-Wallis test for normal and nonnormal continuous variables respectively, and the chi-square test for categorical variables.

The associations of prediabetes and diabetes with CKD progression, composite CV outcome and all-cause mortality were assessed through unadjusted and adjusted Cox proportional hazards models. All adjusted models were stratified by clinic center. Model 1 included age, sex, and race/ethnicity. Model 2 (main model) included those variables in model 1 plus body mass index (BMI), antiplatelet therapy, lipid lowering therapy, systolic blood pressure at baseline, coronary artery disease (CAD, defined as prior MI or prior coronary revascularization), peripheral vascular disease, congestive heart failure, hematocrit, baseline eGFR (CKD-EPI formula), serum albumin, 24-hour urine protein (log-transformed), and inhibition of renin-angiotensin-aldosterone axis (treatment with angiotensin converting enzyme inhibitors, angiotensin receptor blockers, or aldosterone antagonists). The choice of covariates was based on prior knowledge of risk factors/confounders and biological plausibility. The log-rank test was used to compare the survival distribution of outcomes for normoglycemia, prediabetes, and diabetes at baseline, and Kaplan-Meier curves were generated to represent the survival distribution of outcomes by glycemic control at baseline. An additional exploratory model with further adjustment for biomarkers associated with CKD progression and adverse CV outcomes was performed. For this analysis, we included the variables in model 2 plus high-sensitive C reactive protein (hsCRP, log-transformed), brain natriuretic peptide (BNP, log-transformed), and high-sensitivity troponin T (hsTnT, log-transformed), based on prior literature demonstrating the association of these biomarkers with adverse renal and CV outcomes (18–20).

The presence of effect modification (interaction) of the association of glycemic control at baseline with the primary outcomes according to race (white, black, other), sex (women or men), baseline eGFR (as continuous variable), and 24-hour urine protein (as continuous variable, log-transformed) was tested via inclusion of cross-product terms in Cox proportional hazard model, adjusting for the variables in the model 2. Subgroup analyses were performed only if there was evidence for effect modification (P for interaction < 0.1). The proportional hazards assumption was tested for all models. For covariates that violated the proportionality assumption, the corresponding time interaction term was included in the model. An adjusted model (model 2) using a restricted cubic spline with 3 knots was constructed to flexibly display the continuous association between HbA1c or glucose and the hazards of CKD progression, composite CV outcome or all-cause mortality. All analyses were conducted with the statistical software package Stata IC version 14.2 (College Station, TX) using a dataset obtained from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Data Repository. A two-sided P -value < 0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 3701 individuals were included in the present analysis. The mean HbA1c was 5.3% in normoglycemic subjects ($n = 945$), 5.9% in participants with

prediabetes ($n = 847$) and 7.6% in participants with diabetes ($n = 1909$). Relative to those with normoglycemia, participants with diabetes and prediabetes were more likely to be black, and to have a history of CAD, stroke, congestive heart failure, peripheral vascular disease, or hypertension. Participants with diabetes and prediabetes tended to have lower eGFR, higher systolic blood pressure, proteinuria, and BMI at baseline, and were more likely to use antiplatelet and lipid-lowering agents (Table 1).

Association with outcomes

Composite renal outcome. The median follow-up was 7.5 years (25th–75th percentile, 6.2–8.6 years). During this period, 989 had a 50% decline in baseline eGFR to ≤ 15 mL/min/1.73 m² or developed ESRD (896 developed ESRD), and 1238 had a doubling of urine protein to creatinine ratio to ≥ 0.22 g/g creatinine (Table 2). In unadjusted analyses, prediabetes (versus normoglycemia) was not associated with composite renal outcome, whereas diabetes was associated with an increase of the composite renal outcome (hazard ratio [HR] 1.88; 95% confidence interval [CI], 1.66–2.12; $P < 0.001$) (Figure 1A). The pattern of association was similar in adjusted models (model 2 adjusted hazard ratio [aHR] 1.13; 95% CI, 0.96–1.32; $P = 0.14$ for prediabetes; and 1.47; 95% CI, 1.27–1.70; $P < 0.001$ for diabetes). In relation to the components of the composite, prediabetes was not associated with a decline in eGFR $> 50\%$ or ESRD (model 2 aHR 0.88; 95% CI, 0.71–1.10; $P = 0.26$), or ESRD alone (Model 2 aHR 1.01; 95% CI, 0.79–1.29; $P = 0.94$), but was associated with increased risk of proteinuria progression (model 2 aHR 1.23; 95% CI, 1.03–1.47; $P = 0.02$) (Supplementary Table 2) (7). Diabetes was associated with increased risk of ESRD and proteinuria progression (Supplementary Table 2) (7).

Composite cardiovascular outcome. Compared with normoglycemia, prediabetes was associated with an 85% increased risk of the composite CV outcome (HR 1.85; 95% CI, 1.43–2.40; $P < 0.001$), while diabetes was associated with a 3.6-fold increased risk of the composite CV outcome (HR 3.60; 95% CI, 2.89–4.49; $P < 0.001$) in unadjusted models (Figure 1B). In model 2, prediabetes was consistently associated with a 38% increased risk of composite CV outcome (HR 1.38; 95% CI, 1.05–1.82; $P = 0.02$), and diabetes with a 63% increased risk of composite CV outcome (HR 1.63; 95% CI, 1.27–2.11; $P < 0.001$) (Table 2). The association of prediabetes and diabetes with individual components of the composite CV endpoint are presented in

Table 1. Baseline Characteristics of Study Participants

	Normoglycemia	Prediabetes	Diabetes	P-value
	(n = 945)	(n = 847)	(n = 1,909)	
Women	445 (47.1%)	393 (46.4%)	845 (44.3%)	0.30
Age, years	54.4 ± 12.8	59.3 ± 10.4	59.5 ± 9.7	<0.001
Race/ethnicity				<0.001
White	523 (55.3%)	387 (45.7%)	653 (34.2%)	
Black	279 (29.5%)	384 (45.3%)	861 (45.1%)	
Other	143 (15.1%)	76 (9.0 %)	395 (20.7%)	
Myocardial infarction or prior revascularization	111 (11.7%)	155 (18.3%)	530 (27.8%)	<0.001
Prior stroke	64 (6.8 %)	71 (8.4 %)	224 (11.7%)	<0.001
Congestive heart failure	40 (4.2 %)	54 (6.4 %)	255 (13.4%)	<0.001
Peripheral vascular disease	20 (2.1 %)	30 (3.5 %)	196 (10.3%)	<0.001
Hypertension	697 (73.8%)	718 (84.8%)	1,766 (92.5%)	<0.001
Systolic blood pressure, mmHg	121.8 ± 20.1	125.0 ± 20.5	133.2 ± 22.8	<0.001
Body mass index, kg/m ²	29.1 ± 6.8	31.5 ± 7.5	33.8 ± 7.9	<0.001
Inhibitors of RAA axis	511 (54.4%)	535 (63.7%)	1520 (80.1%)	<0.001
Antiplatelet agents	293 (31.2%)	324 (38.6%)	1074 (56.6%)	<0.001
Antidyslipidemic agents	369 (39.3%)	418 (49.8%)	1,405 (74.1%)	<0.001
Oral antidiabetic drugs	0 (0.0 %)	0 (0.0 %)	1,024 (54.0%)	<0.001
Insulin	0 (0.0 %)	0 (0.0 %)	885 (46.7%)	<0.001
Hemoglobin A1c, %	5.3 ± 0.3	5.9 ± 0.3	7.6 ± 1.6	<0.001
Fasting glucose, mg/dL	86.1 ± 7.7	95.4 ± 11.5	137.1 ± 60.7	<0.001
eGFR, mL/min/1.73m ²	47.5 ± 16.4	46.1 ± 14.8	42.1 ± 14.0	<0.001
Urine Protein, g/24h	0.1 (0.1, 0.5)	0.1 (0.1, 0.4)	0.3 (0.1, 1.6)	<0.001
Urine Albumin, mg/24h	30 (7, 271)	26 (7, 201)	150 (20, 973)	<0.001
Serum albumin, g/dL	4.0 ± 0.4	4.1 ± 0.4	3.8 ± 0.5	<0.001
Hematocrit, %	39.0 ± 4.9	39.1 ± 4.8	36.3 ± 4.9	<0.001
High-sensitive CRP, mg/L	2.0 (0.9, 5.0)	2.7 (1.2, 7.1)	2.8 (1.1, 6.9)	<0.001
BNP, pg/mL	30.7 (13.6, 69.4)	30.7 (14.0, 82.2)	50.1 (21.5, 114.7)	<0.001
High-sensitivity TnT, pg/mL	6.9 (1.5, 13.8)	8.9 (4.2, 15.6)	17.6 (9.7, 34.6)	<0.001

Categorical variables are presented as counts (percentages). Continuous variables are presented as mean ± standard deviation or median (25th, 75th percentile). P-values refer to a test for difference (analysis of variance for normally distributed continuous variables; Kruskal-Wallis test for nonnormally distributed continuous variables; and chi-square test for categorical variables).

Abbreviations: BNP, brain natriuretic peptide; CRP, C-reactive protein; TnT, troponin T; eGFR, estimated glomerular filtration rate (CKD-EPI equation); RAA, renin-angiotensin-aldosterone.

Supplementary Table 3 (7). Both prediabetes and diabetes were more strongly associated with an increased risk of heart failure.

In an exploratory model evaluating peripheral artery disease as the outcome of interest, prediabetes and diabetes were associated with a 2.33-fold (HR 2.36; 95% CI, 1.05–5.18; $P = 0.037$) and a 4.34-fold higher risk of peripheral artery disease (HR 4.34; 95% CI, 2.12–8.91; $P < 0.001$), respectively (Supplementary Table 4) (7).

All-cause mortality. Compared with normoglycemic individuals, the risk of all-cause mortality was increased in both prediabetes (HR 1.63; 95% CI, 1.27–2.09; $P < 0.001$) and diabetes (HR 2.56; 95% CI, 2.07–3.17; $P < 0.001$) in unadjusted models (Figure 1C). In model 1, prediabetes was associated with a 36% increased risk of all-cause mortality, while diabetes was associated with a 2.07-fold increased risk. In model 2, there was a trend for increased all-cause mortality with prediabetes (HR 1.28; 95% CI, 0.98–1.66; $P = 0.07$),

while diabetes was associated with a 53% increased risk of this outcome (HR 1.53; 95% CI, 1.20–1.95; $P = 0.001$).

Effect modification and subgroup analyses. There was no evidence for effect modification of the association of glycemic control at baseline with the primary outcomes according to race, sex, or 24-hour urine protein (P -interactions > 0.1). We did find evidence for effect modification for the association of prediabetes/diabetes with all-cause mortality according to baseline eGFR (P -interaction < 0.001). In individuals with eGFR > 45 mL/min/1.73 m², prediabetes at baseline was associated with a 2.2-fold adjusted risk of all-cause mortality (aHR 2.20; 95% CI, 1.31–3.70) and diabetes with a 2.59-fold adjusted risk of all-cause mortality (aHR 2.59; 95% CI, 1.57–4.26); in individuals with eGFR ≤ 45 mL/min/1.73 m², the aHR for all-cause mortality in prediabetes was 1.04 (95% CI, 0.76–1.42) and 1.26 (95% CI, 0.95–1.67) for diabetes.

Table 2. Association of Prediabetes and Diabetes With Outcomes (HR [95% CI])

	Normoglycemia (n = 945)	Prediabetes (n = 847)	Diabetes (n = 1909)
Composite renal outcome			
No. Events/No. Pts	351/896 (39.2%)	340/800 (42.5%)	1094/1781 (61.4%)
Unadjusted HR	(reference)	1.12 (0.96–1.30) <i>P</i> = 0.15	1.88 (1.66–2.12) <i>P</i> < 0.001
Model 1 HR	(reference)	1.12 (0.96–1.31) <i>P</i> = 0.13	1.86 (1.64–2.11) <i>P</i> < 0.001
Model 2 HR	(reference)	1.13 (0.96–1.32) <i>P</i> = 0.14	1.47 (1.27–1.70) <i>P</i> < 0.001
Composite cardiovascular outcome			
No. Events/No. Pts	93/943 (9.9%)	151/844 (17.9%)	579/1901 (30.5%)
Unadjusted HR	(reference)	1.85 (1.43–2.40) <i>P</i> < 0.001	3.60 (2.89–4.49) <i>P</i> < 0.001
Model 1 HR	(reference)	1.59 (1.23–2.07) <i>P</i> < 0.001	2.97 (2.37–3.71) <i>P</i> < 0.001
Model 2 HR	(reference)	1.38 (1.05–1.82) <i>P</i> = 0.021	1.63 (1.27–2.11) <i>P</i> < 0.001
All-cause mortality			
No. Events/No. Pts	104/945 (11.0%)	151/847 (17.8%)	520/1909 (27.2%)
Unadjusted HR	(reference)	1.63 (1.27–2.09) <i>P</i> < 0.001	2.56 (2.07–3.17) <i>P</i> < 0.001
Model 1 HR	(reference)	1.36 (1.06–1.76) <i>P</i> = 0.016	2.07 (1.67–2.57) <i>P</i> < 0.001
Model 2 HR	(reference)	1.28 (0.98–1.66) <i>P</i> = 0.071	1.53 (1.20–1.95) <i>P</i> = 0.001

Composite renal outcome: Development of ESRD (renal transplantation or dialysis initiation), 50% decline in baseline eGFR (CKD-EPI equation) and eGFR ≤ 15 mL/min/1.73 m², or doubling of urine protein to creatinine ratio to ≥ 0.22 g/g creatinine.

Composite cardiovascular outcome: Congestive heart failure, myocardial infarction or stroke.

Model 1: age, sex and race/ethnicity.

Model 2: variables in model 1 plus body mass index, antiplatelet therapy, lipid lowering therapy, systolic blood pressure at baseline, coronary artery disease (defined as prior myocardial infarction or prior coronary revascularization), peripheral vascular disease, congestive heart failure, hematocrit, baseline eGFR, serum albumin and 24-hour urine protein.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HR, hazard ratio; Pts, patients

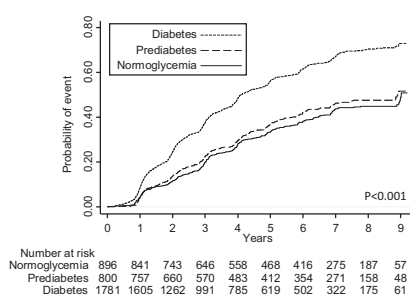
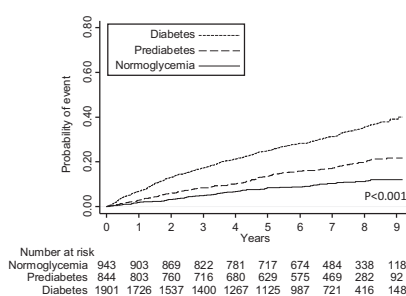
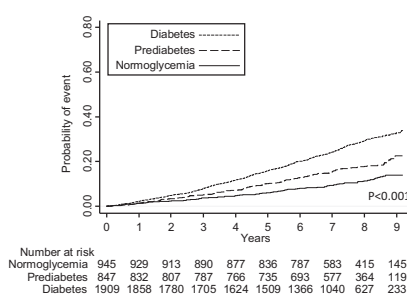
A. Composite renal outcome**B. Composite cardiovascular outcome****C. All-cause mortality**

Figure 1. Kaplan-Meier curves for composite renal outcome (A), composite CV outcome (B), and all-cause mortality (C) in participants with normoglycemia, prediabetes, or diabetes. Composite renal outcome: development of ESRD (renal transplantation or dialysis initiation), a 50% decline in baseline eGFR (CKD-EPI equation) to ≤ 15 mL/min/1.73 m², or doubling of urine protein to creatinine ratio to ≥ 0.22 g/g creatinine. Composite CV outcome: CHF, MI, or stroke. *P* values were calculated with the use of log-rank tests. Abbreviations: CHF, congestive heart failure; CKD, chronic kidney disease; CV, cardiovascular; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; MI, myocardial infarction.

Sensitivity analyses: association with outcomes according to the classification of prediabetes and diabetes by HbA1c or fasting plasma glucose. The number of participants diagnosed with prediabetes and diabetes according to HbA1c and fasting plasma glucose

is shown in Supplementary Table 5 (7). The states of glucose tolerance at the end of follow-up in each baseline group are shown in Supplementary Table 6 (7). In sensitivity analyses that defined prediabetes as baseline HbA1c 5.7% to 6.4% and diabetes as HbA1c $\geq 6.5\%$,

regardless of baseline fasting plasma glucose, the associations of prediabetes and diabetes with clinical outcomes were consistent with those in the main results. On the other hand, when participants were classified only according to fasting plasma glucose levels, there were no significant associations of prediabetes with composite renal outcome, composite CV outcome, or all-cause mortality (Supplementary Table 7) (7). These associations are more clearly apparent in restricted cubic spline analyses, where the associations of HbA1c and fasting plasma glucose are modeled in a continuous fashion (Figure 2).

Exploratory model. Similar patterns of association were noted after adjusting for hsCRP, BNP, and hsTnT, such that prediabetes was associated with a 34% higher risk of the composite CV outcome (HR 1.34; 95% CI, 1.01–1.77; $P = 0.04$), but not with progression of CKD or all-cause mortality (Supplementary Table 8) (7). Diabetes continued to be associated with an increased risk of all outcomes examined.

Discussion

In this cohort of patients with CKD, prediabetes was not associated with increased risk of eGFR decrease or ESRD development, but was associated with an

increased risk of proteinuria progression, increased risk of adverse CV outcomes and a trend towards increased all-cause mortality. These patterns of association persisted when prediabetes was defined according to HbA1c, but not when defined according to fasting plasma glucose. In subgroup analyses, the association of prediabetes with all-cause mortality appeared to be restricted to those with higher baseline eGFR.

Hyperglycemia is known to increase the production of reactive oxygen species, promote the accumulation of advanced glycation end products, activate intracellular signaling molecules such as protein kinase C, and increase the effects of the renin-angiotensin system (21, 22). In patients with diabetes these effects lead to glomerular hyperfiltration, mesangial expansion, glomerular basement membrane thickening, podocyte injury, and glomerular sclerosis, thereby promoting the development of albuminuria and the progression of CKD (22). Whether the milder hyperglycemia of prediabetes results in similar adverse renal effects is uncertain.

The association of prediabetes with the risk of kidney disease has not been consistent across studies. Some studies have suggested an increased risk of kidney disease among participants with prediabetes, although most of those were cross-sectional (23–26). In a prospective cohort study of Korean adults, impaired glucose

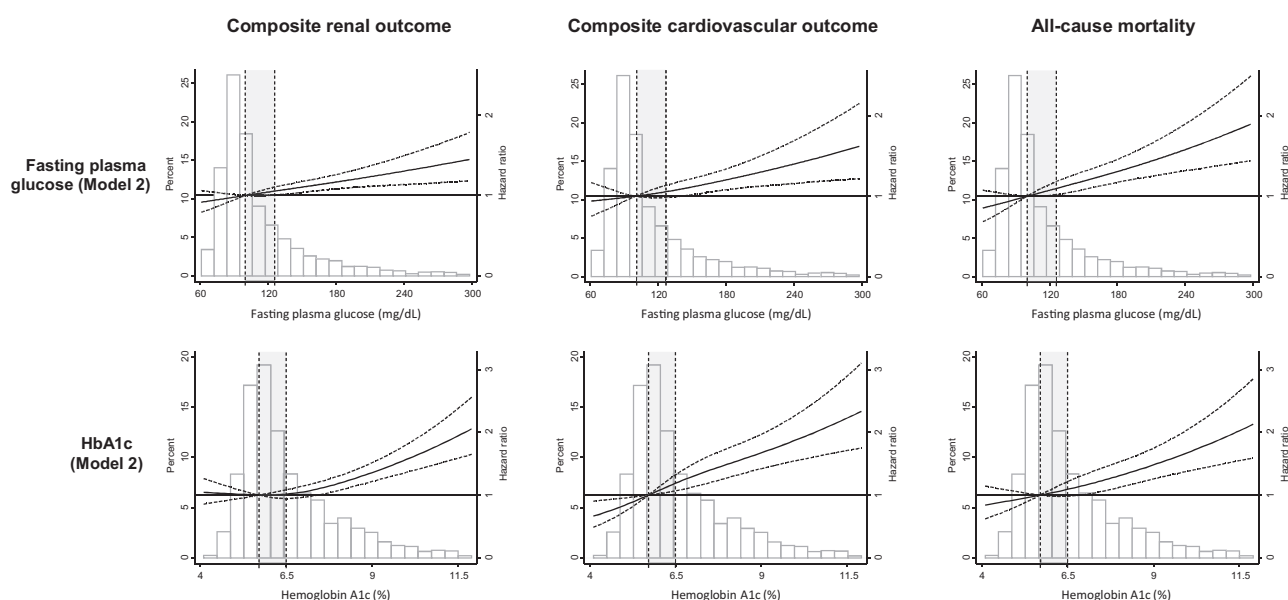


Figure 2. Composite renal outcome and composite CV outcome or all-cause mortality according to baseline fasting plasma glucose and HbA1c levels. Composite renal outcome: development of ESRD (renal transplantation or dialysis initiation), a 50% decline in baseline eGFR (CKD-EPI equation) to ≤ 15 mL/min/1.73 m², or doubling of urine protein to creatinine ratio to ≥ 0.22 g/g creatinine. Composite CV outcome: CHF, MI, or stroke. Model 2: age, sex, race/ethnicity, body mass index, antiplatelet therapy, lipid lowering therapy, systolic blood pressure at baseline, coronary artery disease (defined as prior MI or prior coronary revascularization), peripheral vascular disease, congestive heart failure, hematocrit, baseline eGFR, serum albumin and 24-hour urine protein. Dashed lines indicate the upper and lower 95% CI for the regression line (solid black line). Vertical dashed lines indicate the cutoffs for transition from normoglycemia to prediabetes range and to diabetes range. Prediabetes range is shaded in gray. Abbreviations: CHF, congestive heart failure; CI, confidence interval; CKD, chronic kidney disease; CV, cardiovascular; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; MI, myocardial infarction.

tolerance and HbA1C 5.7% to 6.4%, but not impaired fasting glucose, were independent predictors of incident CKD (10). In a post hoc analysis of the Systolic Blood Pressure Intervention Trial (SPRINT) impaired fasting glucose was also not associated with a higher incidence of CKD, incident albuminuria, or worsening kidney function (27). A meta-analysis of 9 cohort studies reported a modest increased risk of CKD development in participants with prediabetes, but there was a significant heterogeneity across the included studies and a limited adjustment for potential confounders (28).

Few studies have assessed the effects of prediabetes in participants with CKD at baseline. A cohort study of 1165 adults with nondialysis CKD stages 1 to 5 and without diabetes (29) reported that HbA1c in the prediabetes range was not associated with increased progression to ESRD. In our study, although prediabetes was not associated with the composite renal outcome, it was associated with a higher risk of proteinuria progression. The use of change in albuminuria or proteinuria as a surrogate endpoint for progression of CKD and increased risk of ESRD in clinical trials has recently gained support (30). In diabetic kidney disease, hyperfiltration and proteinuria are proposed to be the early clinical manifestations of kidney damage (31). We hypothesize that, in patients with nondiabetic CKD, prediabetes might also contribute to hyperfiltration, glomerular dysfunction, and proteinuria. Although prediabetes was not associated with eGFR decrease or ESRD, with a median follow-up of 7.5 years, it might have contributed to these outcomes if the follow-up had been longer. Furthermore, proteinuria has been associated with increased CV risk (32), suggesting the possibility that the increased risk of adverse CV outcomes in our study may have been partly mediated by the proteinuria progression.

Many mechanistic theories have been postulated to explain the association of prediabetes with higher CV risk. These include associations with endothelial dysfunction (33), changes in myocardial substrate utilization (34), microvascular dysfunction (35), increased in proinflammatory cytokines (36), impaired fibrinolysis and hypercoagulability (37). However, in the general population, the association of prediabetes with adverse CV events is not consistent. Some studies have suggested an increased CV risk (38, 39), while other have not found significant associations (40, 41), suggesting that the CV risk may be dependent on the population studied. Perhaps the most robust observational evidence comes from a meta-analysis including 53 prospective cohort studies, which reported that prediabetes was associated with an increased risk of composite CV events,

coronary heart disease, stroke, and all-cause mortality (42). Importantly, most studies evaluated in this meta-analysis included only a small portion of patients with CKD. Regarding patients with CKD, in a study by Huang et al. including non-dialysis CKD participants without diabetes, HbA1c values in the prediabetes range were associated with increased all-cause mortality (adverse CV events were not assessed in this study) (29). In this study, HbA1c was associated with increased mortality even after adjustment for fasting glucose levels (29). The observation in our study, that prediabetes defined by hemoglobin HbA1c, but not defined by fasting plasma glucose, was associated with increased risk of adverse CV outcomes, suggests that HbA1c may be a better predictor of CV events in patients with CKD. Interestingly, in our study, there was evidence for effect modification of the association of prediabetes with all-cause mortality according to baseline eGFR, such that the association appeared to be restricted to those with higher baseline eGFR. This suggests that while prediabetes may be an important risk factor for all-cause mortality in early phases of CKD, in patients with more advanced CKD (and higher CV risk), prediabetes may not further increase the risk of mortality.

In our study, prediabetes was also associated with a higher risk of peripheral artery disease events, which is in agreement with previous reports in the general population (38). However, the component of the composite CV outcome that was more strongly associated with prediabetes was heart failure. Our results are consistent with the higher risk of heart failure in prediabetes reported in the general population (43). Furthermore, in the Atherosclerosis Risk in the Community Study (ARIC) including participants without prevalent CV disease, prediabetes was associated with increased left ventricular mass, diastolic dysfunction, and subtle reduction in left ventricular systolic function (44). The identification of prediabetes as a risk factor for heart failure in CKD is important given the high incidence of heart failure in this group (45).

From a clinical perspective, our study reports a high prevalence of prediabetes in a representative cohort of participants with CKD, and highlights the risk of CV events in such individuals. Recently, SGLT2 inhibitors have been shown to decrease the risk of adverse CV and renal outcomes in diabetes (46, 47). The mechanism of benefit of SGLT2 inhibitors is likely to be independent of glucose levels and may involve a reduction in intraglomerular pressure. Whether treatment with SGLT2 can also reduce adverse CV and renal outcomes in patients with CKD and prediabetes is unknown. Our finding that diabetes is associated with higher risk for

adverse CV and CKD progression is concordant with most previous studies (48–51).

Regarding the strengths of our study, we performed an analysis of a large prospective cohort with rigorous data collection. Furthermore, we evaluated an ethnically and racially diverse population of participants with varying stages of CKD with adjudicated renal and CV outcomes. There are limitations to our analysis. First, despite the adjustment for several biologically plausible confounders, there may still be residual confounding due to the observational design. We cannot exclude that some associations of prediabetes or diabetes with CV or renal outcomes could have been different if we were able to further reduce residual confounding. Second, the classification of prediabetes and diabetes was performed based on a single baseline analysis. Although this approach is common in similar studies, we cannot exclude the possibility of misclassification of some participants. Third, our classification of glucose metabolism status was based only on the fasting plasma glucose and HbA1c levels. Some participants might have been classified differently if the oral glucose tolerance test had also been evaluated. Finally, our study might have been not powered enough to detect small differences between prediabetes and normoglycemia regarding renal outcomes.

In summary, in participants of CRIC, prediabetes is common, is not associated with an increased risk of eGFR decrease or ESRD development, but is associated with proteinuria progression, and an increased risk of adverse CV outcomes. Given the high CV risk profile of patients with CKD, future studies targeting risk reduction for individuals with CKD and prediabetes are warranted.

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Additional Information

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Disclosure Summary: The authors declare that there are no conflicts of interest relevant to this article.

Data Availability: The dataset analyzed during the current study is available in the National Institute of Diabetes and Digestive and Kidney Diseases Central Repository: <https://repository.niddk.nih.gov/studies/cric/>.

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Supplementary Material

Association of prediabetes with CKD progression and adverse cardiovascular outcomes: an analysis of the CRIC (Chronic Renal Insufficiency Cohort Study)

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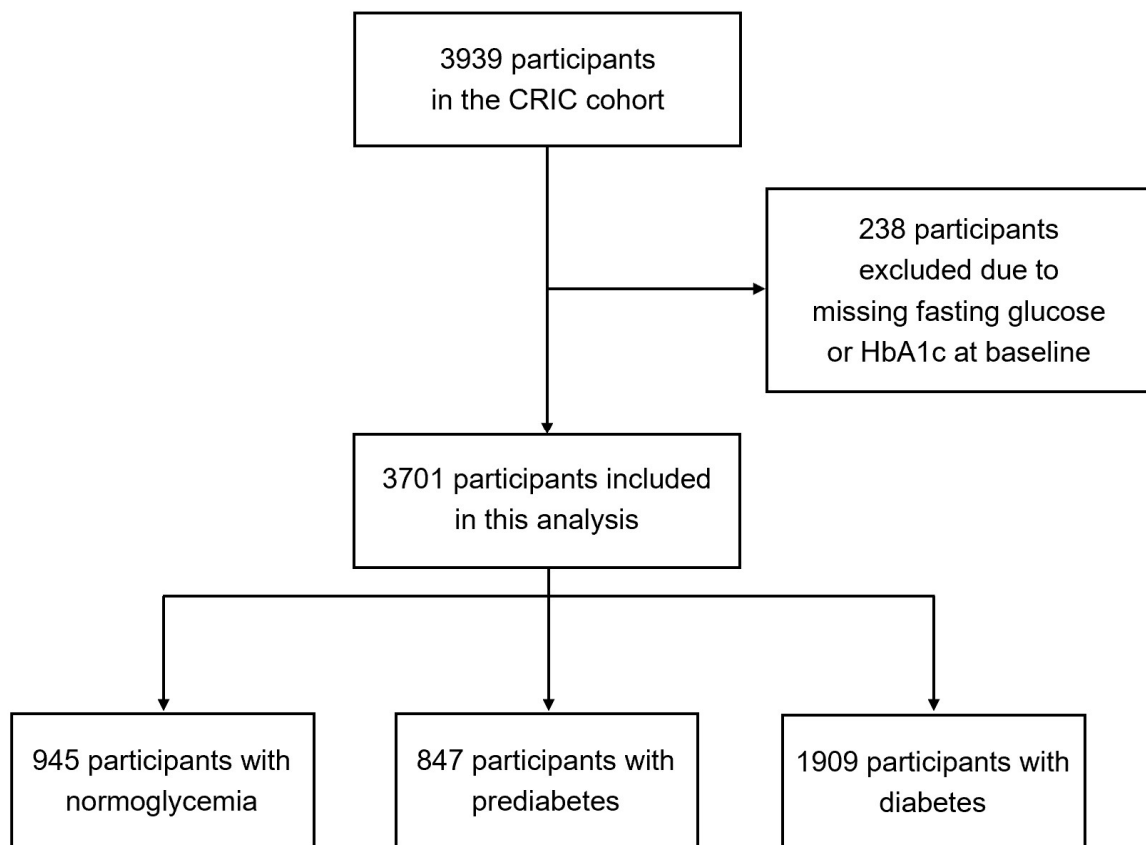
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Supplementary figure 1. Flowchart of the study population

Supplementary table 1. Definition of outcomes (1)

Outcome	Definition
Composite renal outcome *	ESRD (start of chronic dialysis or renal transplantation), OR 50% decline in baseline eGFR and eGFR ≤ 15 ml/min/ 1.73 m ² using the CKD-EPI equation, OR doubling of urine protein to creatinine ratio to ≥ 0.22 g/g creatinine.
CHF	Hospital admission (including prolonged "observation unit" admissions of >24 hours) for new or worsening CHF defined as the presence of a syndrome characterized clinically by breathlessness, pulmonary congestion, effort intolerance, fluid retention and peripheral hypoperfusion. Must have represented a clear abrupt change from the normal clinical state of the patient. Episodes of hospitalized heart failure were adjudicated as to whether the CHF was characterized principally as heart failure with reduced systolic function (based on proximate assessment of LV systolic function), heart failure with preserved systolic function (based on demonstration of intact LV systolic function), or unclassified. These criteria were consistent with the Framingham Heart Study.
MI	Typical rise and gradual fall (troponin) or typical rise and rapid fall (CPK-MB) of cardiac enzymes to >2X upper limit of the normal range in combination with one of the following: 1. Symptoms of myocardial ischemia. 2. Other clinical manifestations of myocardial ischemia (e.g. CHF or new ventricular tachyarrhythmias). 3. ECG changes compatible with ischemia or infarction. <i>Probable MI:</i> Typical clinical setting with chest pain or other findings suggestive of Acute MI in the absence of diagnostic biomarker or ECG changes
Stroke	Ischemic stroke was defined as a fixed (>24 hours) neurologic deficit not explained by another etiology (i.e., primary hemorrhage, trauma, infection, vasculitis, etc.). Confirmatory imaging studies were not essential to the clinical diagnosis of a stroke. Further description was based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) and the CARE Study Classification of Subtypes of Acute Ischemic: 1. Large artery atherosclerosis (embolism/thrombosis) * 2. Cardioembolism (high-risk/medium-risk) * 3. Small-vessel occlusion (lacunae)* 4. Stroke of other determined etiology* 5. Stroke of undetermined etiology <i>*Possible or probable Stroke:</i> depending on results of ancillary studies.
Composite cardiovascular outcome (CHF, MI or Stroke)	CHF (definite/probable), MI (definite/probable/possible), Stroke (definite/probable) Composite (years)
Peripheral artery disease	1. Amputation due to vascular disease 2. Peripheral surgical or percutaneous revascularization

* The original primary renal outcome of the CRIC did not include the proteinuria component.

Abbreviations. CKD: chronic kidney disease, ESRD: end-stage renal disease, eGFR: estimated glomerular filtration rate, CHF: congestive heart failure, LV: left ventricle, CPK-MB: creatine kinase-muscle/brain, ECG: electrocardiogram, MI: myocardial infarction.

Supplementary table 2. Association of prediabetes and diabetes with renal outcomes

	Normoglycemia (n=945)	Prediabetes (n=847)	Diabetes (n=1909)
50% decline in eGFR to ≤ 15 ml/min/1.73 m² or ESRD			
No. Events/No. Pts	172/895 (19.2%)	152/800 (19.0%)	665/1778 (37.4%)
Unadjusted HR	(reference)	1.00 (0.80-1.25) P=0.99	2.13 (1.80-2.52) P<0.001
Model 1 HR	(reference)	1.03 (0.82-1.28) P=0.82	2.22 (1.86-2.65) P<0.001
Model 2 HR	(reference)	0.96 (0.76-1.21) P=0.73	1.38 (1.12-1.70) P=0.003
50% decline in eGFR or ESRD			
No. Events/No. Pts	207/895 (23.1%)	180/800 (22.5%)	774/1778 (43.5%)
Unadjusted HR	(reference)	0.97 (0.79-1.19) P=0.77	2.12 (1.81-2.47) P<0.001
Model 1 HR	(reference)	0.99 (0.81-1.22) P=0.96	2.22 (1.89-2.60) P<0.001
Model 2 HR	(reference)	0.88 (0.71-1.10) P=0.26	1.31 (1.09-1.59) P=0.005
ESRD			
No. Events/No. Pts	158/945 (16.7%)	138/847 (16.3%)	600/1909 (31.4%)
Unadjusted HR	(reference)	1.03 (0.81-1.29) P=0.83	2.09 (1.75-2.50) P<0.001
Model 1 HR	(reference)	1.06 (0.84-1.34) P=0.61	2.25 (1.87-2.71) P<0.001
Model 2 HR	(reference)	1.01 (0.79-1.29) P=0.94	1.30 (1.04-1.62) P=0.019
Proteinuria progression			
No. Events/No. Pts	253/838 (30.2%)	281/769 (36.5%)	704/1606 (43.8%)
Unadjusted HR	(reference)	1.27 (1.07-1.51) P=0.006	1.69 (1.46-1.95) P<0.001
Model 1 HR	(reference)	1.22 (1.03-1.45) P=0.025	1.59 (1.36-1.84) P<0.001
Model 2 HR	(reference)	1.23 (1.03-1.47) P=0.023	1.50 (1.27-1.78) P<0.001

The sum of individual outcomes may be higher than the composite total, as participants may have experienced more than one of the individual composites.

ESRD: renal transplantation or dialysis initiation

Proteinuria progression: doubling of urine protein to creatinine ratio to ≥ 0.22 g/g creatinine

Model 1: age, sex and race/ethnicity.

Model 2: variables in model 1 plus body mass index, antiplatelet therapy, lipid lowering therapy, systolic blood pressure at baseline, coronary artery disease (defined as prior myocardial infarction or prior coronary revascularization), peripheral vascular disease, congestive heart failure, hematocrit, baseline eGFR, serum albumin and 24-hour urine protein.

Abbreviations: HR, hazard ratio; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease.

Supplementary table 3. Association of prediabetes and diabetes with cardiovascular outcomes

	Normoglycemia (n=945)	Prediabetes (n=847)	Diabetes (n=1909)
Myocardial infarction			
No. Events/No. Pts	38/943 (4.0%)	55/844 (6.5%)	210/1901 (11.0%)
Unadjusted HR	(reference)	1.58 (1.04-2.39) P=0.031	3.01 (2.12-4.26) P<0.001
Model 1 HR	(reference)	1.34 (0.88-2.03) P=0.17	2.46 (1.73-3.52) P<0.001
Model 2 HR	(reference)	1.20 (0.78-1.84) P=0.41	1.39 (0.94-2.06) P=0.10
Stroke			
No. Events/No. Pts	24/943 (2.5%)	31/844 (3.7%)	93/1903 (4.9%)
Unadjusted HR	(reference)	1.42 (0.83-2.42) P=0.20	2.16 (1.37-3.40) P=0.001
Model 1 HR	(reference)	1.17 (0.68-2.00) P=0.58	1.62 (1.02-2.58) P=0.041
Model 2 HR	(reference)	1.17 (0.66-2.08) P=0.58	1.23 (0.72-2.11) P=0.44
Heart failure events			
No. Events/No. Pts	49/943 (5.2%)	100/844 (11.8%)	445/1903 (23.4%)
Unadjusted HR	(reference)	2.30 (1.63-3.24) P<0.001	5.08 (3.77-6.83) P<0.001
Model 1 HR	(reference)	2.01 (1.43-2.84) P<0.001	4.28 (3.17-5.79) P<0.001
Model 2 HR	(reference)	1.82 (1.26-2.63) P=0.002	2.20 (1.57-3.09) P<0.001

The sum of individual cardiovascular outcomes may be higher than the composite cardiovascular total, as participants may have experienced more than one of the individual composites.

Model 1: age, sex and race/ethnicity.

Model 2: variables in model 1 plus body mass index, antiplatelet therapy, lipid lowering therapy, systolic blood pressure at baseline, coronary artery disease (defined as prior myocardial infarction or prior coronary revascularization), peripheral vascular disease, congestive heart failure, hematocrit, baseline eGFR, serum albumin and 24-hour urine protein.

Abbreviations: HR, hazard ratio.

Supplementary table 4. Association of prediabetes and diabetes with peripheral artery disease

	Normoglycemia (n=945)	Prediabetes (n=847)	Diabetes (n=1909)
Peripheral artery disease			
No. Events/No. Pts	9/943 (1.0%)	21/844 (2.5%)	136/1903 (7.1%)
Unadjusted HR	(reference)	2.61 (1.19-5.70) P=0.016	8.18 (4.16-16.10) P<0.001
Model 1 HR	(reference)	2.48 (1.13-5.45) P=0.023	7.79 (3.93-15.44) P<0.001
Model 2 HR	(reference)	2.33 (1.05-5.18) P=0.037	4.34 (2.12-8.91) P<0.001

Model 1: age, sex and race/ethnicity.

Model 2: variables in model 1 plus body mass index, antiplatelet therapy, lipid lowering therapy, systolic blood pressure at baseline, coronary artery disease (defined as prior myocardial infarction or prior coronary revascularization), peripheral vascular disease, congestive heart failure, hematocrit, baseline eGFR, serum albumin and 24-hour urine protein.

Abbreviations: HR, hazard ratio.

Supplementary table 5. Diagnosis of prediabetes and diabetes according to HbA1c and fasting plasma glucose

		Fasting plasma glucose			Total
		Normoglycemia	Prediabetes	Diabetes	
HbA1c criteria	Normoglycemia n (%)	945 (25.5)	154 (4.2)	10 (0.3)	1,109 (30.0)
	Prediabetes n (%)	515 (13.9)	178 (4.8)	17 (0.5)	710 (19.2)
	Diabetes n (%)	72 (2.0)	116 (3.1)	1,694 (45.8)	1,882 (50.9)
	Total n (%)	1,532 (41.4)	448 (12.1)	1,721 (46.5)	

Patients treated with anti-diabetic drugs were classified as having diabetes (n=1,603, 43.3%). The remaining patients were defined as having normoglycemia, prediabetes or diabetes according to fasting plasma glucose or hemoglobin A1c.

Supplementary table 6. Glucose tolerance at the end of follow-up in each baseline group

		End of follow-up		
		Normoglycemia	Prediabetes	Diabetes
Baseline	Normoglycemia (n=945)	617 (65.3%)	279 (29.5%)	49 (5.2%)
	Prediabetes (n=847)	190 (22.5%)	544 (64.4%)	111 (13.1%)
	Diabetes (n=1,909)	49 (2.6%)	127 (6.6%)	1,732 (90.8%)

Patients treated with anti-diabetic drugs were classified as having diabetes. The remaining patients were defined as having normoglycemia, prediabetes or diabetes according to fasting plasma glucose and hemoglobin A1c.

Supplementary table 7. Association of prediabetes and diabetes with outcomes, according to classification based on fasting plasma glucose or hemoglobin A1c

Glucose status according to fasting plasma glucose			
	Normoglycemia (n=1532)	Prediabetes (n=448)	Diabetes (n=1721)
Composite renal outcome			
No. Events/No. Pts	593/1445 (41.0%)	180/423 (42.6%)	1012/1609 (62.9%)
Unadjusted HR	(reference)	1.06 (0.90-1.26) P=0.48	1.85 (1.67-2.05) P<0.001
Model 1 HR	(reference)	1.04 (0.88-1.23) P=0.67	1.83 (1.65-2.04) P<0.001
Model 2 HR	(reference)	1.00 (0.84-1.19) P=0.98	1.42 (1.25-1.61) P<0.001
Composite cardiovascular outcome			
No. Events/No. Pts	208/1527 (13.6%)	85/445 (19.1%)	530/1716 (30.9%)
Unadjusted HR	(reference)	1.50 (1.16-1.93) P=0.002	2.65 (2.25-3.13) P<0.001
Model 1 HR	(reference)	1.29 (1.00-1.66) P=0.050	2.31 (1.96-2.73) P<0.001
Model 2 HR	(reference)	1.07 (0.82-1.40) P=0.601	1.32 (1.09-1.60) P=0.005
All-cause mortality			
No. Events/No. Pts	220/1532 (14.4%)	78/448 (17.4%)	477/1721 (27.7%)
Unadjusted HR	(reference)	1.23 (0.95-1.60) P=0.11	2.03 (1.73-2.39) P<0.001
Model 1 HR	(reference)	1.05 (0.81-1.37) P=0.70	1.74 (1.48-2.06) P<0.001
Model 2 HR	(reference)	1.01 (0.77-1.32) P=0.95	1.33 (1.10-1.62) P=0.004
Glucose status according to hemoglobin A1c level			
	Normoglycemia (n=1109)	Prediabetes (n=710)	Diabetes (n=1882)
Composite renal outcome			
No. Events/No. Pts	424/1055 (40.2%)	280/667 (42.0%)	1081/1755 (61.6%)
Unadjusted HR	(reference)	1.06 (0.91-1.23) P=0.46	1.82 (1.63-2.04) P<0.001
Model 1 HR	(reference)	1.07 (0.92-1.25) P=0.39	1.81 (1.61-2.03) P<0.001
Model 2 HR	(reference)	1.07 (0.91-1.25) P=0.42	1.42 (1.24-1.63) P<0.001
Composite cardiovascular outcome			
No. Events/No. Pts	117/1106 (10.6%)	132/708 (18.6%)	574/1874 (30.6%)
Unadjusted HR	(reference)	1.77 (1.38-2.27) P<0.001	3.34 (2.74-4.09) P<0.001
Model 1 HR	(reference)	1.55 (1.20-1.99) P=0.001	2.81 (2.29-3.45) P<0.001
Model 2 HR	(reference)	1.41 (1.09-1.84) P=0.010	1.64 (1.30-2.07) P<0.001
All-cause mortality			
No. Events/No. Pts	134/1109 (12.1%)	130/710 (18.3%)	511/1882 (27.2%)
Unadjusted HR	(reference)	1.51 (1.18-1.92) P=0.001	2.31 (1.91-2.80) P<0.001
Model 1 HR	(reference)	1.30 (1.01-1.65) P=0.038	1.91 (1.57-2.32) P<0.001
Model 2 HR	(reference)	1.21 (0.94-1.56) P=0.141	1.41 (1.13-1.77) P=0.002

Patients treated with anti-diabetic drugs were classified as having diabetes. The remaining patients were defined as having normoglycemia, prediabetes or diabetes according to fasting plasma glucose or hemoglobin A1c. CKD progression: Development of ESRD (renal transplantation or dialysis initiation) or 50% decline in baseline eGFR (CKD-EPI equation) and eGFR ≤ 15 ml/min/1.73 m². Composite cardiovascular outcome: Congestive heart failure, myocardial infarction or stroke. Model 1: age, sex and race/ethnicity. Model 2: variables in model 1 plus body mass index, antiplatelet therapy, lipid lowering therapy, systolic blood pressure at baseline, coronary artery disease (defined as prior myocardial infarction or prior coronary revascularization), peripheral vascular disease, congestive heart failure, hematocrit, baseline eGFR, serum albumin and 24-hour urine protein. Abbreviations: HR, hazard ratio.

Supplementary table 8. Exploratory model

	Normoglycemia (n=945)	Prediabetes (n=847)	Diabetes (n=1909)
Composite renal outcome			
No. Events/No. Pts	351/896 (39.2%)	340/800 (42.5%)	1094/1781 (61.4%)
Unadjusted HR	(reference)	1.12 (0.96-1.30) P=0.15	1.89 (1.67-2.14) P<0.001
Model 3 HR	(reference)	1.11 (0.95-1.30) P=0.20	1.44 (1.24-1.67) P<0.001
Composite cardiovascular outcome			
No. Events/No. Pts	93/943 (9.9%)	151/844 (17.9%)	579/1901 (30.5%)
Unadjusted HR	(reference)	1.85 (1.43-2.40) P<0.001	3.60 (2.89-4.49) P<0.001
Model 3 HR	(reference)	1.34 (1.01-1.77) P=0.044	1.49 (1.15-1.93) P=0.003
All-cause mortality			
No. Events/No. Pts	104/945 (11.0%)	151/847 (17.8%)	520/1909 (27.2%)
Unadjusted HR	(reference)	1.63 (1.27-2.09) P<0.001	2.56 (2.07-3.17) P<0.001
Model 3 HR	(reference)	1.22 (0.93-1.59) P=0.16	1.36 (1.06-1.75) P=0.015

CKD progression: Development of ESRD (renal transplantation or dialysis initiation) or 50% decline in baseline eGFR (CKD-EPI equation) and eGFR ≤ 15 ml/min/1.73 m².

Composite cardiovascular outcome: Congestive heart failure, myocardial infarction or stroke.

Model 3 (exploratory model): variables in model 2 plus high-sensitive C reactive protein, brain natriuretic peptide and high-sensitivity troponin T.

Abbreviations: HR, hazard ratio.

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1. Feldman HI, Appel LJ, Chertow GM, Cifelli D, Cizman B, Daugirdas J, Fink JC, Franklin-Becker ED, Go AS, Hamm LL, He J, Hostetter T, Hsu CY, Jamerson K, Joffe M, Kusek JW, Landis JR, Lash JP, Miller ER, Mohler ER 3rd, Muntner P, Ojo AO, Rahman M, Townsend RR, Wright JT; Chronic Renal Insufficiency Cohort (CRIC) Study Investigators. The Chronic Renal Insufficiency Cohort (CRIC) Study: Design and Methods. *J Am Soc Nephrol* . 2003;14(7 Suppl 2):S148-53.

Chapter 3 | Part C

Cardiovascular effects of GLP-1 receptor activation





Article 9

GLP1 Receptor Agonist Therapy with and without SGLT2 inhibitors in Patients with Type 2 Diabetes

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Short tweet: In T2D with cardiovascular disease, treatment with GLP-1 RA reduced cardiovascular events independently of SGLT2i use. The combined use of GLP-1 RA with SGLT2i should be tested in randomized trials. @UnIC_cardiovasc #T2D #SGLT2i #GLP1-RA

Abstract

Background: Sodium-glucose cotransporter-2 inhibitors (SGLT2i) and GLP-1 receptor agonists (GLP-1 RA) reduce adverse cardiovascular outcomes in type 2 diabetes (T2D). However, the efficacy of combination therapy is unclear.

Objectives: Our aim was to evaluate the effects of GLP-1 RA on cardiovascular outcomes in patients with T2D treated with or without SGLT2i.

Methods: Post hoc analysis of Harmony Outcomes evaluating the effect of albiglutide in T2D with cardiovascular disease by background SGLT2i use. Additionally, a trial-level meta-analysis of Harmony Outcomes and AMPLITUDE-O (which evaluated T2D with cardiovascular or renal disease) was performed combining the treatment effect estimates according to SGLT2i use.

Results: From the 9462 participants in Harmony outcomes, 575 (6.1%) were treated with SGLT2i at baseline. The effect of albiglutide on reducing the composite of cardiovascular death, myocardial infarction or stroke (MACE) was consistent with or without SGLT2i (p-interaction=0.70). The effect of albiglutide on secondary outcomes and adverse events was not modified by SGLT2i. Meta-analysis of Harmony Outcomes and AMPLITUDE-O included 13538 patients, of whom 1193 (8.8%) used SGLT2i. Compared to placebo, GLP1-RA reduced MACE without effect modification by SGLT2i use: HR 0.77, 95%CI 0.76-0.92 without SGLT2i and HR 0.78, 95%CI 0.49-1.24 with SGLT2i (p-interaction=0.95); and reduced heart failure hospitalization: HR 0.72, 95%CI 0.55-0.92 versus HR 0.34, 95%CI 0.12-0.96 (p-interaction=0.18).

Conclusions: In patients with T2D and cardiovascular disease, GLP-1 RA reduced cardiovascular events independently of SGLT2i use. These findings suggest that the

combination of GLP-1 RA with SGLT2i may further reduce cardiovascular risk. Clinical trials with combination therapy are needed.

Keywords: GLP-1 receptor agonist, SGLT2 inhibitor, type 2 diabetes, cardiovascular disease; heart failure

Condensed abstract

Sodium-glucose cotransporter-2 inhibitors (SGLT2i) and GLP-1 receptor agonists (GLP-1 RA) reduce adverse cardiovascular outcomes in type 2 diabetes (T2D). In a post hoc analysis of Harmony Outcomes, and in a meta-analysis with AMPLITUDE-O (13538 patients), we evaluated the effect of GLP-1 RA on cardiovascular outcomes by SGLT2i use (1193 patients, 8.8%, with baseline SGLT2i use in the meta-analysis). Treatment with GLP-1 RA reduced major adverse cardiovascular events (MACE) and heart failure hospitalization independently of SGLT2i use. These findings suggest that combining GLP-1 RA with SGLT2i may further reduce cardiovascular risk in T2D. Clinical trials with combination therapy are needed.

Abbreviations

AMPLITUDE-O, Effect of Efpeglenatide on Cardiovascular Outcomes

ASCVD, atherosclerotic cardiovascular disease

GLP-1 RA, glucagon-like peptide 1 receptor agonists

Harmony Outcomes, Albiglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes and Cardiovascular Disease

HbA1c, glycated hemoglobin

HF, heart failure

HR, hazard ratio

RCT, randomized clinical trials

SGLT2i, sodium-glucose cotransporter-2 inhibitors

T2D, type 2 diabetes

Introduction

Cardiovascular diseases are among the main causes of death and morbidity in people with type 2 diabetes (T2D).¹ Both sodium-glucose cotransporter-2 inhibitors (SGLT2i) and glucagon-like peptide 1 receptor agonists (GLP-1 RA) reduce the cardiovascular risk of patients with type 2 diabetes and atherosclerotic cardiovascular disease (ASCVD).^{2,3} Current guidelines recommend treatment of people with type 2 diabetes and ASCVD with either SGLT2i or GLP-1 RA, but the prioritization of combination routinely in this population is less clear.⁴

Whether the protection from cardiovascular outcomes in people with type 2 diabetes is greater with the combined use of SGLT2i and GLP1-RA (vs either treatment alone) is still uncertain. In an exploratory analysis of the Effect of Efpeglenatide on Cardiovascular Outcomes (AMPLITUDE-O) trial that included 618 (15.2%) participants receiving SGLT2i treatment at baseline, the benefits of using GLP1-RA were similar regardless of background SGLT2i use.⁵ To date, this subgroup analysis is the sole report from a large clinical trial analyzing the effect of GLP-1 RA added to an SGLT2i.

The Harmony Outcomes (Albiglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes and Cardiovascular Disease) evaluated the effect of albiglutide on cardiovascular outcomes among people with type 2 diabetes and cardiovascular disease. This trial included 575 (6.1%) participants treated with SGLT2i at baseline and, as such, represents an important contribution to the evaluation of the effects of GLP-1 RA in patients with and without background SGLT2i treatment. Furthermore, meta-analysis of Harmony outcomes and AMPLITUDE-O allows the evaluation of the effect of GLP-1 RA in more than 1000 similar patients treated with a SGLT2i at baseline. This represents the largest experience of participants treated with SGLT2i in randomized clinical trials (RCTs) testing the effect of GLP-1 RA on cardiovascular outcomes of patients with T2D. The remaining GLP1-RA trials included few participants with baseline SGLT2i use.⁶ We hypothesized that the effects of GLP1-RA on cardiovascular outcomes would be independent of SGLT2i use.

Methods

Harmony Outcomes trial

The design and the main results of the Harmony Outcomes have been previously published.^{7,8} Briefly, Harmony Outcomes was a double-blind RCT that included patients aged 40 years or older with type 2 diabetes with established cardiovascular disease and a glycated hemoglobin (HbA1c) >7.0%, randomized in a 1:1 ratio to once a week subcutaneous injection of albiglutide or matching placebo. The starting dose of the study medication was 30 mg once a week, with possibility to increase to 50 mg once a week after 5 weeks if glucose-lowering intensification was needed. Main exclusion criteria included an estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m², previous pancreatitis or risk factors for pancreatitis.⁸ The trial was conducted between July 2015 and March 2018. GlaxoSmithKline discontinued the marketing of albiglutide in 2018 due to commercial reasons and not related to any safety issues.

The primary outcome of Harmony Outcomes trial was a composite of cardiovascular death, myocardial infarction (MI), or stroke. The median follow-up time was 1.6 (percentile₂₅₋₇₅ 1.3 to 2.0) years. Albiglutide significantly reduced the rate of the primary composite outcome: 338/4731 (7%) events in the albiglutide group vs. 428/4732 (9%) events in the placebo group, HR 0.78 (95%CI 0.68-0.90, P <0.001).

The protocol of the Harmony Outcomes trial was approved by the ethics committee at each participating site and all patients provided written informed consent. Data for this post-hoc analysis was accessed through the Vivli® (Center for Global Clinical Research Data) platform with the Vivli ID: 00008542.

Trial Outcomes

In the present analysis of Harmony Outcomes, we used as main outcome the primary composite outcome of the original trial. We also analyzed as secondary outcomes the following events: the composite of cardiovascular death or heart failure (HF) hospitalization; the composite of cardiovascular death, MI, unstable angina, or stroke; the composite of all-cause mortality, MI or stroke; the individual components of the primary outcome; and all-cause mortality. All outcomes were studied in patients with and without SGLT2i use at baseline.

Body weight, heart rate, systolic blood pressure (SBP), HbA1c, and estimated glomerular filtration rate (eGFR) were used for longitudinal analysis.

Other events were evaluated in Harmony Outcomes, including serious adverse events (any), adverse events leading to permanent drug discontinuation, severe hypoglycemia, diabetic ketoacidosis, urinary tract infection, renal failure, hypersensitivity reaction, pneumonia and pancreatitis.

For the trial-level meta-analysis of Harmony Outcomes with AMPLITUDE-O, we used the published outcomes reported in AMPLITUDE-O SGLT2i subgroup post hoc analysis,⁵ also assessed in the present analysis.

Statistical analysis

The Harmony Outcomes population was divided into two subgroups according to SGLT2i use at baseline (with vs. without SGLT2i), and their characteristics were described as mean (standard deviation) or medians (percentile₂₅₋₇₅) for normal and non-normal continuous variables respectively. Categorical variables were described as numbers and percentage.

Baseline characteristics of the trial population were compared with t-test or Mann-Whitney test for normal and non-normal continuous variables respectively, and with chi-square test for categorical variables.

The effect of albiglutide vs. placebo was tested using Cox models for time-to-first event analyses with treatment group as an explanatory variable and baseline SGLT2i use as a stratification variable, with treatment-by-SGLT2i use interaction term in the model. The results are presented separately for patients with and without baseline SGLT2i use, along with the respective interaction P-value. Analyses were performed according to the intention-to-treat principle. The Kaplan-Meier method was used to estimate event rates.

The effect of treatment on body weight, heart rate, SBP, HbA1c, and eGFR over time, was studied using mixed random effects models with treatment, time, SGLT2i use, baseline value of the variable of interest, treatment-by-time, and treatment-by- SGLT2i interaction terms in the model; random effects were set at the patient id level using an unstructured covariance matrix.

We evaluated adverse events in Harmony Outcomes among patients who received at least one dose of the study drug, using logistic regression models with the treatment group as an explanatory variable and SGLT2i as a stratification variable, and including a treatment-by-SGLT2i interaction term in the model.

For the meta-analysis of Harmony Outcomes with AMPLITUDE-O, we used a random-effect meta-analysis approach with heterogeneity assessed using the Cochran Q test statistic and Higgins and Thompson I^2 .⁹ Heterogeneity was considered to be low, moderate, or high if I^2 was <25%, 25% to 75%, or >75%, respectively.¹⁰ The hazard ratio (HR) and respective 95% confidence intervals (95%CI) were extracted from the treatment effect estimates of SGLT2i

subgroup analyses reported in each trial. Estimates from each trial were combined by the use of inverse variance-weighted averages of logarithmic HR in random-effects analysis.

A two-sided P-value of <0.05 was considered statistically significant. P-values and 95% confidence intervals presented in this report have not been adjusted for multiplicity, and therefore inferences drawn from these statistics may not be reproducible. All analyses were performed using Stata® (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC).

Data and Resource Availability

The Harmony Outcomes database can be fully available from Vivli upon reasonable request.

Results

Baseline patient characteristics

Of the 9462 participants randomized in the Harmony outcomes, 575 (6.1%) were treated with a SGLT2i at baseline. Compared to patients without SGLT2i use at baseline, those using SGLT2i use were younger (62.9 vs 64.2 years), more frequently men (75.7 vs 69.0%), had higher body mass index (BMI) (33.6 vs 32.2 kg/m²), lower HbA1c (8.5 vs 8.8%), more frequent history of coronary artery disease (CAD) (78.6 vs. 70.0%), and less frequent history of HF (15.1 vs 20.6%) and peripheral artery disease (19.5 vs 25.2%). Participants treated with an SGLT2i at baseline, were also more frequently treated with metformin (79.3 vs 73.3%), DPP4i (24.2 vs 14.6%), beta-blockers (71.1 vs 66.4%) and statins (89.6 vs 83.7%), and less frequently treated with sulfonylureas (21.9 vs 29.2%) and anticoagulants (5.9 vs 8.6%) than those not receiving an SGLT2i at baseline (*Table 1*).

The baseline characteristics of patients with and without baseline SGLT2i use were well balanced within albiglutide and placebo groups (*Supplemental Table 1*). During the trial period, more participants in the placebo group started SGLT2i than in the albiglutide group (361 vs 240 participants).

Effects of albiglutide vs. placebo on outcomes according to SGLT2 inhibition

The effect of albiglutide (vs. placebo) on the primary outcome was consistent regardless of SGLT2i use at baseline: HR 0.78, 95%CI 0.67-0.90 in patients without baseline SGLT2i use and HR 0.89, 95%CI 0.45-1.77 in patients with baseline SGLT2i use, interaction P =0.70. A similar pattern was observed for cardiovascular death or HF hospitalization: HR 0.87, 95%CI 0.71-1.06 in patients without baseline SGLT2i use and HR 0.68, 95%CI 0.28-1.64 in patients with baseline SGLT2i use, interaction P =0.60. The effect of albiglutide on cardiovascular death, HF hospitalization, the composite of cardiovascular death, MI, unstable angina, or

stroke, the composite of all-cause mortality, MI or stroke, and all-cause mortality was also not modified by baseline SGLT2i use (interaction $P > 0.1$ for all) (*Table 2*).

Effects on Heart Rate, Blood Pressure, HbA1c, Body Weight, and eGFR

During the trial period, compared to placebo, albiglutide increased heart rate by 1 to 2 bpm, decreased weight by 1 to 2 kg, and decreased HbA1c by approximately 0.5%, without significant differences by baseline SGLT2i use. The effects of albiglutide on eGFR and SBP were small and did not differ by baseline SGLT2i use (interaction $P > 0.1$ for all) (*Supplemental Figure 1*).

Adverse events with albiglutide vs. placebo by baseline SGLT2i use

Overall, albiglutide was well-tolerated regardless of baseline SGLT2i use (*Table 3*). Treatment with albiglutide was not associated with an increased risk of serious adverse events but was associated with more permanent drug discontinuation than placebo, without no difference according to baseline SGLT2i use. Severe hypoglycemia, diabetic retinopathy, renal failure, atrial fibrillation/flutter, hypersensitivity reactions, pneumonia, pancreatitis, urinary tract infection and diabetic ketoacidosis were not significantly different between treatment groups, and did not vary according to baseline SGLT2i use (interaction $P > 0.1$ for all) (*Table 3*).

Meta-analysis of Harmony Outcomes and AMPLITUDE-O

A total of 13538 patients with type 2 diabetes from the Harmony outcomes and AMPLITUDE-O were included in this analysis, of whom 1193 (8.8%) were using SGLT2i at baseline. Compared to placebo, GLP1-RA reduced the composite of cardiovascular death,

MI, or stroke (HR 0.77, 95%CI 0.76-0.92) without significant interaction between participants with and without baseline SGLT2i use (interaction P =0.95): HR 0.77, 95%CI 0.76-0.92, in patients without baseline SGLT2i use, and HR 0.78, 95%CI 0.49-1.24, in patients with baseline SGLT2i use. A similar pattern was observed for the composite outcomes of cardiovascular death, MI, unstable angina or stroke, and the composite of all-cause death, MI or stroke (*Central illustration*).

Similarly, compared to placebo, GLP1-RA reduced the risk of HF hospitalization (HR 0.69, 95%CI 0.54-0.88) without significant interaction between participants with and without baseline SGLT2i use (interaction P =0.18): HR 0.72, 95%CI 0.55-0.92, in patients without baseline SGLT2i use, and HR 0.34, 95%CI 0.12-0.96, in patients with baseline SGLT2i use (*Central illustration*).

Discussion

In Harmony Outcomes, treatment with albiglutide reduced the composite of cardiovascular death, MI or stroke, and the risk of heart failure hospitalization, and this benefit was not modified by baseline treatment with an SGLT2i. Meta-analysis of Harmony Outcomes and AMPLITUDE-O showed consistent findings.

A previous meta-analysis of RCTs with GLP-1 RA in type 2 diabetes showed that treatment with GLP-1 RA decreased the risk of MI, stroke or cardiovascular death by 14%, the risk of cardiovascular death by 12%, and the risk of HF hospitalization by 12%.³ These benefits appeared to be independent of whether the GLP-1 RA had structural homology with the human GLP-1 (e.g. albiglutide) or exendin-4 (e.g. efpeglenatide).³

Previous RCTs had shown that the combination of GLP-1 RA with SGLT2i was associated with additive benefits on metabolic outcomes. In the DURATION-8 (Exenatide once weekly plus dapagliflozin once daily versus exenatide or dapagliflozin alone in patients with type 2 diabetes inadequately controlled with metformin monotherapy),¹¹ AWARD-10 (Dulaglutide as add-on therapy to SGLT2 inhibitors in patients with inadequately controlled type 2 diabetes),¹² and SUSTAIN 9 (Semaglutide once weekly as add on to SGLT-2 inhibitor therapy in type 2 diabetes) trials,¹³ the combination of a GLP-1 RA with an SGLT2i led to additional reductions in HbA1c, weight and blood pressure. The fact that GLP-1 RA and SGLT2i have complementary mechanisms of action supports the hypothesis that their combination may also have additive effects on cardiovascular events.¹⁴ The cardiovascular benefits of SGLT2i may be related to renal, hemodynamic, and metabolic effects.¹⁵ SGLT2i positively influence cardiac function and metabolism, enhance erythropoiesis, and reduce inflammation.^{15,16} The cardiovascular benefits of GLP-1 RA may be related to the improvement of cardiovascular risk factors, such as HbA1c, body weight, blood pressure, and lipid profile, as well as direct anti-atherosclerotic effects.^{17,18}

However, before the present analysis, only the AMPLITUDE-O trial provided information on the effects of a GLP-1 RA (exendin-4-based) on cardiovascular outcomes according to baseline SGLT2i use.⁵ Thus, our results from the Harmony Outcomes trial with a different GLP-1 RA (human GLP-1-based) and the meta-analysis of both Harmony Outcomes and AMPLITUDE-O, almost doubling the number of SGLT2i treated participants, supports and extends the evidence that GLP-1 RA are efficacious when added to an SGLT2i. Importantly, with individual participant data from Harmony Outcomes we were also able to examine the safety of combining these therapies, finding no significant cause for concern. Specifically, the safety profile of GLP-1 RA was similar among those with and without SGLT2i treatment. Of note, the risk of serious adverse events, hypoglycemia and diabetic retinopathy was not increased by albiglutide and the risk of urinary tract infections and diabetic ketoacidosis was very low overall.

Current guidelines from the American Diabetes Association (ADA)/European Association for the Study of Diabetes (EASD),¹⁹ and from the American Heart Association (AHA)/American College of Cardiology (ACC)²⁰ recommend that all patients with type 2 diabetes and established cardiovascular disease should be treated with a GLP-1 RA or an SGLT2i. The ADA/EASD consensus algorithm recommends the combination of a GLP-1 RA and an SGLT2i if HbA1c is above target after the initiation of one of these classes.¹⁹ The ACC consensus recommends reassessing patients after treatment with a GLP-1 RA or an SGLT2i and considering the addition of the alternative class if the benefits outweigh the risks.²⁰ However, both guidelines do not recommend routinely adding a GLP-1 RA to those already on an SGLT2i for further reduction of the risk of adverse cardiovascular outcomes. The approach used in these recommendations is in disagreement with the recommendation for other conditions with a high risk of adverse cardiovascular outcomes (e.g. treatment of acute myocardial infarction or treatment of HF with reduced ejection fraction), where the

concomitant use of all interventions with clear evidence of benefit on major outcomes is recommended.²¹⁻²³ Clearly, cost considerations are relevant here but each of these treatments, individually, is cost-effective in high-risk patients, in part because prevention of cardiovascular events offsets the cost of therapy.

Our study has limitations that we should acknowledge. This analysis was not pre-specified and the randomized treatment assignment in the Harmony Outcomes was not stratified according to baseline SGLT2i use. Given the exploratory nature of this study, we did not perform statistical correction for test multiplicity. The small proportion of participants in the subgroup with baseline SGLT2i use (6.1% in Harmony Outcomes and 8.8% in the meta-analysis) reduced the precision of the treatment effect estimates and the statistical power of the interaction tests. However, it is important to note that this still represents a sample of more than 1000 patients who were treated with an SGLT2i at baseline, and the consistency of the point estimates for all outcomes suggests that the benefits of GLP-1 RA on cardiovascular outcomes are independent of the use of SGLT2i. Harmony Outcomes did not collect data on albuminuria and, as such, we could not evaluate the effects of albiglutide on composite renal outcomes according to baseline SGLT2i use. Albiglutide and efpeglenatide are not currently available for clinical use. However, as the ASCVD risk reduction with GLP-1 RA was also observed with other drugs in the class (liraglutide, semaglutide and dulaglutide),³ our findings are likely to apply to other GLP-1 RA. Our findings do not apply to patients with advanced HF with reduced ejection fraction (HFrEF) where GLP-1 RA may increase the risk of adverse cardiovascular events.²⁴⁻²⁶ Despite these limitations, our meta-analysis describes the largest number of patients with baseline SGLT2i use randomized to treatment with a GLP-1 RA or placebo and thus the best evidence to date on the effects of the addition of GLP-1 RA to patients treated with SGLT2i.³

Conclusions

In patients with type 2 diabetes and cardiovascular disease enrolled in Harmony Outcomes, and in a meta-analysis of Harmony Outcomes and AMPLITUDE-O, treatment with a GLP-1 RA reduced the risk of adverse cardiovascular events independently of background SGLT2i use without increased risk of serious adverse events. These findings suggest that the benefits of a GLP-1 RA in patients with type 2 diabetes and cardiovascular disease were consistent in patients receiving or not background treatment with an SGLT2i. Randomized trials are needed to evaluate the potential added value of combining these treatments.

Clinical perspectives

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS

In patients with type 2 diabetes and cardiovascular disease, treatment with GLP-1 receptor agonists reduce cardiovascular events independently of treatment with SGLT2 inhibitors, supporting combination therapy.

TRANSLATIONAL OUTLOOK

Randomized trials are needed to evaluate the potential advantages and disadvantages of combining GLP1 receptor agonists with SGLT2 inhibitors.

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Table 1. Baseline patient characteristics by baseline SGLT2i use

	No SGLT2i at baseline n=8887	SGLT2i at baseline n=575	P-value
Age, years	64.2 ± 8.7	62.9 ± 8.2	<0.001
Male sex	6133 (69.0%)	435 (75.7%)	<0.001
Race			<0.001
Asian	457 (5.1 %)	13 (2.3 %)	
Hispanic	1936 (21.8%)	57 (9.9 %)	
Black or African American	214 (2.4 %)	11 (1.9 %)	
White	6091 (68.5%)	491 (85.4%)	
Other	189 (2.1 %)	3 (0.5 %)	
Region			<0.001
Asia Pacific	368 (4.1 %)	8 (1.4 %)	
Eastern Europe	1974 (22.2%)	73 (12.7%)	
Latin America	1684 (18.9%)	19 (3.3 %)	
North America	1765 (19.9%)	179 (31.1%)	
Western Europe	3096 (34.8%)	296 (51.5%)	
Smoking			<0.001
Current smoker	1398 (15.7%)	90 (15.7%)	
Former smoker	3788 (42.6%)	293 (51.0%)	
Never smoked	3699 (41.6%)	192 (33.4%)	
Hypertension	7673 (86.3%)	511 (88.9%)	0.09
CAD	6225 (70.0%)	452 (78.6%)	<0.001
Myocardial infarction	4160 (46.8%)	298 (51.8%)	0.02
Heart failure	1835 (20.6%)	87 (15.1%)	0.001
PAD	2242 (25.2%)	112 (19.5%)	0.002
Stroke	1592 (17.9%)	89 (15.5%)	0.14
Atrial fibrillation/flutter	802 (9.0 %)	40 (7.0 %)	0.09
BMI, kg/m²	32.2 ± 5.9	33.6 ± 5.8	<0.001
Heart rate, bpm	72.3 ± 10.6	72.0 ± 10.7	0.52
Systolic BP, mmHg	134.9 ± 16.6	132.2 ± 16.3	<0.001
Diastolic BP, mmHg	76.9 ± 10.1	75.3 ± 9.5	<0.001
HbA1c, %	8.8 ± 1.5	8.5 ± 1.2	<0.001
eGFR, ml/min/1.73m²	79.0 ± 25.6	80.1 ± 23.8	0.28

Nephropathy	1641 (18.5%)	97 (16.9%)	0.34
Diabetic eye disease	1820 (20.5%)	117 (20.3%)	0.94
Diabetes duration, years	14.1 ± 8.7	14.7 ± 8.3	0.08
Insulin	5261 (59.2%)	335 (58.3%)	0.66
Metformin	6512 (73.3%)	456 (79.3%)	0.001
DPP4i	1298 (14.6%)	139 (24.2%)	<0.001
Sulfonylureas	2599 (29.2%)	126 (21.9%)	<0.001
Thiazolidinediones	174 (2.0 %)	20 (3.5 %)	0.013
ACEi/ARBs	7056 (79.4%)	468 (81.4%)	0.25
Beta-blockers	5901 (66.4%)	409 (71.1%)	0.02
MRAs	765 (8.6 %)	43 (7.5 %)	0.35
Statins	7440 (83.7%)	515 (89.6%)	<0.001
Antiplatelet agents	6836 (76.9%)	454 (79.0%)	0.26
Anticoagulants	763 (8.6 %)	34 (5.9 %)	0.025

Legend: Data are mean (standard deviation), median (percentile25-75) or number (%). No corrections for multiple testing were applied. CAD, coronary artery disease; PAD, peripheral artery disease; BMI, body mass index; HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate; DPP4i, dipeptidyl peptidase 4 inhibitors; SGLT2i, sodium-glucose co-transporter 2 inhibitors; ACEi/ARBs, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; MRAs, mineralocorticoid receptor antagonists.

Table 2. Effect of albiglutide vs. placebo on the trial outcomes by baseline SGLT2i use

Outcome/SGLT2i baseline status	Albiglutide		Placebo		HR (95%CI)	Inter. P
	n/N. (%)	Inc.-rate per 100py	n/N. (%)	Inc.-rate per 100py		
Cardiovascular death, MI, or stroke						
No SGLT2i	318/4420 (7.2)	4.6	407/4467 (9.1)	6.0	0.78 (0.67-0.90)	0.70
SGLT2i	17/310 (5.5)	3.5	16/265 (6.0)	3.9	0.89 (0.45-1.77)	
Cardiovascular death or HF hospitalization						
No SGLT2i	179/4420 (4.0)	2.5	207/4467 (4.6)	2.9	0.87 (0.71-1.06)	0.60
SGLT2i	9/310 (2.9)	1.8	11/265 (4.2)	2.6	0.68 (0.28-1.64)	
Cardiovascular death						
No SGLT2i	116/4420 (2.6)	1.6	122/4467 (2.7)	1.7	0.96 (0.74-1.23)	0.46
SGLT2i	6/310 (1.9)	1.2	8/265 (3.0)	1.9	0.63 (0.22-1.82)	
HF hospitalization						
No SGLT2i	76/4420 (1.7)	1.1	106/4467 (2.4)	1.5	0.72 (0.54-0.97)	0.62
SGLT2i	3/310 (1.0)	0.6	5/265 (1.9)	1.2	0.50 (0.12-2.08)	
Cardiovascular death, MI, unstable angina, or stroke						
No SGLT2i	364/4420 (8.2)	5.3	463/4467 (10.4)	6.8	0.78 (0.68-0.90)	0.82
SGLT2i	20/310 (6.5)	4.1	20/265 (7.5)	4.9	0.84 (0.45-1.56)	
All-cause mortality, MI or stroke						
No SGLT2i	382/4420 (8.6)	5.6	465/4467 (10.4)	6.8	0.82 (0.71-0.94)	0.57
SGLT2i	20/310 (6.5)	4.1	17/265 (6.4)	4.1	0.99 (0.52-1.89)	
All-cause mortality						
No SGLT2i	187/4420 (4.2)	2.6	195/4467 (4.4)	2.7	0.97 (0.79-1.18)	0.60
SGLT2i	9/310 (2.9)	1.8	10/265 (3.8)	2.4	0.76 (0.31-1.86)	

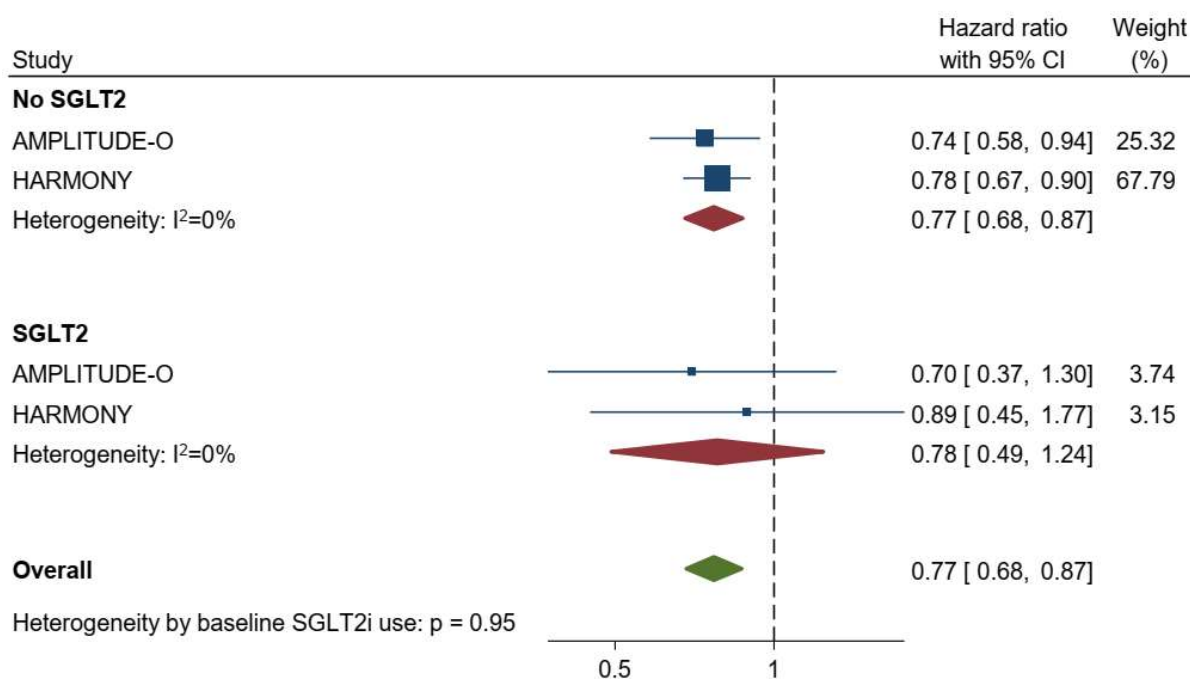
No corrections for multiple testing were applied. Legend: IR, incidence rate per 100 person-years; HF, heart failure; MI, myocardial infarction; SGLT2i, SGLT2 inhibitor; Inter., interaction

Table 3. Adverse events with albiglutide vs. placebo by baseline SGLT2i use

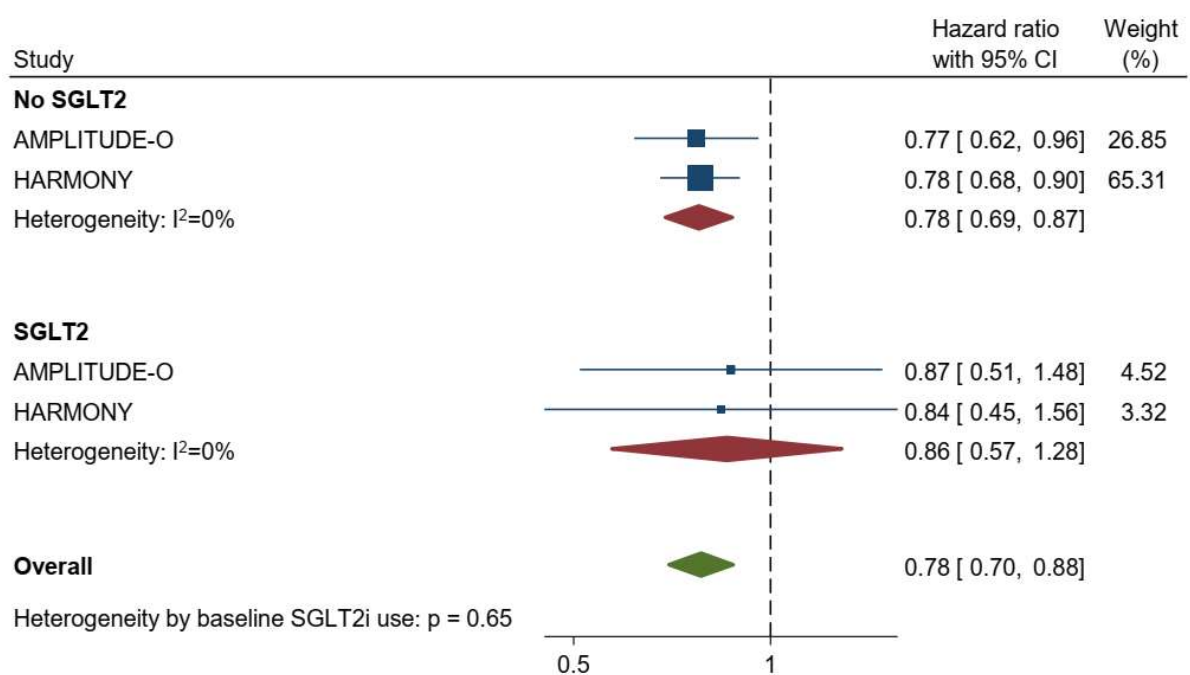
Outcome/SGLT2i baseline status	Albiglutide n. (%)	Placebo n. (%)	OR (95%CI)	Inter. P
Serious adverse event (any)				0.29
No SGLT2i	964/4406 (21.9)	1052/4450 (23.6)	0.90 (0.82-1.00)	
SGLT2i	56/310 (18.1)	62/265 (23.4)	0.72 (0.48-1.08)	
Adverse event leading to permanent study drug discontinuation				0.92
No SGLT2i	403/4406 (9.1)	322/4450 (7.2)	1.29 (1.11-1.50)	
SGLT2i	26/310 (8.4)	17/265 (6.4)	1.34 (0.71-2.52)	
Severe hypoglycemia				0.77
No SGLT2i	30/4406 (0.7)	54/4450 (1.2)	0.56 (0.36-0.87)	
SGLT2i	1/310 (0.3)	1/265 (0.4)	0.85 (0.05-13.73)	
Diabetic retinopathy				0.79
No SGLT2i	72/4406 (1.6)	84/4450 (1.9)	0.86 (0.63-1.19)	
SGLT2i	6/310 (1.9)	5/265 (1.9)	1.03 (0.31-3.40)	
Renal failure				0.57
No SGLT2i	265/4406 (6.0)	308/4450 (6.9)	0.86 (0.73-1.02)	
SGLT2i	14/310 (4.5)	11/265 (4.2)	1.09 (0.49-2.45)	
Atrial fibrillation/flutter (new onset)				0.24
No SGLT2i	99/4406 (2.2)	126/4450 (2.8)	0.79 (0.60-1.03)	
SGLT2i	9/310 (2.9)	5/265 (1.9)	1.55 (0.51-4.70)	
Hypersensitivity reaction				0.91
No SGLT2i	42/4406 (1.0)	45/4450 (1.0)	0.94 (0.62-1.44)	
SGLT2i	3/310 (1.0)	3/265 (1.1)	0.85 (0.17-4.26)	
Pneumonia				0.84
No SGLT2i	124/4406 (2.8)	131/4450 (2.9)	0.95 (0.74-1.22)	
SGLT2i	7/310 (2.3)	7/265 (2.6)	0.85 (0.29-2.46)	
Pancreatitis				_*
No SGLT2i	10/4406 (0.2)	7/4450 (0.2)	1.44 (0.55-3.80)	
SGLT2i	0/310 (0)	0/265 (0)	_*	
Urinary tract infection				_*
No SGLT2i	1/4406 (0.02)	1/4450 (0.02)	1.01 (0.06-16.16)	
SGLT2i	0/310 (0)	0/265 (0)	_*	
Diabetic ketoacidosis				_*
No SGLT2i	2/4406 (0.05)	1/4450 (0.02)	2.02 (0.18-22.30)	
SGLT2i	0/310 (0)	0/265 (0)	_*	

Analysis was restricted to participants that received at least one dose of the study drug. No corrections for multiple testing were applied. *OR and interaction p-value not calculated due to no events in the subgroup with baseline SGLT2i use. Legend: SGLT2i, SGLT2 inhibitor; OR, odds ratio; Inter., interaction.

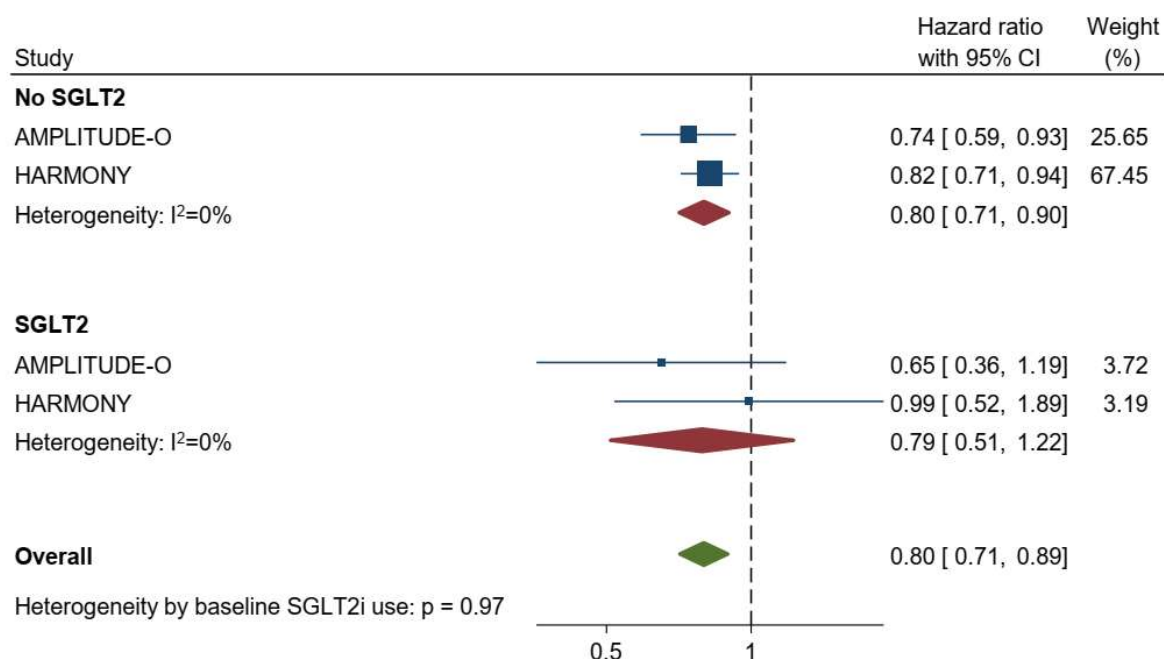
A. Cardiovascular death, MI, or stroke



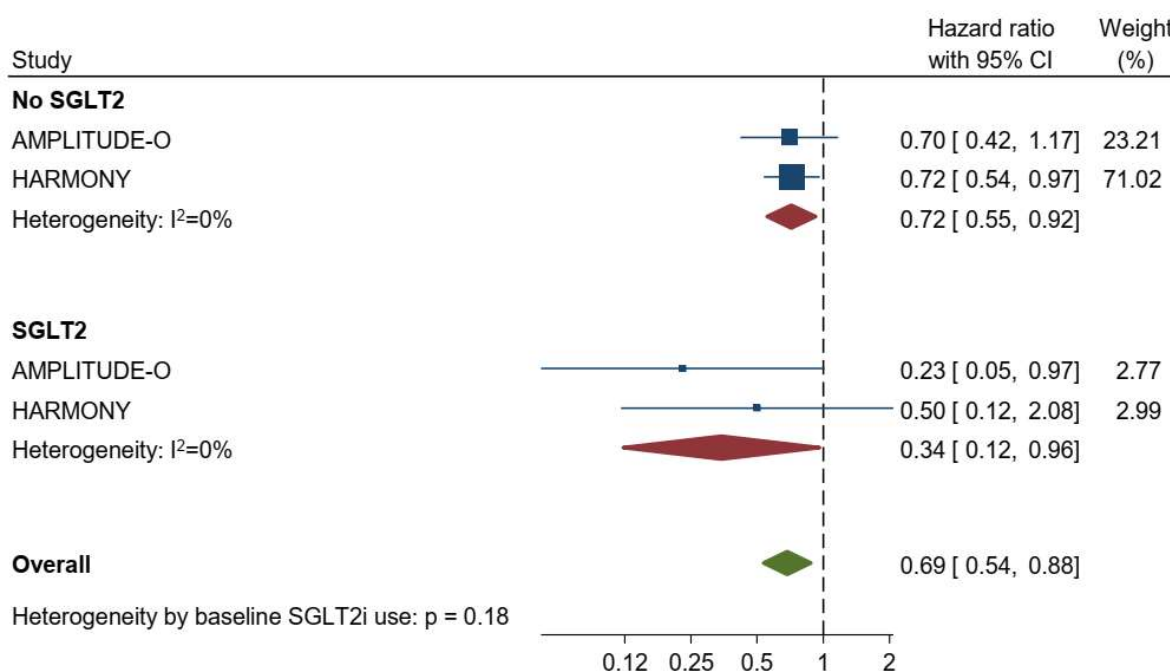
B. Cardiovascular death, MI, unstable angina, or stroke



C. All-cause death, MI or stroke



D. HF Hospitalization



Central illustration. Meta-analysis of cardiovascular outcomes of GLP-1 receptor agonist by baseline SGLT2i use. No corrections for multiple testing were applied. AMPLITUDE-O, Effect of Efpeglenatide on Cardiovascular Outcomes; HARMONY, Albiglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes and Cardiovascular Disease; HF, heart failure; MI, myocardial infarction; SGLT2i, SGLT2 inhibitor.

GLP1 Receptor Agonist Therapy with and without SGLT2 inhibitors in Patients with Type 2 Diabetes

Supplementary Material

Supplemental Table 1. Baseline patient's characteristics by treatment group stratified by baseline SGLT2i use	2

Supplemental Figure 1. Effect of albiglutide on longitudinal measures by baseline SGLT2i use	5

Table S1. Baseline patient's characteristics by treatment group stratified by baseline SGLT2i use

	No SGLT2i at baseline		SGLT2i at baseline	
	Placebo	Albiglutide	Placebo	Albiglutide
Age, years	n=4467	n=4420	n=265	n=310
	64.2 ± 8.6	64.2 ± 8.7	62.7 ± 8.4	63.1 ± 8.0
Male sex	3059 (68.5%)	3074 (69.5%)	206 (77.7%)	229 (73.9%)
Race				
Asian	236 (5.3 %)	221 (5.0 %)	6 (2.3 %)	7 (2.3 %)
Hispanic	959 (21.5%)	977 (22.1%)	29 (10.9%)	28 (9.0 %)
Black or African American	107 (2.4 %)	107 (2.4 %)	7 (2.6 %)	4 (1.3 %)
White	3067 (68.7%)	3024 (68.4%)	221 (83.4%)	270 (87.1%)
Other	98 (2.2 %)	91 (2.1 %)	2 (0.8 %)	1 (0.3 %)
Region				
Asia Pacific	187 (4.2 %)	181 (4.1 %)	4 (1.5 %)	4 (1.3 %)
Eastern Europe	977 (21.9%)	997 (22.6%)	33 (12.5%)	40 (12.9%)
Latin America	834 (18.7%)	850 (19.2%)	11 (4.2 %)	8 (2.6 %)
North America	892 (20.0%)	873 (19.8%)	86 (32.5%)	93 (30.0%)
Western Europe	1577 (35.3%)	1519 (34.4%)	131 (49.4%)	165 (53.2%)
Smoking				
Current smoker	711 (15.9%)	687 (15.5%)	40 (15.1%)	50 (16.1%)
Former smoker	1861 (41.7%)	1927 (43.6%)	138 (52.1%)	155 (50.0%)
Never smoked	1894 (42.4%)	1805 (40.8%)	87 (32.8%)	105 (33.9%)

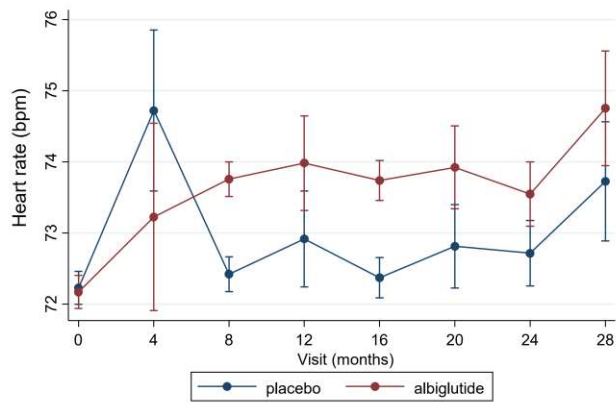
Hypertension	3862 (86.5%)	3811 (86.2%)	233 (87.9%)	278 (89.7%)
CAD	3137 (70.2%)	3088 (69.9%)	208 (78.5%)	244 (78.7%)
Myocardial infarction	2097 (47.0%)	2063 (46.7%)	139 (52.5%)	159 (51.3%)
Heart failure	931 (20.8%)	904 (20.5%)	37 (14.0%)	50 (16.1%)
PAD	1106 (24.8%)	1136 (25.7%)	53 (20.0%)	59 (19.0%)
Stroke	812 (18.2%)	780 (17.6%)	42 (15.8%)	47 (15.2%)
Atrial fibrillation/flutter	403 (9.0 %)	399 (9.0 %)	18 (6.8 %)	22 (7.1 %)
BMI, kg/m²	32.2 ± 5.9	32.2 ± 5.9	33.8 ± 5.7	33.4 ± 5.9
Heart rate, bpm	72.4 ± 10.5	72.3 ± 10.6	73.1 ± 11.3	71.1 ± 10.2
Systolic BP, mmHg	134.9 ± 16.5	134.9 ± 16.6	132.0 ± 16.0	132.3 ± 16.5
Diastolic BP, mmHg	77.0 ± 10.2	76.9 ± 10.1	75.0 ± 9.4	75.5 ± 9.6
HbA1c, %	8.7 ± 1.5	8.8 ± 1.5	8.5 ± 1.2	8.5 ± 1.2
eGFR, ml/min/1.73m²	78.8 ± 25.4	79.1 ± 25.7	80.4 ± 24.5	80.0 ± 23.2
Nephropathy	798 (17.9%)	843 (19.1%)	42 (15.8%)	55 (17.7%)
Diabetic eye disease	899 (20.1%)	921 (20.8%)	56 (21.1%)	61 (19.7%)
Diabetes duration, years	14.1 ± 8.9	14.0 ± 8.6	14.5 ± 7.9	15.0 ± 8.6
Insulin	2587 (57.9%)	2674 (60.5%)	150 (56.6%)	185 (59.7%)
Metformin	3297 (73.8%)	3215 (72.7%)	209 (78.9%)	247 (79.7%)
DPP4i	674 (15.1%)	624 (14.1%)	65 (24.5%)	74 (23.9%)
Sulfonylureas	1318 (29.5%)	1281 (29.0%)	61 (23.0%)	65 (21.0%)
Thiazolidinediones	91 (2.0 %)	83 (1.9 %)	11 (4.2 %)	9 (2.9 %)
ACEi/ARBs	3551 (79.5%)	3505 (79.3%)	216 (81.5%)	252 (81.3%)

Beta-blockers	2992 (67.0%)	2909 (65.8%)	190 (71.7%)	219 (70.6%)
MRAs	376 (8.4 %)	389 (8.8 %)	19 (7.2 %)	24 (7.7 %)
Statins	3751 (84.0%)	3689 (83.5%)	237 (89.4%)	278 (89.7%)
Antiplatelet agents	3424 (76.7%)	3412 (77.2%)	2215 (81.1%)	239 (77.1%)
Anticoagulants	403 (9.0 %)	360 (8.1 %)	11 (4.2 %)	23 (7.4 %)

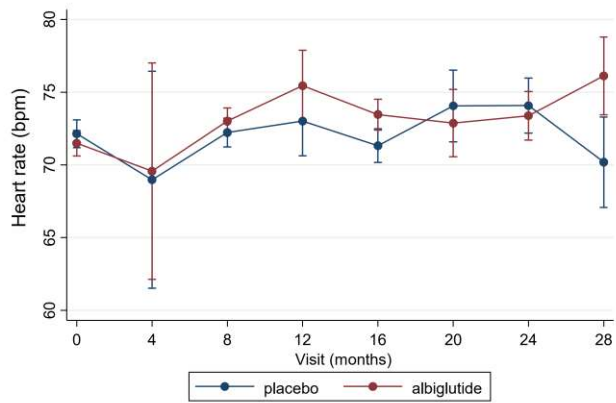
Legend: Data are mean (standard deviation), median (percentile25-75) or number (%). CAD, coronary artery disease; PAD, peripheral artery disease; BMI, body mass index; HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate; DPP4i, dipeptidyl peptidase 4 inhibitors; SGLT2i, sodium glucose co-transporter 2 inhibitors; ACEi/ARBs, angiotensin converting enzyme inhibitors/angiotensin receptor blockers; MRAs, mineralocorticoid receptor antagonists.

Heart rate

No SGLT2i

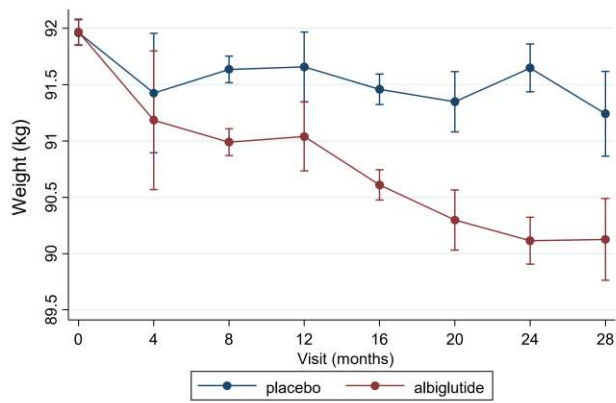


SGLT2i

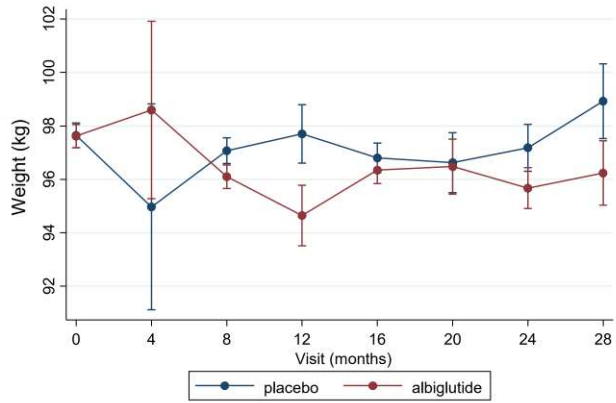


Weight

No SGLT2i

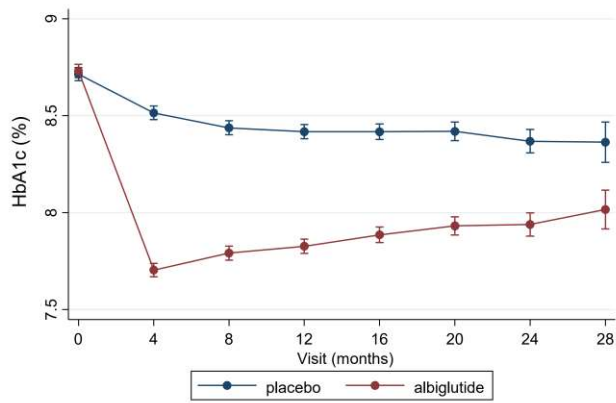


SGLT2i

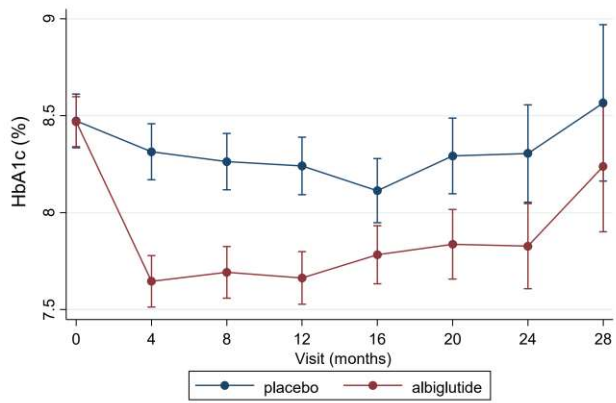


HbA1c

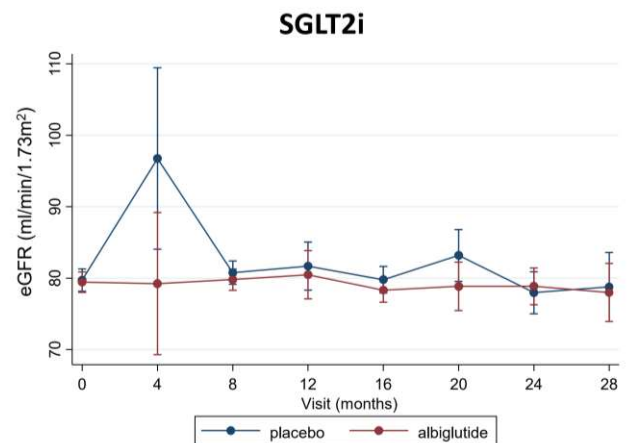
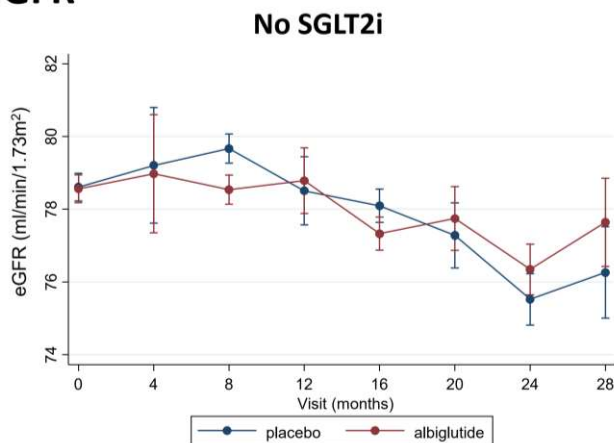
No SGLT2i



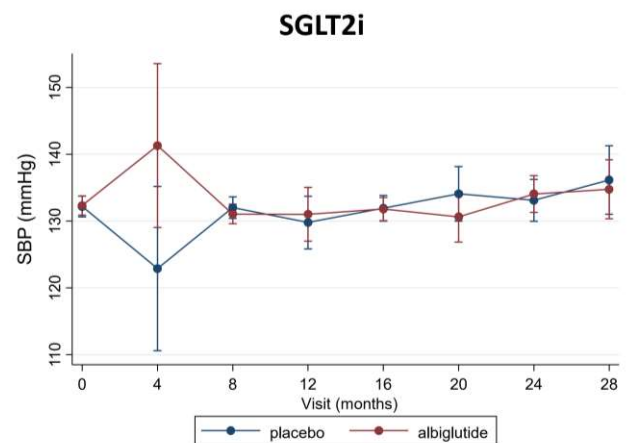
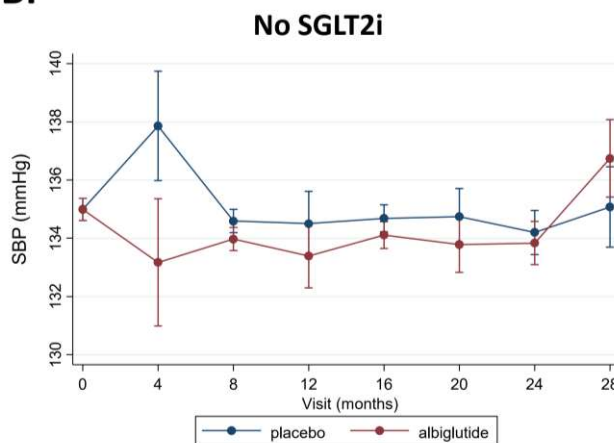
SGLT2i



eGFR



SBP



Supplementary Figure 1. Effect of albiglutide on longitudinal measures by baseline SGLT2i use.

Legend: SGLT2i, SGLT2 inhibitor; HbA1c%, glycated hemoglobin; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.

Caption: Difference between albiglutide and placebo: 1) heart rate: No SGLT2i: 8 months +1.3 (1.0 to 1.7) bpm, 16 months +1.4 (1.0 to 1.8), 24 months +0.8 (0.2 to 1.5) bpm; SGLT2i: 8 months +0.8 (-0.6 to 2.1) bpm, 16 months +2.1 (0.6 to 3.7) bpm, 24 months -0.7 (-3.2 to 1.8) bpm; interaction $P=0.39$; 2) weight: No SGLT2i: 8 months -0.6 (-0.8 to -0.5) Kg, 16 months -0.8 (-1.0 to -0.7) Kg, 24 months -1.5 (-1.8 to -1.2) Kg; SGLT2i: 8 months -1.0 (-1.6 to -0.3) Kg, 16 months -0.5 (-1.2 to 0.3) Kg, 24 months -1.5 (-2.7 to -0.3) Kg; interaction $P=0.93$; 3) HbA1c: No SGLT2i: 8 months -0.6 (-0.7 to -0.6) %, 16 months -0.5 (-0.6 to -0.5) %, 24 months -0.4 (-0.5 to -0.3) %; SGLT2i: 8 months -0.6 (-0.8 to -0.4) %, 16 months -0.3 (-0.6 to -0.1) %, 24 months -0.5 (-0.8 to -0.1) %; interaction $P=0.85$; 4) eGFR: No SGLT2i: 8 months -1.1 (-1.7 to -0.6) ml/min/1.73m², 16 months -0.8 (-1.4 to -0.1) ml/min/1.73m², 24 months 0.8 (-0.2 to 1.8) ml/min/1.73m²; SGLT2i: 8 months -1.0 (-3.2 to 1.2) ml/min/1.73m², 16 months -1.5 (-4.0 to 1.1) ml/min/1.73m², 24 months 0.9 (-3.0 to 4.8) ml/min/1.73m²; interaction $P=0.83$; 5) SBP: No SGLT2i: 8 months -0.6 (-1.2 to -0.1) mmHg, 16 months -0.6 (-1.2 to 0.1) mmHg, 24 months -0.4 (-1.4 to 0.7) mmHg; SGLT2i: 8 months -1.0 (-3.2 to 1.2) mmHg, 16 months -0.1 (-2.7 to 2.5) mmHg, 24 months +1.0 (-3.2 to 5.1) mmHg; interaction $P=0.91$.



Article 10

Albiglutide in patients with type 2 diabetes and heart failure: a post-hoc analysis from Harmony Outcomes

Ferreira JP, Sharma A, Vasques-Nóvoa F, Angélico-Gonçalves A, Leite AR, Borges-Canha M, Carvalho D, Packer M, Zannad F, Leite-Moreira A, Neves JS.

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Albiglutide in patients with type 2 diabetes and heart failure: a post-hoc analysis from Harmony Outcomes

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Aim

Glucagon-like peptide-1 receptor agonists (GLP1-RA) improve cardiovascular outcomes in patients with type 2 diabetes (T2D). However, some studies suggest that their effects in patients with heart failure (HF) may be attenuated. We aimed to explore the effects of the GLP1-RA albiglutide on HF outcomes in patients with and without HF history enrolled in the Harmony Outcomes trial.

Methods and results

Harmony Outcomes enrolled patients with T2D and cardiovascular disease randomized to either albiglutide or placebo over a median follow-up of 1.6 years. A total of 9462 patients were included, of whom 1922 (20%) had HF history. Patients with HF had more cardiovascular comorbidities, poorer renal function, and had a three to four-fold higher risk of HF events compared to patients without HF. Compared to placebo, the effect of albiglutide on the composite of cardiovascular death or HF hospitalization was more pronounced among patients without HF (hazard ratio [HR] 0.73, 95% confidence interval [CI] 0.56–0.95) than in patients with HF (HR 1.06, 95% CI 0.79–1.43) (interaction $p = 0.062$). A similar pattern was observed for HF hospitalizations (interaction $p = 0.025$). The effect of albiglutide on cardiovascular death, sudden death or 'pump failure' death, and all-cause mortality was also attenuated among patients with HF history, but without significant interaction ($p > 0.1$). The benefit of albiglutide to reduce atherosclerotic events was consistent regardless of HF history.

Conclusions

In patients with T2D and cardiovascular disease, albiglutide appeared to have no effect in reducing HF-related events among patients with HF history. These findings, placed in the context of other trials, suggest that GLP1-RA may not improve HF outcomes in patients with HF.

Keywords

Albiglutide • Glucagon-like peptide-1 receptor agonists • Type 2 diabetes • Heart failure

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Introduction

Glucagon-like peptide-1 receptor agonists (GLP1-RA) reduced the incidence of cardiovascular events in patients with type 2 diabetes (T2D) and constitute a preferential drug class for reducing the cardiovascular risk of patients with T2D.^{1,2}

Despite the robust evidence supporting the use of GLP1-RA to reduce the risk of atherosclerotic events in patients with T2D, the impact of GLP1-RA on heart failure (HF) outcomes is less well established.² Furthermore, the effect of GLP1-RA in patients with overt HF is uncertain.³ Dedicated outcome trials with GLP1-RA in HF are scarce, and those performed enrolled a few hundred of patients having limited power to study the effect of GLP1-RA on morbidity and mortality of HF patients.⁴ Moreover, among patients with HF and a reduced ejection fraction (HFrEF), GLP1-RA have no clear benefit and may even be harmful.^{5,6} Also, an analysis by HF subgroups from the EXSCeL (Exenatide Study of Cardiovascular Event Lowering) study suggested that the benefit of exenatide to reduce mortality and HF hospitalizations was present only in patients without prior HF, with no benefit (for any of the studied outcomes) among patients with HF.⁷ The existing evidence with GLP1-RA in patients with HF contrasts with that using sodium–glucose cotransporter 2 inhibitors (SGLT2i), a drug class that showed a consistent benefit to reduce HF hospitalizations in patients with T2D with or without established HF, regardless of ejection fraction.^{8–10}

Generating further evidence around the use of GLP1-RA in HF patients is extremely important since 10–20% of patients with T2D suffer from HF (and a high proportion may be undiagnosed) and 40–50% of patients with HF have T2D.^{11–13}

The present study aims to assess the effect of the GLP1-RA albiglutide according to HF history in patients with T2D and cardiovascular disease enrolled in the Harmony Outcomes (albiglutide and cardiovascular outcomes in patients with type 2 diabetes and cardiovascular disease) trial.¹⁴

Methods

Study design, participants, and procedures

The design and baseline characteristics of Harmony Outcomes have been previously published.¹⁵ In short, Harmony Outcomes was a double-blind, randomized, placebo-controlled, event-driven trial including patients with T2D with a glycated haemoglobin (HbA1c) above 7.0%, aged 40 years or older, with established cardiovascular disease (coronary: myocardial infarction [MI], at least 50% stenosis in one coronary artery or more, or previous coronary revascularization; cerebrovascular: ischaemic stroke, at least 50% carotid artery stenosis, or a previous carotid vascular procedure; and/or peripheral arterial: intermittent claudication and an ankle to brachial index <0.9, non-traumatic amputation, or a previous peripheral vascular procedure), randomized in a 1:1 ratio to either once a week subcutaneous injections of albiglutide or matching placebo. The starting dose of the study medication was 30 mg in 0.5 ml once a week, with possibility to increase to 50 mg once a week after 5 weeks if glucose-lowering intensification was required. Patients with an estimated glomerular

filtration rate (eGFR) <30 ml/min/1.73 m², previous pancreatitis or risk factors for pancreatitis were excluded from the trial.¹⁴

The protocol was approved by the ethics committee at each participating site and all patients provided written informed consent. An independent data and safety monitoring committee, with access to unblinded data, performed regular safety surveillance.

Outcomes

The primary outcome of Harmony Outcomes was a composite of cardiovascular death, MI, or stroke, analysed as time-to-first event according to the intention-to-treat principle.

The median follow-up time was 1.6 (25th–75th percentile 1.3–2.0) years.

In the present post-hoc analysis of Harmony Outcomes, the main outcome was a composite of cardiovascular death or HF hospitalization. Cardiovascular death, HF hospitalization (first and total), sudden death or HF ('pump failure') death, and all-cause death were also studied, as well as the addition of unstable angina to the composite of cardiovascular death, myocardial infarction, or stroke. All outcomes were studied in patients with and without HF history.

All outcomes were independently adjudicated by an adjudication committee blinded to treatment allocation. A HF hospitalization was adjudicated only when HF was the primary cause of hospitalization, lasting more than 24 h along with signs and symptoms of HF.¹⁴

Statistical analysis

The Harmony Outcomes population was divided into two subgroups according to prior HF history (with vs. without HF history), and their characteristics were described as medians (25th–75th percentile) for continuous variables and number (%) for categorical variables and compared through means of Mann–Whitney and Chi² tests, as appropriate. Normality testing was performed using Kolmogorov–Smirnov tests. The associations of HF history with outcomes were assessed using Cox models for time-to-first event analyses and negative binomial models for total HF event analysis, adjusting for age, sex, race, coronary artery disease (CAD), body mass index (BMI), systolic blood pressure (SBP), HbA1c, and eGFR. The choice of these covariates was based on their established association with HF and cardiovascular outcomes.¹⁶ The effect of albiglutide versus placebo was tested using Cox models for time-to-first event analyses and negative binomial models for total HF event analysis with treatment group as explanatory variable and HF history as stratification variable, with treatment-by-HF history interaction term in the model. The results are presented separately for patients with and without HF history, along with the respective interaction *p*-value. Analyses were performed according to the intention-to-treat principle and, additionally, including patients who received at least one dose of the study treatment. The Kaplan–Meier method was used to estimate event rates. The effect of treatment on body weight, heart rate, SBP, HbA1c, and eGFR over time, was studied using mixed random effects models with treatment, time, HF history, baseline value of the variable of interest, treatment-by-time, and treatment-by-HF history interaction terms in the model; random effects were set at the patient id level using an unstructured covariance matrix. Adverse events were studied among patients who received at least one dose of the study drug using logistic regression models with treatment group as explanatory variable and HF history as stratification variable, with treatment-by-HF history interaction term in the model. No data imputation was performed. No adjustment for multiplicity was performed

Table 1 Baseline patient characteristics by heart failure history status

Characteristic	HF	No HF	p-value
n	1922	7540	
Age, years	65.0 (58.0–70.0)	65.0 (58.0–70.0)	0.40
Women	671 (34.9%)	2223 (29.5%)	<0.001
NYHA class I/II	1635 (86.8%)	0	<0.001
Race			
Asian	88 (4.6%)	382 (5.1%)	<0.001
Hispanic	171 (8.9%)	1822 (24.2%)	
Black or African American	35 (1.8%)	190 (2.5%)	
White	1603 (83.4%)	4979 (66.0%)	
Other	25 (1.3%)	167 (2.2%)	
Region			
Asia Pacific	74 (3.9%)	302 (4.0%)	<0.001
Eastern Europe	930 (48.4%)	1117 (14.8%)	
Latin America	111 (5.8%)	1592 (21.1%)	
North America	283 (14.7%)	1661 (22.0%)	
Western Europe	524 (27.3%)	2868 (38.0%)	
Current smoker	258 (13.4%)	1230 (16.3%)	<0.001
CAD	1564 (81.4%)	5113 (67.8%)	<0.001
CABG	433 (22.5%)	1299 (17.2%)	<0.001
PAD	395 (20.6%)	1959 (26.0%)	<0.001
Cerebrovascular disease	499 (26.0%)	1843 (24.4%)	0.17
Atrial fibrillation/flutter	374 (19.5%)	468 (6.2%)	<0.001
Weight, kg	93.0 (81.0–107.2)	89.7 (78.0–102.8)	<0.001
BMI, kg/m ²	32.6 (29.0–37.0)	31.2 (28.0–35.2)	<0.001
Heart rate, bpm	72 (65–78)	72 (65–79)	0.82
Systolic BP, mmHg	134 (123–143)	135 (124–145)	0.005
Diastolic BP, mmHg	79 (70–85)	78 (70–83)	<0.001
HbA1c, %	8.5 (7.7–9.6)	8.3 (7.6–9.4)	<0.001
eGFR, ml/min/1.73 m ²	72 (56–89)	78 (62–95)	<0.001
eGFR <60 ml/min/1.73 m ²	593 (30.9%)	1564 (20.7%)	<0.001
Diabetic eye disease	615 (32.0%)	1322 (17.5%)	<0.001
Diabetes duration, years	12.4 (7.1–18.4)	13.0 (7.8–19.5)	<0.001
Insulin	1176 (61.2%)	4420 (58.6%)	0.041
Metformin	1309 (68.1%)	5659 (75.1%)	<0.001
DPP4i	231 (12.0%)	1206 (16.0%)	<0.001
Sulfonylureas	608 (31.6%)	2117 (28.1%)	0.002
SGLT2i	87 (4.5%)	488 (6.5%)	0.001
Thiazolidinediones	20 (1.0%)	174 (2.3%)	<0.001
ACEi/ARBs	1647 (85.7%)	5877 (77.9%)	<0.001
Beta-blockers	1572 (81.8%)	4738 (62.8%)	<0.001
MRAs	394 (20.5%)	414 (5.5%)	<0.001
CCBs	624 (32.5%)	2235 (29.6%)	0.016
Nitrates	417 (21.7%)	1138 (15.1%)	<0.001
Thiazides	425 (22.1%)	1701 (22.6%)	0.67
Loop diuretics	728 (37.9%)	1066 (14.1%)	<0.001
Statins	1692 (88.0%)	6263 (83.1%)	<0.001
Fibrates	124 (6.5%)	591 (7.8%)	0.040
Ezetimibe	78 (4.1%)	538 (7.1%)	<0.001
Antiplatelet agents	1521 (79.1%)	5769 (76.5%)	0.015
Anticoagulants	291 (15.1%)	506 (6.7%)	<0.001

Continuous variables are reported as medians (25th–75th percentile).

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; CABG, coronary artery bypass graft; CAD, coronary artery disease; CCB, calcium channel blocker; DPP4i, dipeptidyl peptidase 4 inhibitor; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HF, heart failure; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PAD, peripheral arterial disease; SGLT2i, sodium–glucose cotransporter 2 inhibitor.

given the exploratory nature of this work. A two-sided p -value <0.05 was considered statistically significant for main effects, and a two-sided p -value <0.10 was considered statistically significant for interaction tests, which have less statistical power. These statistical thresholds were used solely for data interpretation and hypothesis generation and were placed in the clinical context along with the work from others in the discussion. Analyses were performed using Stata® (StataCorp. 2021, Stata Statistical Software: release 17; StataCorp LLC, College Station, TX, USA).

Data access

All data packages were assessed through the Vivli® (Center for Global Clinical Research Data) platform with the Vivli ID: 00007609.

Results

Main report summary

As shown in the main report of Harmony Outcomes,¹⁴ in the overall population albiglutide reduced the composite of cardiovascular death, myocardial infarction, or stroke: 338/4731 (7%) events in the albiglutide group versus 428/4732 (9%) events in the placebo group (hazard ratio [HR] 0.78, 95% confidence interval [CI] 0.68–0.90, $p < 0.001$); and the composite of cardiovascular death, unstable angina, myocardial infarction, or stroke: 373/4731 (8%) events in the albiglutide group versus 468/4732 (10%) events in the placebo group (HR 0.78, 95% CI 0.69–0.90, $p = 0.001$). Albiglutide did not reduce the composite of cardiovascular death or HF hospitalization: 188/4731 (4%) events in the albiglutide group versus 218/4732 (5%) events in the placebo group (HR 0.85, 95% CI 0.70–1.04, $p = 0.11$); cardiovascular death: 122/4731 (3%) events in the

albiglutide group versus 130/4732 (3%) events in the placebo group (HR 0.93, 95% CI 0.73–1.19, $p = 0.58$); nor all-cause mortality: 196/4731 (4%) events in the albiglutide group versus 205/4732 (5%) events in the placebo group (HR 0.95, 95% CI 0.79–1.16, $p = 0.64$).

Patient characteristics by heart failure history status

From the 9462 patients randomized into the trial, 1922 (20.3%) had HF history. Compared to patients without HF history, those with HF were more frequently women (34.9% vs. 29.5%), White (83.4% vs. 66.0%), had more frequent history of CAD (81.4% vs. 67.8%), atrial fibrillation/flutter (19.5% vs. 6.2%), and diabetic eye disease (32% vs. 17.5%), had higher BMI (32.6 vs. 31.2 kg/m²), lower eGFR (72 vs. 78 ml/min/1.73 m²), and used angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEi/ARBs), beta-blockers, mineralocorticoid receptor antagonists (MRAs), loop diuretics and statins more frequently (all $p < 0.05$). Most patients with HF history were in New York Heart Association (NYHA) class I/II (86.8%) at the time of inclusion (Table 1). The patient's description with further splitting by randomized treatment is presented in online supplementary Table S1.

Association of heart failure history with events

Compared to patients without HF history, those with HF history had a higher risk of cardiovascular events and all-cause mortality. For example, the risk of cardiovascular death or HF hospitalization was 2.6-fold higher (HR 2.64, 95% CI 2.15–3.23), the risk of HF hospitalizations was 3.6-fold higher (HR 3.59, 95% CI 2.67–4.81),

Table 2 Associations of heart failure history with cardiovascular events and mortality

Event	HF, n (%) IR per 100 py	No HF, n (%) IR per 100 py	HR (95% CI) ^a p -value
Cardiovascular death or HF hospitalization	179/1922 (9.3) IR = 5.9	227/7540 (3.0) IR = 1.9	2.64 (2.15–3.23) $p < 0.001$
Cardiovascular death	96/1922 (5.0) IR = 3.1	156/7540 (2.1) IR = 1.3	2.11 (1.62–2.74) $p < 0.001$
Sudden death or 'pump failure' death	60/1922 (3.1) IR = 1.9	78/7540 (1.0) IR = 0.6	2.58 (1.82–3.65) $p < 0.001$
HF hospitalization (first)	103/1922 (5.4) IR = 3.4	87/7540 (1.2) IR = 0.7	3.59 (2.67–4.81) $p < 0.001$
HF hospitalizations (total) ^b	153/1922	106/7540	4.41 (3.17–6.15) $p < 0.001$
Cardiovascular death, MI, or stroke	246/1922 (12.8) IR = 8.3	512/7540 (6.8) IR = 4.4	1.66 (1.42–1.94) $p < 0.001$
Cardiovascular death, MI, unstable angina, or stroke	284/1922 (14.8) IR = 9.6	628/7540 (8.3) IR = 5.4	1.56 (1.35–1.80) $p < 0.001$
All-cause mortality	141/1922 (7.3) IR = 4.5	260/7540 (3.4) IR = 2.2	1.92 (1.55–2.37) $p < 0.001$

CI, confidence interval; HF, heart failure; HR, hazard ratio; IR, incidence rate; MI, myocardial infarction; py, person-years.

^aHR adjusted for age, sex, race, coronary artery disease, body mass index, systolic blood pressure, glycated haemoglobin, and estimated glomerular filtration rate.

^bTotal events and IR ratio obtained from a negative binomial model.

the risk of cardiovascular death, MI, or stroke was 1.7-fold higher (HR 1.66, 95% CI 1.42–1.94), and the risk of all-cause mortality was 1.9-fold higher (HR 1.92, 95%CI 1.55–2.37) (Table 2). The breakdown of event-rates by NYHA class is presented in online supplementary Table S2 and shows a gradual increase in risk with higher NYHA classes.

Effect of albiglutide on the study outcomes by heart failure history status

Compared to placebo, the effect of albiglutide on the composite of cardiovascular death or HF hospitalization was more pronounced

among patients without HF history (HR 0.73, 95% CI 0.56–0.95 in patients without HF history and HR 1.06, 95% CI 0.79–1.43 in patients with HF history, interaction $p = 0.062$). A similar pattern was observed for HF hospitalizations (HR 0.49, 95% CI 0.31–0.77 in patients without HF history and HR 0.97, 95% CI 0.66–1.42 in patients with HF history, interaction $p = 0.025$). The effect of albiglutide on cardiovascular death, sudden death or ‘pump failure’ death, and all-cause mortality also showed an attenuated effect among patients with HF history, although without significant treatment-by-HF history interaction (interaction $p > 0.1$). The benefit of albiglutide to reduce the composite of cardiovascular death, MI, or stroke (including the extended composite incorporating

Table 3 Effect of albiglutide versus placebo on the study outcomes by heart failure history

Outcome/HF history status	Albiglutide, n (%) IR per 100 py	Placebo, n (%) IR per 100 py	HR (95% CI)	Interaction p-value
Cardiovascular death or HF hospitalization				
No HF	97/3776 (2.6) IR = 1.6	130/3764 (3.5) IR = 2.2	0.73 (0.56–0.95)	0.062
HF	91/954 (9.5) IR = 6.1	88/968 (9.1) IR = 5.7	1.06 (0.79–1.43)	
Cardiovascular death				
No HF	74/3776 (2.0) IR = 1.2	82/3764 (2.2) IR = 1.4	0.89 (0.65–1.21)	0.54
HF	48/954 (5.0) IR = 3.1	48/968 (5.0) IR = 3.0	1.04 (0.70–1.55)	
Sudden death or ‘pump failure’ death				
No HF	32/3776 (0.8) IR = 0.5	46/3764 (1.2) IR = 0.8	0.69 (0.44–1.08)	0.32
HF	29/954 (3.0) IR = 1.9	31/968 (3.2) IR = 2.0	0.97 (0.58–1.61)	
HF hospitalization (first)				
No HF	29/3776 (0.8) IR = 0.5	58/3764 (1.5) IR = 1.0	0.49 (0.31–0.77)	0.025
HF	50/954 (5.2) IR = 3.3	53/968 (5.5) IR = 3.4	0.97 (0.66–1.42)	
HF hospitalizations (total) ^a				
No HF	33/3776	71/3764	0.47 (0.30–0.75)	0.096
HF	72/954	81/968	0.85 (0.51–1.40)	
Cardiovascular death, MI, or stroke				
No HF	234/3776 (6.2) IR = 4.0	278/3764 (7.4) IR = 4.8	0.82 (0.69–0.98)	0.30
HF	101/954 (10.6) IR = 6.8	145/968 (15.0) IR = 9.8	0.70 (0.54–0.90)	
Cardiovascular death, MI, unstable angina, or stroke				
No HF	282/3776 (7.5) IR = 4.8	346/3764 (9.2) IR = 6.0	0.80 (0.68–0.93)	0.64
HF	121/954 (12.7) IR = 8.2	163/968 (16.8) IR = 11.0	0.75 (0.59–0.94)	
All-cause mortality				
No HF	125/3776 (3.3) IR = 2.1	135/3764 (3.6) IR = 2.3	0.91 (0.71–1.16)	0.48
HF	71/954 (7.4) IR = 4.6	70/968 (7.2) IR = 4.4	1.06 (0.76–1.47)	

CI, confidence interval; HF, heart failure; HR, hazard ratio; IR, incidence rate; MI, myocardial infarction; py, person-years.

^aTotal events and IR ratio obtained from a negative binomial model.

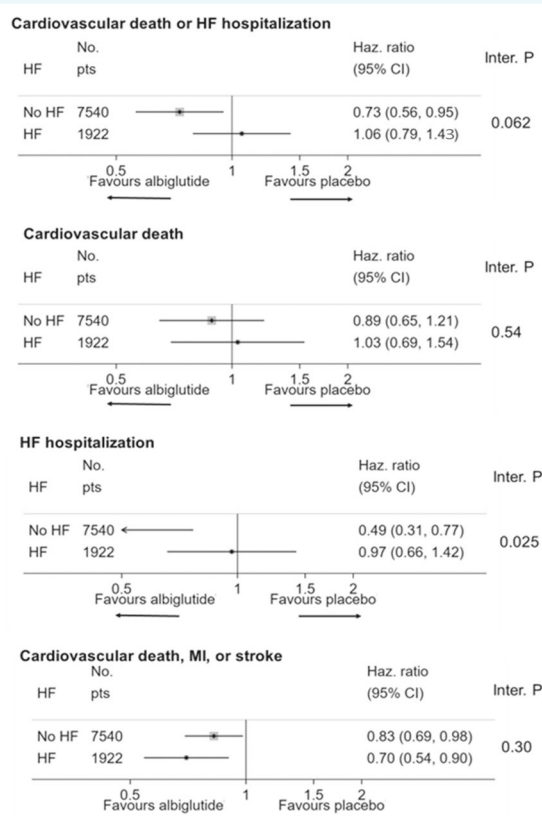


Figure 1 Forest plots: effect of albiglutide versus placebo by heart failure (HF) history status. CI, confidence interval; Inter., interaction; Haz., hazard; MI, myocardial infarction.

unstable angina) was consistent regardless of HF history (interaction $p > 0.1$) (Table 3, Figure 1 and online supplementary Figure S1 for Kaplan–Meier curves).

The treatment effect remained similar when considering only the patients who received at least one dose of study drug (online supplementary Table S3).

Effect of albiglutide on longitudinal measures by heart failure history status

On average, albiglutide increased heart rate by 1 to 2 bpm, decreased weight by 1 to 2 kg, and decreased HbA1c by 0.5% to 1%, without significant differences according to HF history. In addition, the effect of albiglutide on eGFR and SBP was of small magnitude without differences according to HF history (interaction $p > 0.1$ for all) (Figure 2).

Adverse events by heart failure history status

Overall, albiglutide was well-tolerated regardless of HF history status (Table 4). Permanent drug discontinuation was more frequent

among patients taking albiglutide, without difference by HF history (interaction $p = 0.73$). Incidence of pneumonia and atrial fibrillation/flutter might have been less frequent among patients with HF history taking albiglutide (interaction $p = 0.062$ and 0.072 , respectively) (Table 4).

Discussion

The present study shows that among almost 9500 patients with T2D and established cardiovascular disease, around 20% had HF history. These were more frequently women, had more frequent cardiovascular comorbidities (e.g. coronary artery disease, atrial fibrillation/flutter, diabetic eye disease), poorer renal function, and were more frequently treated with renin–angiotensin–aldosterone system inhibitors and loop diuretics. Patients with HF history were mildly symptomatic with the vast majority in NYHA classes I or II. Despite mildly symptomatic, compared to patients without HF history, those with HF had an increased risk of cardiovascular events and mortality, including a 3.6-fold higher risk of subsequent HF hospitalization, 2.6-fold higher risk of sudden or ‘pump failure’ death, 1.7-fold higher risk of cardiovascular death, MI or stroke, and 1.9-fold higher risk of death. Notwithstanding the high risk of events, particularly HF-related, albiglutide (compared to placebo) appeared to have no effect in reducing cardiovascular death and HF hospitalizations among patients with HF history, but appeared to reduce HF hospitalizations in patients without HF history, who had a low risk for such events (placebo group: 1.0 events per 100 person-years in patients without HF vs. 3.4 events per 100 person-years in patients with HF history), with a significant treatment-by-HF history interaction p -value of 0.025. Interestingly, the differences in treatment effect observed for HF outcomes contrast with the effect seen on atherosclerotic cardiovascular events such as MI, unstable angina, or stroke with a similar event reduction irrespective of HF history status. In addition, albiglutide reduced weight regardless of HF status. Overall, albiglutide was well tolerated without major differences between patients with and without HF history. These results are of clinical importance, because they suggest that in patients with T2D and HF history, GLP1-RA may not be the ideal drugs for reducing HF-related events, albeit that may still be considered to reduce atherosclerotic events regardless of HF. However, as the risk of HF-related events is highly increased in patients with concomitant T2D and HF, SGLT2i may constitute a better option for treating patients with T2D and HF, as SGLT2i have consistently shown to improve HF outcomes, including in patients with severe HF.^{8,9,17–19}

Our findings replicate to a great extent those from the EXSCCEL study in 14 752 patients with T2D with and without cardiovascular disease of whom 16% had HF history,⁷ where the effect of exenatide to reduce the composite of cardiovascular death or HF hospitalization and all-cause death or HF hospitalization was more pronounced among patients without HF history (HR 0.86, 95% CI 0.74–1.01 and HR 0.81, 95% CI 0.71–0.93, respectively) than in patients with HF history in whom the effect was neutral (HR 1.05, 95% CI 0.86–1.29 and HR 1.07, 95% CI 0.89–1.29; interaction

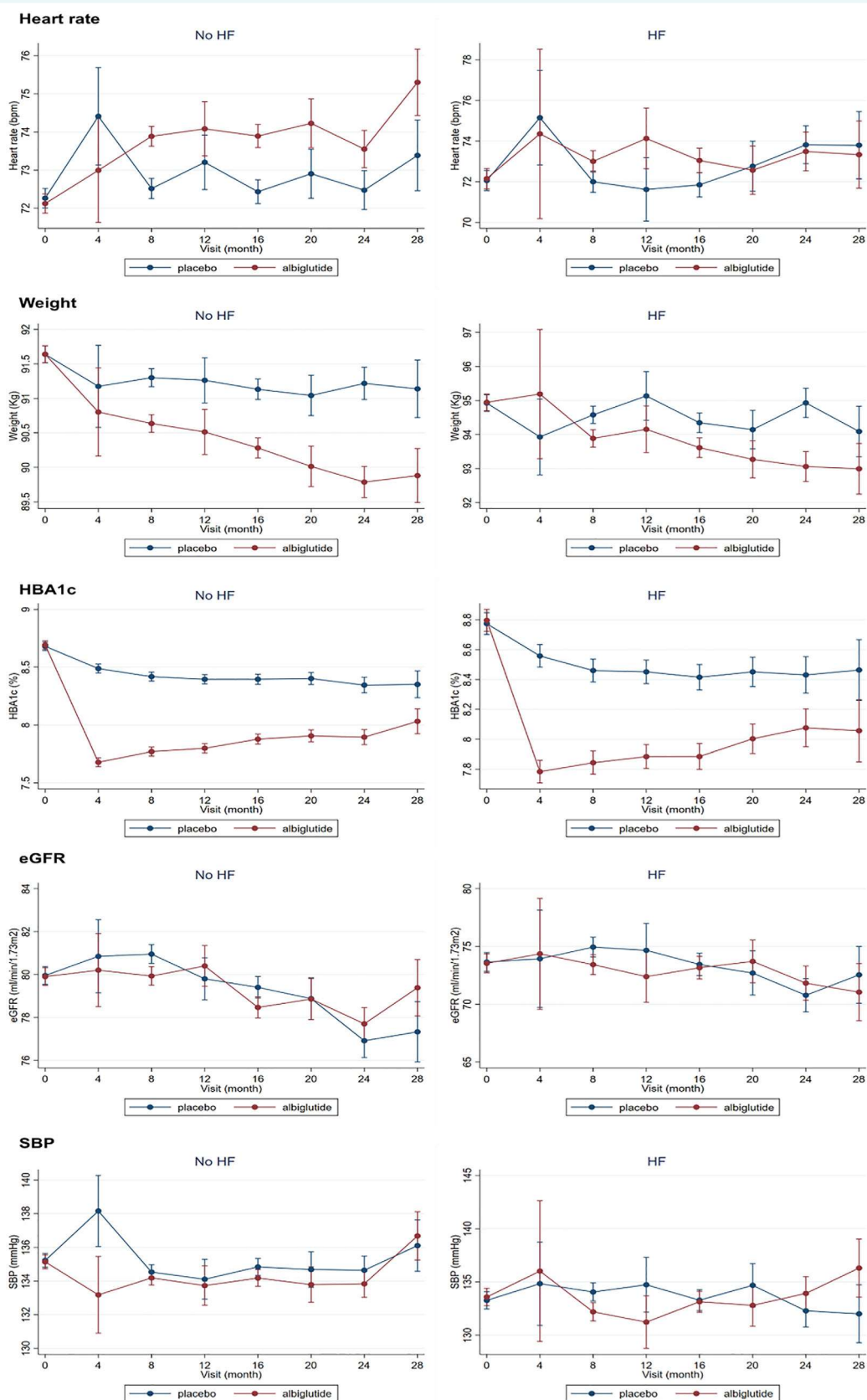


Figure 2 Legend on Next Page.

Table 4 Adverse events with albiglutide versus placebo by heart failure history status

AE/HF history status	Albiglutide, n (%)	Placebo, n (%)	OR (95% CI)	Interaction p-value
Serious adverse event (any)				0.52
No HF	756/3764 (20.1)	812/3749 (21.7)	0.91 (0.81–1.02)	
HF	264/952 (27.7)	302/966 (31.3)	0.84 (0.69–1.03)	
Adverse event leading to permanent drug discontinuation				0.73
No HF	324/3764 (8.6)	258/3749 (6.9)	1.27 (1.07–1.51)	
HF	105/952 (11.0)	81/966 (8.4)	1.35 (1.00–1.84)	
Severe hypoglycaemia				0.71
No HF	32/3764 (0.9)	47/3749 (1.3)	0.68 (0.43–1.06)	
HF	9/952 (0.9)	16/966 (1.7)	0.57 (0.25–1.29)	
Diabetic retinopathy				0.59
No HF	717/3764 (19.0)	699/3749 (18.6)	1.03 (0.91–1.15)	
HF	325/952 (34.1)	311/966 (32.2)	1.09 (0.90–1.32)	
Renal failure				0.12
No HF	170/3764 (4.5)	178/3749 (4.7)	0.95 (0.77–1.18)	
HF	72/952 (7.6)	101/966 (10.5)	0.70 (0.51–0.96)	
Hypersensitivity reaction				0.65
No HF	36/3764 (1.0)	40/3749 (1.1)	0.90 (0.57–1.41)	
HF	10/952 (1.1)	9/966 (0.9)	1.13 (0.46–2.79)	
Pneumonia				0.062
No HF	115/3764 (3.1)	104/3749 (2.8)	1.10 (0.84–1.45)	
HF	37/952 (3.9)	54/966 (5.6)	0.68 (0.45–1.05)	
Pancreatitis				0.80
No HF	13/3764 (0.3)	11/3749 (0.3)	1.18 (0.53–2.63)	
HF	11/952 (1.2)	11/966 (1.1)	1.01 (0.44–2.35)	
Atrial fibrillation/flutter (new onset)				0.072
No HF	57/3531 (1.6)	55/3518 (1.6)	1.03 (0.71–1.50)	
HF	21/767 (2.7)	37/778 (4.8)	0.56 (0.33–0.97)	

AE, adverse event; CI, confidence interval; HF, heart failure; OR, odds ratio.

$p = 0.13$ and $= 0.015$, respectively). The benefit of exenatide to reduce MI or stroke events was similar in patients with and without HF history.

Our findings also align with the results from the LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) trial in 9370 patients with T2D and a high cardiovascular risk of whom 18% had HF history,²⁰ where the effect of liraglutide to reduce the composite of cardiovascular death or HF hospitalization was more pronounced among patients

without HF history (HR 0.77, 95% CI 0.65–0.91) than in patients with HF history in whom the effect was neutral (HR 0.92, 95% CI 0.74–1.15; interaction $p = 0.19$). Again, the effect to reduce cardiovascular death, MI, or stroke was similar in patients with and without HF history.²¹

In the SUSTAIN-6 (Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes) trial, HF outcomes according to HF history status have not been reported yet; however, and in contrast with Harmony Outcomes, EXSCEL and LEADER, the

Figure 2 Effect of albiglutide on longitudinal measures by heart failure (HF) history status. Difference between albiglutide and placebo: (1) heart rate: No HF: 8 months +1.4 (1.0 to 1.7) bpm, 16 months +1.5 (1.0 to 1.9) bpm, 24 months +1.1 (0.4 to 1.8) bpm; HF: 8 months +1.0 (0.3 to 1.7) bpm, 16 months +1.2 (0.3 to 2.0) bpm, 24 months –0.3 (–1.6 to 1.0) bpm; interaction $p = 0.56$; (2) weight: No HF: 8 months –0.7 (–0.8 to –0.5) kg, 16 months –0.9 (–1.1 to –0.6) kg, 24 months –1.4 (–1.8 to –1.1) kg; HF: 8 months –0.7 (–1.1 to –0.3) kg, 16 months –0.7 (–1.1 to –0.3) kg, 24 months –1.9 (–2.5 to –1.3) kg; interaction $p = 0.92$; (3) HBA1c: No HF: 8 months –0.6 (–0.7 to –0.6)%, 16 months –0.5 (–0.6 to –0.5)%, 24 months –0.5 (–0.5 to –0.4)%; HF: 8 months –0.6 (–0.7 to –0.6)%, 16 months –0.5 (–0.7 to –0.4)%, 24 months –0.4 (–0.5 to –0.2)%; interaction $p = 0.86$; (4) eGFR: No HF: 8 months –1.0 (–1.6 to –0.4) ml/min/1.73 m², 16 months –0.9 (–1.6 to –0.2) ml/min/1.73 m², 24 months +0.8 (–0.3 to +1.9) ml/min/1.73 m²; HF: 8 months –1.5 (–2.7 to –0.3) ml/min/1.73 m², 16 months –0.3 (–1.7 to +1.1) ml/min/1.73 m², 24 months +1.1 (–1.0 to +3.1) ml/min/1.73 m²; interaction $p = 0.94$; (5) SBP: No HF: 8 months –0.3 (–1.0 to +0.3) mmHg, 16 months –0.7 (–1.3 to +0.4) mmHg, 24 months –0.8 (–1.9 to +0.4) mmHg; HF: 8 months –1.9 (–3.1 to –0.6) mmHg, 16 months –0.1 (–1.5 to +1.2) mmHg, 24 months +1.6 (–0.6 to +3.8) mmHg; interaction $p = 0.54$. eGFR, estimated glomerular filtration rate; HBA1c, glycated haemoglobin; SBP, systolic blood pressure.

effect of semaglutide to reduce the primary composite outcome of cardiovascular death, MI, or stroke was more pronounced in patients without HF history (HR 0.64, 95% CI 0.48–0.86) than in patients with HF history in whom the effect was neutral (HR 1.03, 95% CI 0.64–1.66; interaction $p = 0.09$).²² These findings were reinforced in the individual patient combined analysis of SUSTAIN-6 and PIONEER-6 (Oral Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes), where the HRs for the composite of cardiovascular death, MI, or stroke were <1.0 in all subgroups, except for those with HF history (HR 1.06, 95% CI 0.72–1.57; interaction $p = 0.046$). Moreover, in the combined trials semaglutide did not reduce HF hospitalizations (HR 1.03, 95% CI 0.75–1.40).²³

No HF outcomes by HF history status or HF subgroup analysis have been reported in AMPLITUDE-O (Cardiovascular and Renal Outcomes with Efglenatide in Type 2 Diabetes), with only 71 patients experiencing an HF event in this trial.²⁴

Together, these findings suggest that GLP1-RA do not improve HF-related outcomes among patients with HF history that is, those who are at highest risk for HF events.

While SGLT2i are effective for prevention and treatment of HF in patients with T2D, GLP1-RA may prevent the development of HF in T2D but do not reduce HF events in those with established HF. The mechanisms underlying the effect attenuation of GLP1-RA in patients with HF history are not well established, but they may be related to an increase in heart rate which may be particularly deleterious to HF patients.²⁵ Furthermore, the glucagon-like peptide-1 receptor, expressed in cardiomyocytes and sinoatrial node cells, signals through a cAMP-dependent pathway which may induce intracellular calcium overload that may be deleterious in HF patients, particularly those with advanced HFrEF (which were excluded from Harmony Outcomes).^{3,26,27} In this regard, in the FIGHT (Functional Impact of GLP-1 for Heart Failure Treatment) trial, liraglutide might have increased the risk of emergency department visit, HF hospitalization, or all-cause death compared to placebo (HR 1.36, 95% CI 0.99–1.85, $p = 0.05$).⁵ Still, it is important to highlight that patients with symptomatic HFrEF were most likely not included (or only in residual numbers) in T2D trials, where most patients with HF history were mildly symptomatic outpatients, likely with preserved ejection fraction. On the other hand, atherosclerotic events may be reduced regardless of HF history, because these may be more dependent on glycaemic control, weight reduction and improvements in metabolic profile, mechanisms that may also contribute to reduce the incidence of new-onset HF.²⁸

Limitations

Several limitations should be acknowledged in this study. First, this is a post-hoc analysis of a randomized trial without adjustment for test multiplicity and these findings should be regarded as exploratory. Second, details about HF were incomplete and not standardized across T2D trials. Third, natriuretic peptide values were not available and left ventricular ejection fraction or other echocardiographic parameters not reported. Fourth, in the main Harmony Outcomes publication,¹⁴ a total of 9463 patients were

included in the intention-to-treat analysis, whereas in our dataset 9462 patients were available. Moreover, in the main Harmony Outcomes publication, 766 primary outcome events (composite of cardiovascular death, MI, or stroke) were reported, whereas we found 758 events. Numbers regarding HF and mortality outcomes, and all estimates of treatment effects were similar in the two datasets. Fifth, only 13% ($n = 287$) patients had reported NYHA class III HF, which does not allow to provide reliable estimates within this subgroup. Most patients with HF history were White and these findings cannot be generalized to other ethnicities. Discontinuation of concomitant treatments (such as ACEi/ARBs and beta-blockers) was not consistently reported throughout the follow-up; thus, we cannot speculate about their potential association with HF risk. Finally, the extensive replication of the results suggests that there is a true attenuation of GLP1-RA effect among patients with HF, particularly regarding HF-related outcomes; still, dedicated large-scale outcome trials with GLP1-RA in patients with HF are warranted.

Conclusions

In patients with T2D and cardiovascular disease enrolled in Harmony Outcomes, albiglutide appeared to reduce HF-related events among patients without HF history but not among those with HF history in whom the effect was neutral. These findings placed in the context of other trials suggest that GLP1-RA may not improve HF outcomes in patients with HF, although they may be used to reduce atherosclerotic events regardless of HF history. Dedicated trials with GLP1-RA in HF patients are necessary.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplemental Material

Supplemental Table 1. Patient's characteristics by heart failure history status and randomized treatment

Characteristic	HF		No HF	
	Placebo	Albiglutide	Placebo	Albiglutide
N.	968	954	3764	3776
Age, years	65.0 (58.0, 70.0)	65.0 (59.0, 71.0)	65.0 (58.0, 70.0)	64.0 (58.0, 70.0)
Women	352 (36.4%)	319 (33.4%)	1115 (29.6%)	1108 (29.3%)
NYHA I/II	820 (86.3%)	815 (87.4%)	0 (0.0%)	0 (0.0%)
Asian	42 (4.3%)	46 (4.8%)	200 (5.3%)	182 (4.8%)
Hispanic	93 (9.6%)	78 (8.2%)	895 (23.8%)	927 (24.5%)
Black or African American	21 (2.2%)	14 (1.5%)	93 (2.5%)	97 (2.6%)
White	798 (82.4%)	805 (84.4%)	2490 (66.2%)	2489 (65.9%)
Other	14 (1.4%)	11 (1.2%)	86 (2.3%)	81 (2.1%)
Asia Pacific	33 (3.4%)	41 (4.3%)	158 (4.2%)	144 (3.8%)
Eastern Europe	464 (47.9%)	466 (48.8%)	546 (14.5%)	571 (15.1%)
Latin America	62 (6.4%)	49 (5.1%)	783 (20.8%)	809 (21.4%)
North America	152 (15.7%)	131 (13.7%)	826 (21.9%)	835 (22.1%)
Western Europe	257 (26.5%)	267 (28.0%)	1451 (38.5%)	1417 (37.5%)
Current Smoker	125 (12.9%)	133 (13.9%)	626 (16.6%)	604 (16.0%)
CAD	790 (81.6%)	774 (81.1%)	2555 (67.9%)	2558 (67.7%)
CABG	208 (21.5%)	225 (23.6%)	634 (16.8%)	665 (17.6%)
PAD	192 (19.8%)	203 (21.3%)	967 (25.7%)	992 (26.3%)
Cerebrovascular disease	250 (25.8%)	249 (26.1%)	921 (24.5%)	922 (24.4%)
Atrial fibrillation/flutter	188 (19.4%)	186 (19.5%)	233 (6.2%)	235 (6.2%)
Weight, kg	92.3 (80.9, 105.6)	93.9 (81.9, 109.0)	89.9 (78.2, 103.0)	89.5 (78.0, 102.2)
BMI, Kg/m ²	32.4 (29.0, 36.7)	32.7 (29.1, 37.1)	31.2 (28.0, 35.2)	31.2 (28.0, 35.1)
Heart rate, bpm	72.0 (65.0, 78.0)	72.0 (66.0, 79.0)	72.0 (65.0, 79.0)	72.0 (65.0, 79.0)
Systolic BP, mmHg	134.0 (122.0, 143.0)	134.0 (123.0, 144.0)	135.0 (124.0, 145.0)	134.0 (124.0, 145.0)
Diastolic BP, mmHg	78.0 (70.0, 85.0)	79.0 (70.0, 85.0)	78.0 (70.0, 83.0)	77.0 (70.0, 83.0)
HbA1c, %	8.4 (7.7, 9.5)	8.5 (7.7, 9.6)	8.3 (7.6, 9.4)	8.3 (7.6, 9.5)
eGFR, ml/min/1.73m ²	72.0 (57.0, 88.0)	71.0 (55.0, 89.0)	79.0 (62.0, 95.0)	78.0 (63.0, 95.0)
eGFR <60 ml/min/1.73m ²	286 (29.5%)	307 (32.2%)	806 (21.4%)	758 (20.1%)
Diabetic eye disease	300 (31.0%)	315 (33.0%)	655 (17.4%)	667 (17.7%)
Diabetes duration, years	12.2 (6.9, 18.1)	12.6 (7.4, 18.7)	13.1 (7.8, 19.7)	13.0 (7.9, 19.3)
Insulin	565 (58.4%)	611 (64.0%)	2172 (57.7%)	2248 (59.5%)
Metformin	658 (68.0%)	651 (68.2%)	2848 (75.7%)	2811 (74.4%)
DPP4i	115 (11.9%)	116 (12.2%)	624 (16.6%)	582 (15.4%)

Sulfonylureas	325 (33.6%)	283 (29.7%)	1054 (28.0%)	1063 (28.2%)
SGLT2i	37 (3.8%)	50 (5.2%)	228 (6.1%)	260 (6.9%)
Thiazolidinediones	13 (1.3%)	7 (0.7%)	89 (2.4%)	85 (2.3%)
ACEi/ARBs	830 (85.7%)	817 (85.6%)	2937 (78.0%)	2940 (77.9%)
Beta-blockers	806 (83.3%)	766 (80.3%)	2376 (63.1%)	2362 (62.6%)
MRAs	188 (19.4%)	206 (21.6%)	207 (5.5%)	207 (5.5%)
CCBs	308 (31.8%)	316 (33.1%)	1123 (29.8%)	1112 (29.4%)
Nitrates	222 (22.9%)	195 (20.4%)	568 (15.1%)	570 (15.1%)
Thiazides	200 (20.7%)	225 (23.6%)	837 (22.2%)	864 (22.9%)
Loop diuretics	378 (39.0%)	350 (36.7%)	521 (13.8%)	545 (14.4%)
Statins	851 (87.9%)	841 (88.2%)	3137 (83.3%)	3126 (82.8%)
Fibrates	62 (6.4%)	62 (6.5%)	283 (7.5%)	308 (8.2%)
Ezetimibe	36 (3.7%)	42 (4.4%)	255 (6.8%)	283 (7.5%)
Antiplatelet agents	768 (79.3%)	753 (78.9%)	2871 (76.3%)	2898 (76.7%)
Anticoagulants	147 (15.2%)	144 (15.1%)	267 (7.1%)	239 (6.3%)

Legend: CAD, coronary artery disease; CABG, coronary artery bypass grafting; PAD, peripheral artery disease; BMI, body mass index; HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate; DPP4i, dipeptidyl peptidase 4 inhibitors; SGLT2i, sodium glucose co-transporter 2 inhibitors; ACEi/ARBs, angiotensin converting enzyme inhibitors/angiotensin receptor blockers; MRAs, mineralocorticoid receptor antagonists; CCBs, calcium channel blockers.

Continuous variables are reported as medians and respective 25th to 75th percentiles.

Supplemental Table 2. Associations of NYHA class with cardiovascular events and mortality

Event	NYHA III/IV n. (%) IR 100py	NYHA I/II n. (%) IR 100py	No HF n. (%) IR 100py	HR (95%CI) * P-value NYHA I/II vs. No HF	HR (95%CI) * P-value NYHA III/IV vs. No HF
Cardiovascular death or HF hospitalization	46/248 (18.5) IR=12.7	123/1635 (7.5) IR=4.7	227/7540 (3.0) IR=1.9	2.16 (1.72-2.70) P <0.001	5.08 (3.65-7.06) P <0.001
Cardiovascular death, MI, or stroke	51/248 (20.6) IR =14.4	184/1635 (11.3) IR=7.2	512/7540 (6.8) IR =4.4	1.46 (1.23-1.74) P <0.001	2.67 (1.98-3.60) P <0.001
All-cause mortality	33/248 (13.3) IR=8.5	103/1635 (6.3) IR=3.8	260/7540 (3.4) IR =2.2	1.65 (1.31-2.09) P <0.001	3.63 (2.49-5.29) P <0.001

*Hazard ratio adjusted for age, sex, race, coronary artery disease, body mass index, systolic blood pressure, HbA1c, and eGFR.

Legend: IR, incidence-rate per 100 person-years; HF, heart failure; MI, myocardial infarction.

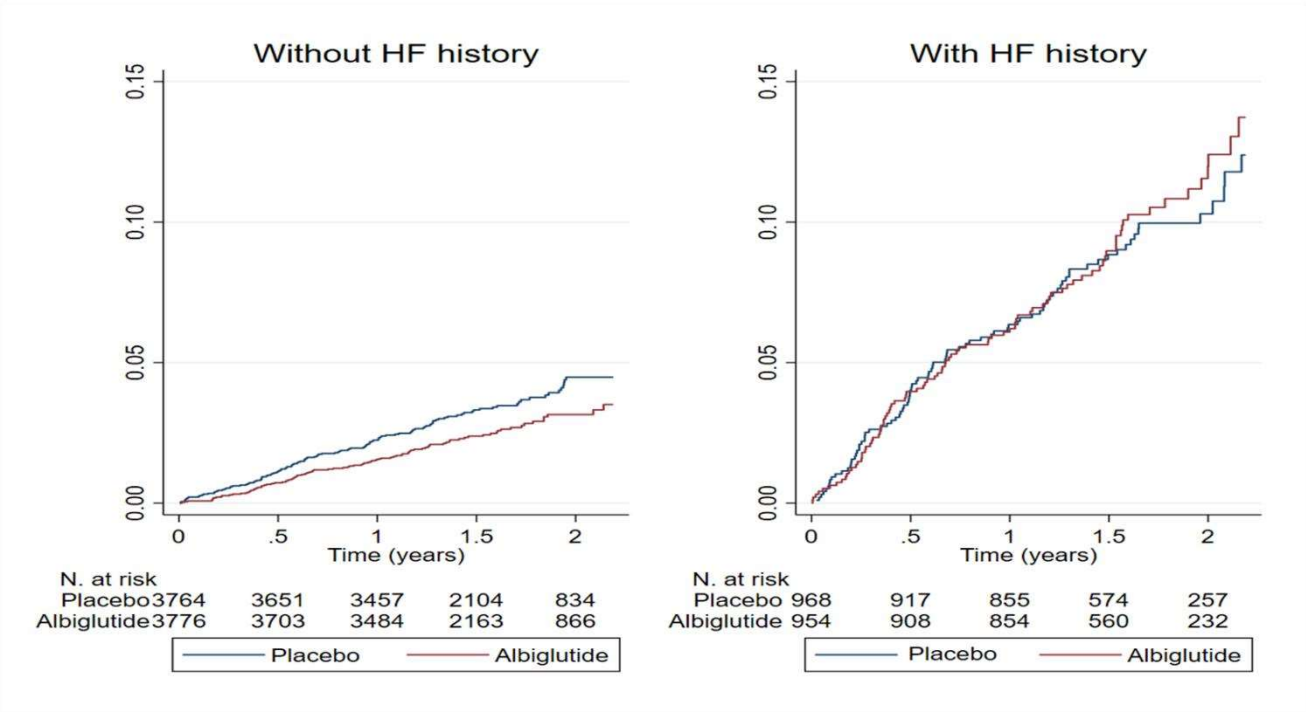
Supplemental Table 3. Effect of albiglutide vs. placebo on the study outcomes by HF history among patients who received at least one dose of the study drug

Outcome/HF history status	Albiglutide n. (%)	Placebo n. (%)	HR (95%CI)	Inter.P
Cardiovascular death or HF hospitalization				
No HF	97/3764 (2.6)	130/3749 (3.5)	0.73 (0.56-0.95)	0.063
HF	91/952 (9.6)	88/966 (9.1)	1.06 (0.79-1.42)	
Cardiovascular death				
No HF	74/3764 (2.0)	82/3749 (2.2)	0.89 (0.65-1.21)	0.54
HF	48/952 (5.0)	48/966 (5.0)	1.04 (0.70-1.55)	
HF hospitalization (first)				
No HF	29/3764 (0.8)	58/3749 (1.5)	0.49 (0.31-0.77)	0.025
HF	50/952 (5.3)	53/966 (5.5)	0.96 (0.66-1.42)	
Cardiovascular death, MI, or stroke				
No HF	234/3764 (6.2)	277/3749 (7.4)	0.83 (0.69-0.98)	0.29
HF	101/952 (10.6)	145/966 (15.0)	0.70 (0.54-0.90)	
All-cause mortality				
No HF	123/3764 (3.3)	135/3749 (3.6)	0.90 (0.70-1.14)	0.43
HF	71/952 (7.5)	70/966 (7.2)	1.06 (0.76-1.47)	

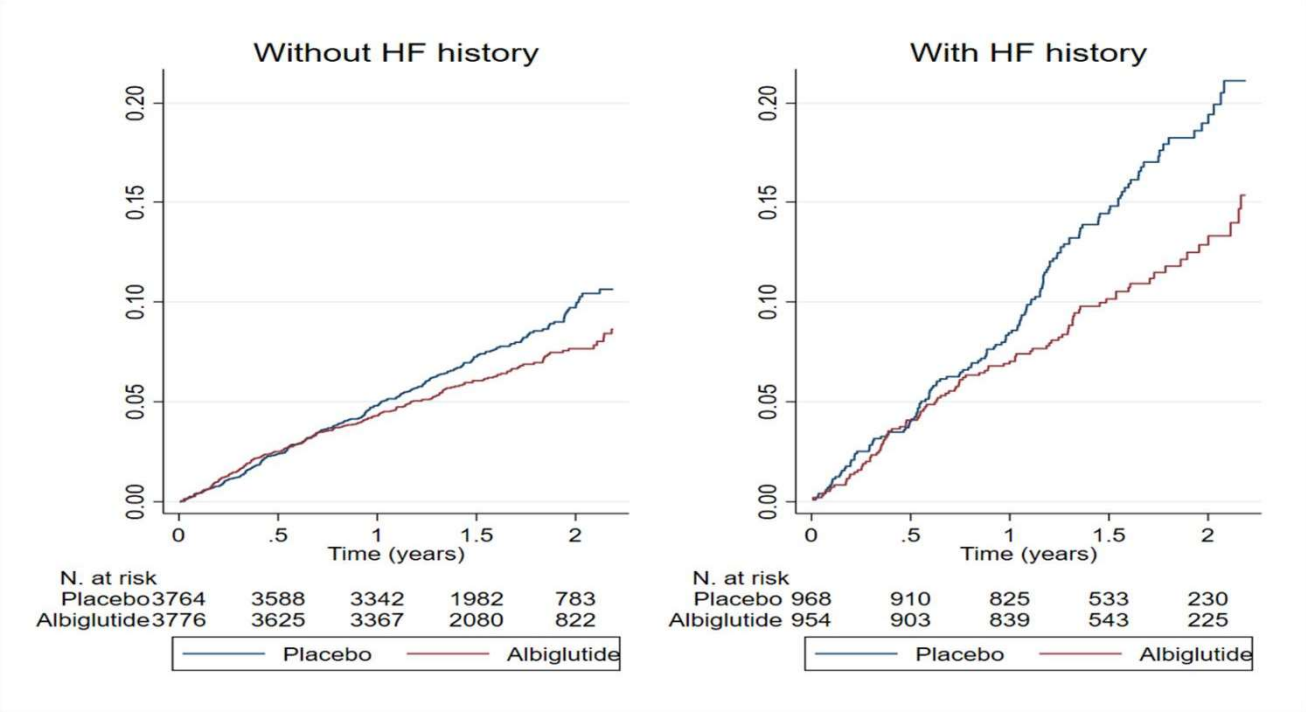
Legend: HF, heart failure; MI, myocardial infarction.

Supplemental Figure 1. Kaplan-Meier curves for the composite outcomes of cardiovascular death or heart failure hospitalization and cardiovascular death, myocardial infarction, or stroke

Cardiovascular death or HF hospitalization



Cardiovascular death, MI or Stroke



Legend: HF, heart failure; MI, myocardial infarction.



Article 11

Glucagon-like peptide 1 receptor agonists in patients with type 2 diabetes with and without heart failure: a meta-analysis of randomized placebo-controlled outcome trials

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ORIGINAL ARTICLE

Glucagon-like peptide 1 receptor agonists in patients with type 2 diabetes with and without chronic heart failure: A meta-analysis of randomized placebo-controlled outcome trials

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Abstract

Aim: Glucagon-like peptide 1 receptor agonists (GLP1-RA) reduce atherosclerotic events in patients with type 2 diabetes (T2D) and a high cardiovascular risk. The effect of GLP1-RA to reduce heart failure (HF) has been inconsistent across T2D trials, and individual trials were underpowered to assess the effect of GLP1-RA according to HF history. In this meta-analysis we aim to assess the effect of GLP1-RA in patients with and without HF history in stable ambulatory patients with T2D.

Methods: Random-effects meta-analysis of placebo-controlled trials. The hazard ratio (HR) and 95% confidence intervals (95% CI) were extracted from the treatment effect estimates of HF subgroup analyses reported in each individual study. The primary outcome was a composite of HF hospitalization or cardiovascular death.

Results: In total, 54 092 patients with T2D from seven randomized controlled trials were included, of whom 8460 (16%) had HF history. Compared with placebo, GLP1-RA did not reduce the composite of HF hospitalization or cardiovascular death in patients with HF history: HR 0.96, 95% CI: 0.84-1.08, but reduced this outcome in patients without HF history: HR 0.84, 95% CI: 0.76-0.92. GLP1-RA did not reduce all-cause death in patients with HF history: HR 0.98, 95% CI: 0.86-1.11, but reduced mortality in patients without HF history: HR 0.85, 95% CI: 0.79-0.92. GLP1-RA reduced atherosclerotic events regardless of HF history: HR 0.85, 95% CI: 0.75-0.97 with HF, and HR 0.88, 95% CI: 0.83-0.93 without HF.

Conclusions: Treatment with GLP1-RA did not reduce HF hospitalizations and mortality in patients with concomitant T2D and HF, but may prevent new-onset HF and mortality in patients with T2D without HF. The reduction of atherosclerotic events with GLP1-RA was not influenced by HF history status.

KEYWORDS

glucagon-like peptide 1 receptor agonists, heart failure, meta-analysis, treatment effects

1 | INTRODUCTION

Glucagon-like peptide 1 receptor agonists (GLP1-RA) reduce atherosclerotic events in patients with type 2 diabetes (T2D) and a high cardiovascular risk.¹⁻³ The effect of GLP1-RA to reduce heart failure (HF) events has been modest and inconsistent across T2D trials.⁴

Small trials in patients with HF with reduced ejection fraction (HFrEF) with and without T2D, suggest that GLP1-RA may be harmful to these patients because of a potential increase in HF hospitalizations and ventricular arrhythmias.^{5,6} Still, patients with HFrEF have been largely excluded from T2D trials, where only a minority of patients with mildly symptomatic stable HF and a presumably preserved EF (HFpEF) were included.⁷

Patients with HF represent between 10% and 20% of the population in T2D trials with GLP1-RA⁸⁻¹⁵; thus, each individual trial was underpowered to assess the effect of GLP1-RA in patients with HF. A meta-analysis of large outcome randomized controlled trials (RCTs) provides more power to study the effect of GLP1-RA among patients with T2D with and without HF history.

The aim of this meta-analysis is to study the effect of GLP1-RA versus placebo on HF outcomes in stable ambulatory patients with T2D according to HF history.

2 | METHODS

2.1 | Search strategy and selection criteria

This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guideline.¹⁶ We defined both MeSH terms (controlled language) and free text terms to express each component of PICO expression: (P) Population, stable ambulatory patients with T2D; (I) Intervention, GLP1-RA; (C) Comparison, placebo and (O) Outcomes, cardiovascular events. The PubMed search query included the following and it was last searched on 8 September 2022: ("diabetes mellitus" [Mesh Terms] OR "type 2 diabetes") AND ("Glucagon-Like Peptide 1" [MeSH Terms] OR GLP1RA OR "glucagon-like peptide 1 receptor agonists") AND ("placebos" [MeSH Terms] OR placebo) AND ("randomized controlled trial" [Publication Type] OR "randomized controlled trial" OR random × [Title/Abstract]) AND ("cardiovascular death" OR "cardiovascular events" OR death) NOT (("meta-analysis" [Publication Type]) OR ("review" [Publication Type])). We did not restrict the search by language, date or publication status. Sixty-five studies were initially identified, of which 58 were excluded because of inadequate study design, outcomes, or topic of interest, as we were only interested in large outcome placebo-controlled RCTs. Then, we have assessed whether the identified trials displayed subgroup analyses in patients with and without HF history, resulting in seven selected RCTs: (a) Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER; NCT01179048)^{9,17}; (b) Trial to Evaluate Cardiovascular and Other Long-term Outcomes With Semaglutide in Subjects With Type 2

Diabetes (SUSTAIN-6; NCT01720446)^{10,18}; (c) Exenatide Study of Cardiovascular Event Lowering Trial (EXSCeL; NCT01144338)^{7,11}; (d) Effect of Albiglutide, When Added to Standard Blood Glucose Lowering Therapies, on Major Cardiovascular Events in Subjects With Type 2 Diabetes Mellitus (Harmony Outcomes; NCT02465515)^{12,19}; (e) Researching Cardiovascular Events With a Weekly Incretin in Diabetes (REWIND; NCT01394952)^{13,20}; (f) A Trial Investigating the Cardiovascular Safety of Oral Semaglutide in Subjects With Type 2 Diabetes (PIONEER-6; NCT02692716)^{14,18}; and (g) A Study to Evaluate Cardiovascular Outcomes in Patients With Type 2 Diabetes Treated With ITCA 650 (FREEDOM; NCT01455896).²¹ Trial eligibility was confirmed by two independent reviewers (J.P.F., F.A.S.), data extraction was performed by J.P.F., and the study quality assessment was performed by J.P.F., F.A.S. and J.S.N. In addition, the risk of bias in randomized trials was assessed with the RoB 2 tool.²² We have also cross-checked the RCTs selected to the present analysis with other recently published meta-analyses and the agreement was perfect.¹⁻³ We have completed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.

The Effect of Efglenatide on Cardiovascular Outcomes (AMPLITUDE-O; NCT03496298) trial¹⁵ did not report treatment effects in HF subgroups and was not included in this analysis. Moreover, only 71 first HF events were reported in AMPLITUDE-O, which would not weight much in the present meta-analysis.¹⁵

The Evaluation of Cardiovascular Outcomes in Patients with Type 2 Diabetes After Acute Coronary Syndrome During Treatment with Lixisenatide (ELIXA; NCT01147250)⁸ was an acute myocardial infarction trial in patients with T2D; thus, it was not included in our main analysis, which is focused on stable ambulatory patients with T2D. Still, HF outcomes were reported in ELIXA, and we display these in the Supporting Information.

A detailed description of each individual trial is provided in the respective main reports as referenced above.

The analyses were performed to assess whether the HF history could modify the GLP1-RA effect in outcomes in patients with and without HF history at baseline, that is, before any randomized treatment administration.

2.2 | Outcomes

The primary outcome in the present meta-analysis was a composite of first HF hospitalization or cardiovascular death stratified by HF status. Secondary outcomes included the individual components of the primary outcome (i.e. HF hospitalization and cardiovascular death), all-cause death, and composite of time-to-first of myocardial infarction, stroke, or cardiovascular death (MACE) stratified by HF status.

All the studied outcomes were independently adjudicated in the respective trials. In the REWIND trial, HF hospitalizations included Emergency Room visits for worsening HF. In the FREEDOM trial, MACE also included hospitalization for unstable angina.

The studied trials and the respective studied outcomes are displayed in Table S1.

TABLE 1 Main baseline characteristics of the patients in the studied trials

Characteristic	LEADER	SUSTAIN-6	EXSCEL	HARMONY	PIONEER-6	REWIND	FREEDOM
Total, N	9340	3297	14 752	9463	3183	9901	4156
GLP1-RA	Liraglutide	Semaglutide	Exenatide	Albiglutide	Semaglutide	Dulaglutide	Exenatide
HF history	1667 (18)	573 (17)	2389 (16)	1922 (20)	388 (12)	853 (9)	668 (16)
NYHA I/II	1439 (86)	–	2071 (87)	1635 (87)	–	–	–
Median F-U, year	3.8	2.1	3.2	1.6	1.3	5.4	1.3
Men	6003 (64)	2002 (61)	9149 (62)	6569 (69)	2176 (68)	5312 (54)	2631 (63)
Mean/median age, years	64	65	62	64	66	66	63
White race	7238 (77)	2736 (83)	11 175 (76)	6583 (70)	2300 (72)	7498 (76)	3822 (92)
Mean/median HbA1c, %	8.7	8.7	8.0	8.7	8.2	7.4	8.0
Established CVD	7598 (81)	2735 (83) ^a	10 781 (73)	9462 (100)	2695 (85) ^a	3114 (31)	3159 (76)
CKD	2307 (25)	939 (29)	3191 (22)	2157 (23)	856 (27)	2199 (22)	408 (10)
ACEi/ARB	7741 (83)	2753 (84)	11 788 (80)	7726 (82)	–	8068 (81)	3154 (76)
Beta-blocker	5181 (55)	1894 (57)	8211 (56)	6310 (67)	–	4511 (46)	2206 (53)
SGLT2i	0	5 (<1)	77 (<1)	575 (6)	305 (10)	–	–
Metformin	7144 (76)	2414 (73)	11 295 (77)	6969 (74)	2463 (77)	8037 (81)	3526 (85)
DPP4i	6 (<1)	5 (<1)	2203 (15)	1437 (15)	2 (<1)	564 (6)	–
Insulin	4169 (45)	1913 (58)	6836 (46)	5597 (59)	1930 (61)	2363 (24)	1474 (35)

Note: Results presented as n (%), unless otherwise specified.

Abbreviations: –, not reported; ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; CKD, chronic kidney disease or kidney impairment as displayed in each trial; CVD, cardiovascular disease; DPP4i, dipeptidyl peptidase 4 inhibitors; EXSCEL, Exenatide Study of Cardiovascular Event Lowering; FREEDOM, A Study to Evaluate Cardiovascular Outcomes in Patients With Type 2 Diabetes Treated With ITCA 650; F-U, follow-up; HARMONY, Albiglutide and Cardiovascular Outcomes in Patients With Type 2 Diabetes and Cardiovascular Disease; HbA1c, glycated haemoglobin; HF, heart failure; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome; NYHA, New York Heart Association Functional Class; PIONEER-6, Peptide Innovation for Early Diabetes Treatment; REWIND, Researching Cardiovascular Events with a Weekly Incretin in Diabetes; SGLT2i, sodium-glucose cotransporter 2 inhibitor; SUSTAIN-6, Trial to Evaluate Cardiovascular and Other Long Term Outcomes with Semaglutide in Subjects with Type 2 Diabetes.

^aEstablished cardiovascular disease or chronic kidney disease.

2.3 | Statistical analysis

A random-effect meta-analysis approach was used with heterogeneity assessed using the Cochran Q test statistic and Higgins and Thompson I^2 .²³ Heterogeneity was considered low, moderate or high if I^2 was <25%, 25%-75% or >75%, respectively. The hazard ratio (HR) and respective 95% confidence intervals (95% CI) were extracted from the treatment effect estimates of HF subgroup analyses reported in the publications of each individual study. Estimates from each study were combined by use of inverse variance-weighted averages of logarithmic HR in random-effects analysis. We assessed publication bias by visual inspection of funnel plots, with the ascertainment for potential asymmetry of published results by Egger's regression test and Duval and Tweedie's trim-and-fill method. The statistical analyses were performed using Stata[®] software version 17 (StataCorp LLC, College Station, TX, USA).

3 | RESULTS

3.1 | Patient's characteristics

In total, patients with T2D from seven RCTs were included in this analysis, of whom 8460 (16%) had HF history. SUSTAIN-6 (N = 3297),

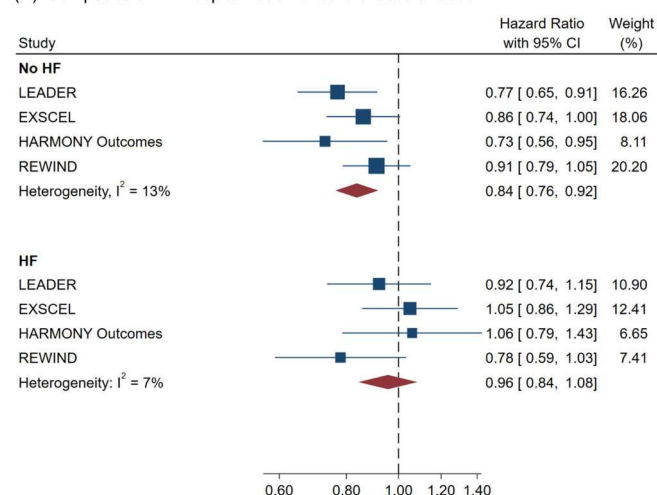
PIONEER-6 (N = 3183) and FREEDOM (N = 4156) reported MACE by HF history status but did not report HF hospitalizations or mortality by HF status; thus, for HF-related outcomes, in total, 43 456 patients were included (as available in LEADER, EXSCEL, Harmony Outcomes, and REWIND), of whom 6831 (16%) had HF history.

The distribution of patients with HF history at baseline was 1667 (18%) in LEADER, 2389 (16%) in EXSCEL, 1922 (20%) in HARMONY and 853 (9%) in REWIND, plus 573 (17%) in SUSTAIN-6, 388 (12%) in PIONEER-6 and 668 (16%) in FREEDOM. The median follow-up in years was: 3.8 in LEADER; 3.2 in EXSCEL; 1.6 in HARMONY; 5.4 in REWIND; 2.1 in SUSTAIN-6, 1.3 in PIONEER-6; and 1.3 in FREEDOM. The median age ranged from 62 years in EXSCEL to 66 years in REWIND. Most patients were White (>70%) and men (>60%). Nearly one-quarter of the patients had chronic kidney disease. The majority were treated with angiotensin-converting enzyme inhibitor/angiotensin receptor blockers and β -blockers, and only a small minority was treated with sodium glucose co-transporter 2 inhibitors (Table 1).

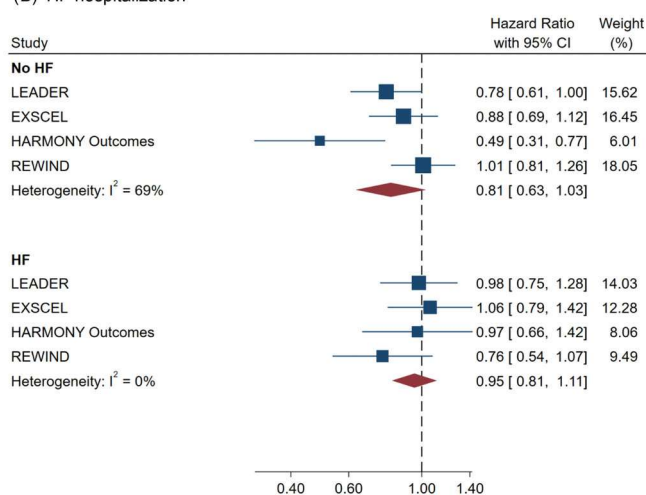
3.2 | Risk associated with history of heart failure

In the placebo group of the respective trials, compared with patients without HF history, those with HF had a $\approx$ 3-fold higher risk of

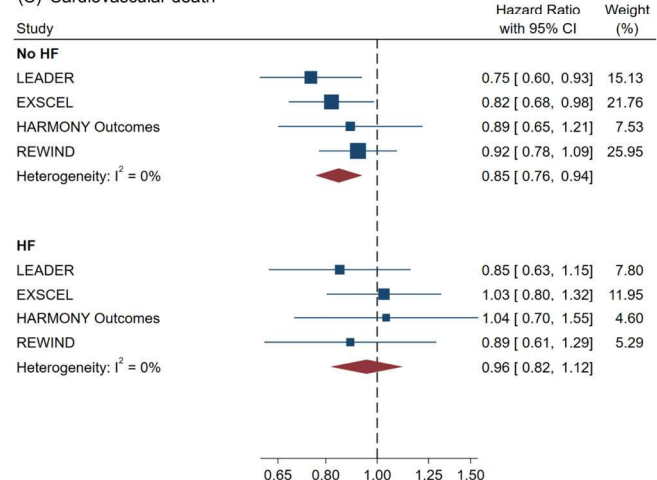
(A) Composite of HF hospitalization or cardiovascular death



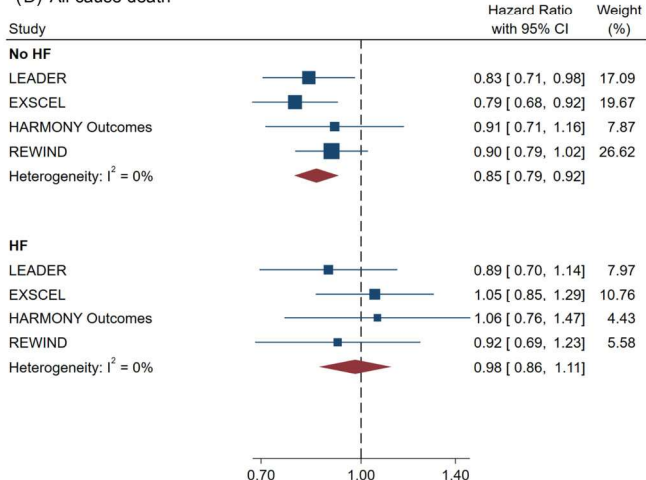
(B) HF hospitalization



(C) Cardiovascular death



(D) All-cause death



(E) MACE

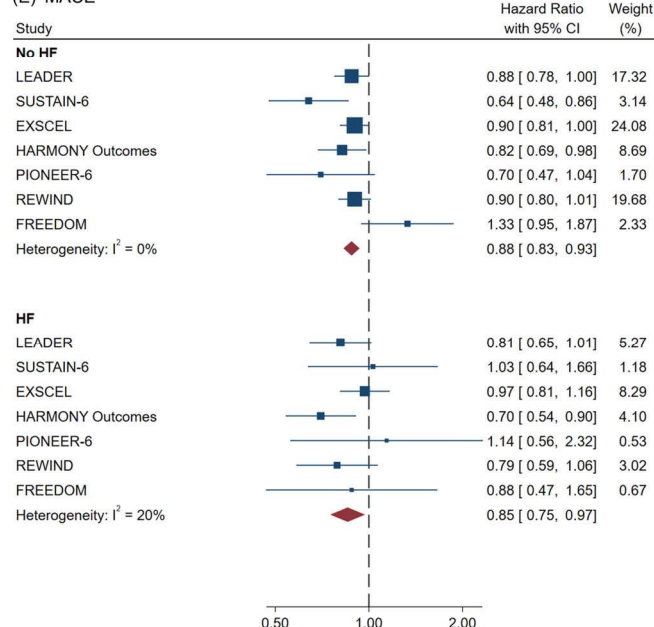


FIGURE 1 Legend on next page.

experiencing HF hospitalization or cardiovascular death, a ≈ 3 - to 5-fold higher risk of experiencing HF hospitalization, a ≈ 2 -fold higher risk of cardiovascular death and all-cause death, and a ≈ 1.5 - to 2-fold higher risk of experiencing a MACE event during the follow-up (Figure S1).

3.3 | Effect of glucagon-like peptide 1 receptor agonist versus placebo according to history of heart failure

The event number and treatment effects in each individual trial are displayed in the Table S2.

Compared with placebo, GLP1-RA did not reduce the composite of HF hospitalization or cardiovascular death in patients with HF history: HR 0.96, 95% CI: 0.84-1.08 ($I^2 = 7\%$) but reduced the composite of HF hospitalization or cardiovascular death in patients without HF history: HR 0.84, 95% CI: 0.76-0.92 ($I^2 = 13\%$); treatment-by-HF interaction, $p = .09$ (Figure 1A).

GLP1-RA did not reduce HF hospitalizations in patients with HF history: HR 0.95, 95% CI: 0.81-1.11 ($I^2 = 0\%$) but showed a tendency for reduction of HF hospitalizations in patients without HF history, despite moderate between-trial heterogeneity: HR 0.81, 95% CI: 0.63-1.03 ($I^2 = 69\%$); treatment-by-HF interaction, $p = .26$ (Figure 1B). Including the ELIXA trial in the analysis of HF hospitalizations did not modify the estimates as ELIXA had a neutral effect on HF hospitalizations in patients with and without HF history. (Figure S2).

GLP1-RA did not reduce cardiovascular death in the subgroup with HF history: HR 0.96, 95% CI: 0.82-1.12 ($I^2 = 0\%$) but reduced cardiovascular death in patients without HF history: HR 0.85, 95% CI: 0.76-0.94 ($I^2 = 0\%$); treatment-by-HF interaction, $p = .20$ (Figure 1C).

GLP1-RA did not reduce all-cause death in patients with HF history: HR 0.98, 95% CI: 0.86-1.11 ($I^2 = 0\%$) but did reduce all-cause death in patients without HF history: HR 0.85, 95% CI: 0.79-0.92 ($I^2 = 0\%$); treatment-by-HF interaction, $p = .07$ (Figure 1D).

GLP1-RA reduced MACE regardless of HF history: HR 0.85, 95% CI: 0.75-0.97 in patients with HF history ($I^2 = 20\%$) and HR 0.88, 95% CI: 0.83-0.93 in patients without HF history ($I^2 = 0\%$); treatment-by-HF interaction, $p = .69$ (Figure 1E).

The assessment of publication bias by funnel plots, Egger's regression test and trim-and-fill method support the absence of bias (Figure S3).

4 | DISCUSSION

The present meta-analysis, including 54 092 ambulatory patients with T2D of whom 8460 (16%) had HF history, showed that patients with HF history have a ≈ 3 -fold higher risk of being hospitalized for worsening HF or dying from cardiovascular causes compared with patients without HF history. However, compared with placebo, treatment with GLP1-RA did not reduce HF hospitalizations or cardiovascular death in patients with HF history (i.e. those with a high risk of HF-related events) but reduced HF hospitalizations or cardiovascular death (16% relative reduction) in patients without HF history (i.e. those with a low risk of HF-related events). Similarly, GLP1-RA did not reduce cardiovascular and all-cause death in patients with HF history but did reduce mortality among patients without HF history. The differences in GLP1-RA treatment effect by HF history on HF and mortality outcomes were not seen for atherosclerotic events, which were reduced irrespective of HF history status (with a relative reduction of $\approx 15\%$). These findings suggest that GLP1-RA may not reduce HF hospitalizations and mortality in patients with T2D and HF but may prevent new-onset HF and mortality in patients with T2D without HF. In addition, GLP1-RA may be considered to reduce atherosclerotic events irrespective of HF status.

These results are clinically relevant highlighting that in patients with T2D and no HF, treatment with GLP1-RA may prevent new-onset HF, reduce atherosclerotic events, and mortality. On the other hand, in patients with T2D and HF who are at high risk of subsequent HF hospitalizations and mortality, the use of GLP1-RA may not be useful to reduce such events.

These results contrast with those from sodium glucose co-transporter 2 inhibitors, which showed a marked reduction of HF-related events in patients with and without HF.²⁴⁻²⁷ Despite this meta-analysis not suggesting a harmful effect of GLP1-RA in patients with HF, it should be highlighted that patients with advanced HF, a recent HF decompensation or severely reduced left ventricular EF (LVEF) were excluded from these trials. Such patients with severely symptomatic HF, an reduced LVEF, and who had been recently hospitalized for worsening HF were included in the FIGHT trial (the Heart Failure Network Functional Impact of GLP-1 for Heart Failure Treatment) and randomized to either liraglutide or placebo (N = 300). In FIGHT, liraglutide might have increased the risk of emergency department visit, HF hospitalization, or all-cause death compared with placebo (HR 1.36, 95% CI: 0.99-1.85, $p = .05$).⁵ The LIVE trial (Effect of Liraglutide on Left Ventricular Function in Stable Chronic Heart Failure Patients with and without Diabetes), in which 241 patients with

FIGURE 1 Treatment effects according to HF history status. (A) Composite of HF hospitalization or cardiovascular death. (B) HF hospitalization. (C) Cardiovascular death. (D) All-cause death. (E) MACE. EXSCEL, Exenatide Study of Cardiovascular Event Lowering; HARMONY, Albiglutide and Cardiovascular Outcomes in Patients With Type 2 Diabetes and Cardiovascular Disease; HF, heart failure; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome; PIONEER-6, Peptide Innovation for Early Diabetes Treatment; REWIND, Researching Cardiovascular Events with a Weekly Incretin in Diabetes; SUSTAIN-6, Trial to Evaluate Cardiovascular and Other Long Term Outcomes with Semaglutide in Subjects with Type 2 Diabetes. Treatment-by-HF interaction p -value: (A) $p = .09$; (B) $p = .26$; (C) $p = .20$; (D) $p = .07$; (E) $p = .69$

stable HFrEF were enrolled, suggested an increased risk of adverse cardiac events with GLP1-RA. In particular, the risk of serious cardiac events was higher in the group treated with liraglutide as compared with placebo, which was driven by ventricular tachycardias and atrial fibrillation.⁶

The mechanisms underlying the GLP1-RA effect attenuation in patients with HF history are probably multifactorial. GLP1-RA are known to increase heart rate, which may be deleterious to patients with HF.²⁸ Furthermore, GLP1 receptors, expressed in cardiomyocytes and sinoatrial node cells, signal through a cyclic AMP-dependent pathway, which may induce calcium overload in the cardiomyocytes and may increase the risk of ventricular ectopy in patients at high risk.^{4,29,30} In fact, the effect of GLP1-RA may differ between HFrEF and HFpEF. Among the studies included in this meta-analysis, only EXSCeL reported LVEF. In EXSCeL LVEF was available in 33% ($n = 4892$) of the patients, and of these only 10% ($n = 469$) had an LVEF $<40\%$, that is, $\geq 90\%$ of the patients with echocardiographic data available had an EF of $\geq 40\%$.⁷ These findings from EXSCeL support the notion that most patients with HF included in T2D trials had a preserved LVEF. Given the central role of cyclic AMP-dependent pathways in the progression of HFrEF, it is plausible that the risk of adverse events with GLP1-RA may be higher in HFrEF than in HFpEF.³¹

Whether similar effects are observed with the dual glucose-dependent insulinotropic polypeptide and GLP-1 receptor agonist tirzepatide is uncertain. In this regard, the ongoing SUMMIT (A Study of Tirzepatide in Participants with Heart Failure with Preserved Ejection Fraction and Obesity; NCT04847557) trial will assess the effect of tirzepatide in 700 patients with HFpEF and obesity. Despite the clinical importance of this trial, it will have a short follow-up (52 weeks) and will be underpowered to assess the impact of tirzepatide on HF hospitalizations or mortality. Further large outcome RCTs should test the efficacy and safety of GLP1-RA in patients with HF across a wide range of EFs.

Some limitations should be acknowledged in this study. Differences in eligibility criteria, patient characteristics, baseline risk, HF prevalence, HF definition, trial duration and outcome definition may have influenced the treatment effects and contributed to some of the heterogeneity found in the meta-analysis. Particularly, the REWIND trial analysed patients with HF at baseline (9%, $n = 853$), but ignored patients having HF history (10%, $n = 915$). This explains the small proportion of patients with HF reported in REWIND, which was the lowest among all studied trials, reporting 15%-20% of patients with HF. The low proportion of patients considered as having HF in REWIND contributed to the low precision of the estimates observed in this HF subgroup and may have introduced bias in this analysis. The absence of data on LVEF and other measures of severity of HF for most RCTs is also a limitation of this analysis. We report treatment-by-HF interaction p values in the results section of this article; however, these tests lack statistical power and were not adjusted for multiplicity, and they should be interpreted with caution and not replace clinical judgement. The trials SUSTAIN-6, PIONEER-6 and FREEDOM did not report HF outcomes by HF history status. More than 70% of the patients included in the analysis were White, which

limits the generalizability of these results to other populations. The HF subgroups were not stratified at randomization and were imbalanced in the trials; therefore, these subgroup analyses do not portend the same level of evidence as a dedicated and adequately powered outcome RCT, which should be performed.

5 | CONCLUSIONS

Treatment with GLP1-RA did not reduce HF hospitalizations and mortality in patients with concomitant T2D and HF, but may prevent new-onset HF and mortality in patients with T2D but without HF. The reduction of atherosclerotic events with GLP1-RA was not influenced by HF history status.

AUTHOR CONTRIBUTIONS

JPF and JSN had the idea, conceptualized the study, supervised data collection, and wrote the first and subsequent drafts of the manuscript. All other authors provided critical input and edits to the manuscript.

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CONFLICT OF INTEREST

JPF is a consultant for Boehringer Ingelheim and AstraZeneca. He has received research support from Novartis, Boehringer Ingelheim, AstraZeneca and Bayer through his institution. MBC has received speaker fees from AstraZeneca. DC received consulting or speaker fees from AstraZeneca, BIAL; Boehringer Ingelheim, Eli-Lilly, Merck, Novo Nordisk, Servier. JSN has received consulting or speaker fees from AstraZeneca, BIAL, Boehringer Ingelheim, Lilly, Merck and Novo Nordisk. FZ reports personal fees from Boehringer Ingelheim, during the conduct of the study; personal fees from Janssen, Novartis, Boston Scientific, Amgen, CVRx, AstraZeneca, Vifor Fresenius, Cardior, Cereno Pharmaceutical, Applied Therapeutics, Merck, Bayer and, Cellprothera, other from CVCT, and Cardiorenal, outside the submitted work. MP reports personal fees from Boehringer Ingelheim, during the conduct of the study; personal fees from AbbVie, Actavis, Amgen, Amarin, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, CSL Behring, Cytokinetics, Johnson & Johnson, Eli Lilly & Company, Moderna, Novartis, ParatusRx, Pfizer, Relypsa, Salamandra, Synthetic Biologics, Theravance and Casana, outside the submitted work. MS and TI are employees of Boehringer Ingelheim. AS is supported by the McGill University Health Centre (MUHC) Foundation, Montreal General Hospital (MGH) Foundation, Sarah Louise King Award, Marjorie Cadham Award, Inez and Willena Beaton award, Fonds de Recherche Santé

Quebec (FRSQ) Junior 1 clinician scholars' program, Canada Institute for Health Research grant no. 175 095 and reports receiving support from the European Society of Cardiology young investigator grant, Roche Diagnostics, Boehringer-Ingelheim, Novartis and Takeda. All other authors have no conflicts of interest to declare.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/dom.14997>.

DATA AVAILABILITY STATEMENT

The data here presented are publicly available in the respective publications.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplementary Material

Supplementary Table 1. Analyzed trials and respective outcomes

Trial	Trial primary outcome	HF subgroup meta-analysis outcomes
LEADER	3P-MACE	HF hospitalization or CV death HF hospitalization CV death All-cause death 3P-MACE
SUSTAIN-6	3P-MACE	3P-MACE
EXSCEL	3P-MACE	HF hospitalization or CV death HF hospitalization CV death All-cause death 3P-MACE
HARMONY Outcomes	3P-MACE	HF hospitalization or CV death HF hospitalization CV death All-cause death 3P-MACE
PIONEER-6	3P-MACE	3P-MACE
REWIND*	3P-MACE	HF hospitalization or CV death HF hospitalization CV death All-cause death 3P-MACE
FREEDOM	4P-MACE	4P-MACE

*REWIND included visits to the emergency room for worsening HF as equivalent to HF hospitalizations.

Legend: 3P-MACE, 3-point major adverse cardiovascular events with a composite of time-to-first of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke; 4P-MACE major adverse cardiovascular events with a composite of time-to-first of death from cardiovascular causes, nonfatal myocardial infarction, nonfatal stroke, or unstable angina. HF, heart failure; CV, cardiovascular; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome; SUSTAIN-6, Trial to Evaluate Cardiovascular and Other Long Term Outcomes with Semaglutide in Subjects with Type 2 Diabetes; EXSCEL, Exenatide Study of Cardiovascular Event Lowering; HARMONY, Albiglutide and Cardiovascular Outcomes in Patients With Type 2 Diabetes and Cardiovascular Disease; PIONEER-6, Peptide Innovation for Early Diabetes Treatment; REWIND, Researching Cardiovascular Events with a Weekly Incretin in Diabetes; FREEDOM, A Study to Evaluate Cardiovascular Outcomes in Patients With Type 2 Diabetes Treated With ITCA 650.

Supplementary Table 2. Events and treatment effect by HF subgroups

Trial/outcome	HF	GLP1-RA		Placebo		HR (95%CI)
		Event	Non-event	Event	Non-event	
Heart failure hospitalization or cardiovascular death						
LEADER	No	235	3598	305	3535	0.77 (0.65-0.91)
	Yes	157	678	167	665	0.92 (0.74-1.15)
EXSCEL	No	287	5907	326	5842	0.86 (0.74-1.01)
	Yes	187	974	182	1046	1.05 (0.86-1.29)
HARMONY	No	97	3679	130	3634	0.73 (0.56-0.95)
	Yes	91	863	88	880	1.06 (0.79-1.43)
REWIND	No	369	4140	400	4100	0.91 (0.79-1.05)
	Yes	87	334	110	322	0.78 (0.59-1.03)
Heart failure hospitalization						
LEADER	No	110	3723	140	3700	0.78 (0.61-1.00)
	Yes	108	727	108	724	0.98 (0.75-1.28)
EXSCEL	No	129	6065	144	6024	0.88 (0.69-1.11)
	Yes	90	1071	87	1141	1.06 (0.79-1.42)
HARMONY	No	29	3747	58	3706	0.49 (0.31-0.77)
	Yes	50	904	53	915	0.97 (0.66-1.42)
REWIND	No	158	4351	155	4345	1.01 (0.81-1.26)
	Yes	55	366	71	361	0.76 (0.54-1.08)
Cardiovascular death						
LEADER	No	143	3690	190	3650	0.75 (0.60-0.93)
	Yes	76	759	88	744	0.85 (0.63-1.16)
EXSCEL	No	212	5982	256	5912	0.82 (0.68-0.98)
	Yes	128	1033	127	1101	1.03 (0.80-1.31)
HARMONY	No	74	3702	82	3682	0.89 (0.65-1.21)
	Yes	48	906	48	920	1.04 (0.70-1.55)
REWIND	No	263	4246	283	4217	0.92 (0.78-1.09)
	Yes	53	368	61	371	0.89 (0.61-1.28)
All-cause death						
LEADER	No	262	3571	315	3525	0.83 (0.70-0.97)
	Yes	119	716	132	700	0.89 (0.70-1.14)
EXSCEL	No	328	5866	410	5758	0.79 (0.68-0.92)
	Yes	179	982	174	1054	1.05 (0.85-1.29)
HARMONY	No	125	3651	135	3629	0.91 (0.71-1.16)
	Yes	71	883	70	898	1.06 (0.76-1.47)
REWIND	No	450	4059	495	4005	0.90 (0.79-1.02)
	Yes	84	337	93	339	0.92 (0.69-1.24)
MACE						
LEADER	No	466	3367	524	3316	0.88 (0.78-1.00)
	Yes	142	693	170	662	0.81 (0.65-1.02)
SUSTAIN-6	No	73	1290	112	1249	0.64 (0.48-0.86)
	Yes	35	250	34	254	1.03 (0.64-1.66)
EXSCEL	No	612	5582	668	5500	0.90 (0.81-1.00)
	Yes	227	934	237	991	0.97 (0.81-1.16)
HARMONY	No	234	3542	278	3486	0.82 (0.69-0.98)
	Yes	101	853	145	823	0.70 (0.54-0.90)

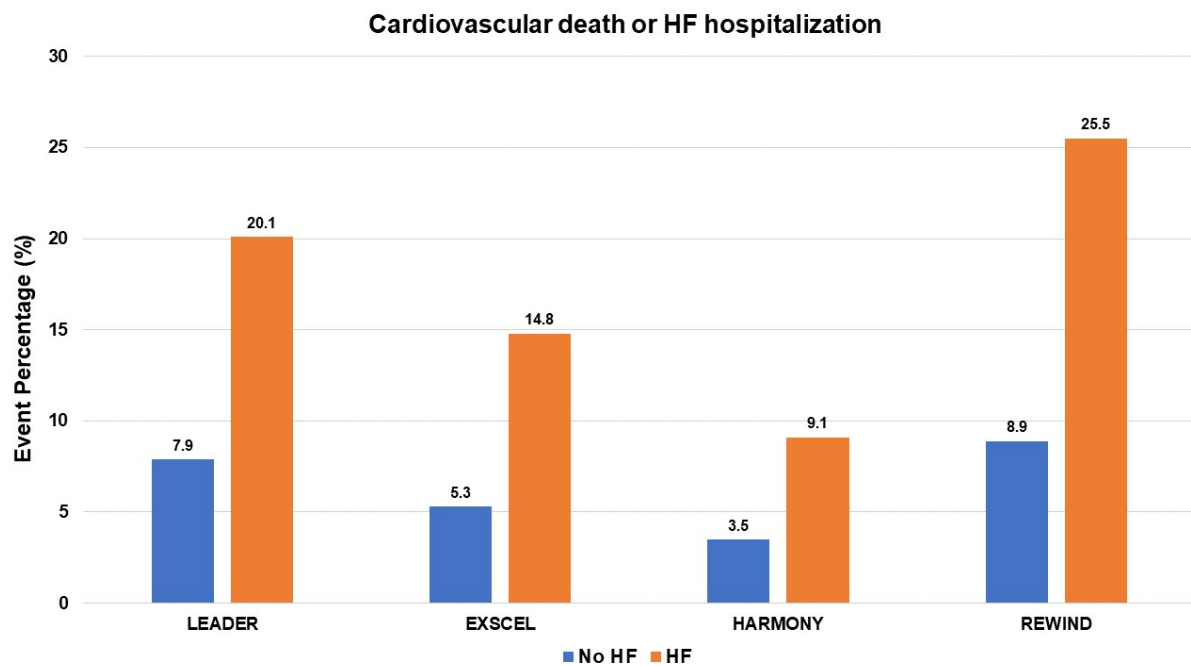
PIONEER-6	No	43	1341	60	1318	0.70 (0.47-1.04)
	Yes	16	172	15	185	1.14 (0.56-2.31)
REWIND	No	516	3993	563	3937	0.90 (0.80-1.01)
	Yes	76	345	96	336	0.79 (0.59-1.07)
FREEDOM	No	77	1673	58	1680	1.33 (0.95-1.87)
	Yes	18	307	21	322	0.88 (0.47-1.66)

Legend: MACE, major adverse cardiovascular events with a composite of time-to-first of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke. In the FREEDOM trial MACE also included hospitalization for unstable angina

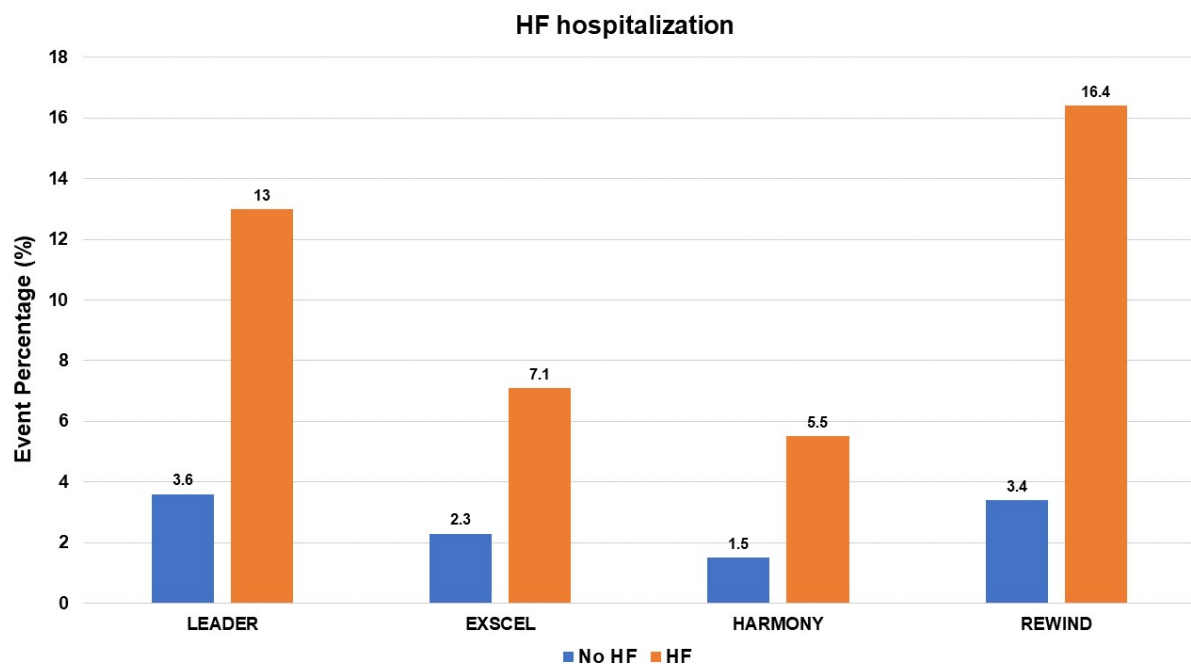
LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome; SUSTAIN-6, Trial to Evaluate Cardiovascular and Other Long Term Outcomes with Semaglutide in Subjects with Type 2 Diabetes; EXSCEL, Exenatide Study of Cardiovascular Event Lowering; HARMONY, Albiglutide and Cardiovascular Outcomes in Patients With Type 2 Diabetes and Cardiovascular Disease; PIONEER-6, Peptide Innovation for Early Diabetes Treatment; REWIND, Researching Cardiovascular Events with a Weekly Incretin in Diabetes; FREEDOM, A Study to Evaluate Cardiovascular Outcomes in Patients With Type 2 Diabetes Treated With ITCA 650.

Supplementary Figure 1. Proportion of events in the placebo group of the studied trials

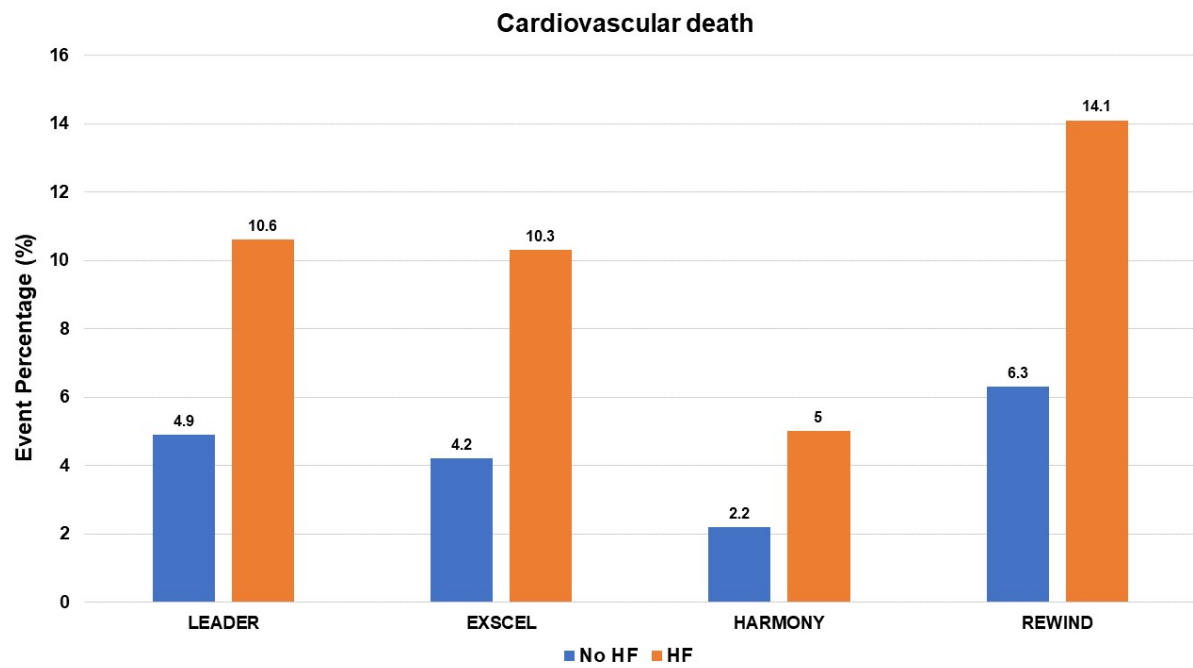
A) Composite of HF hospitalization or cardiovascular death



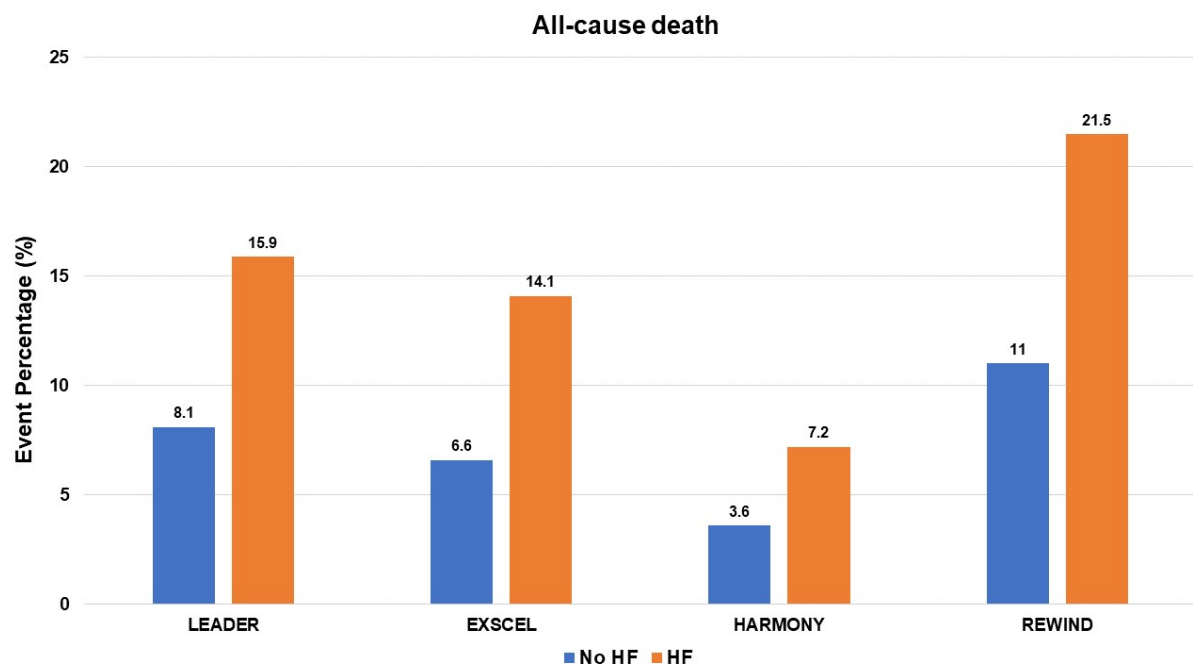
B) HF hospitalization



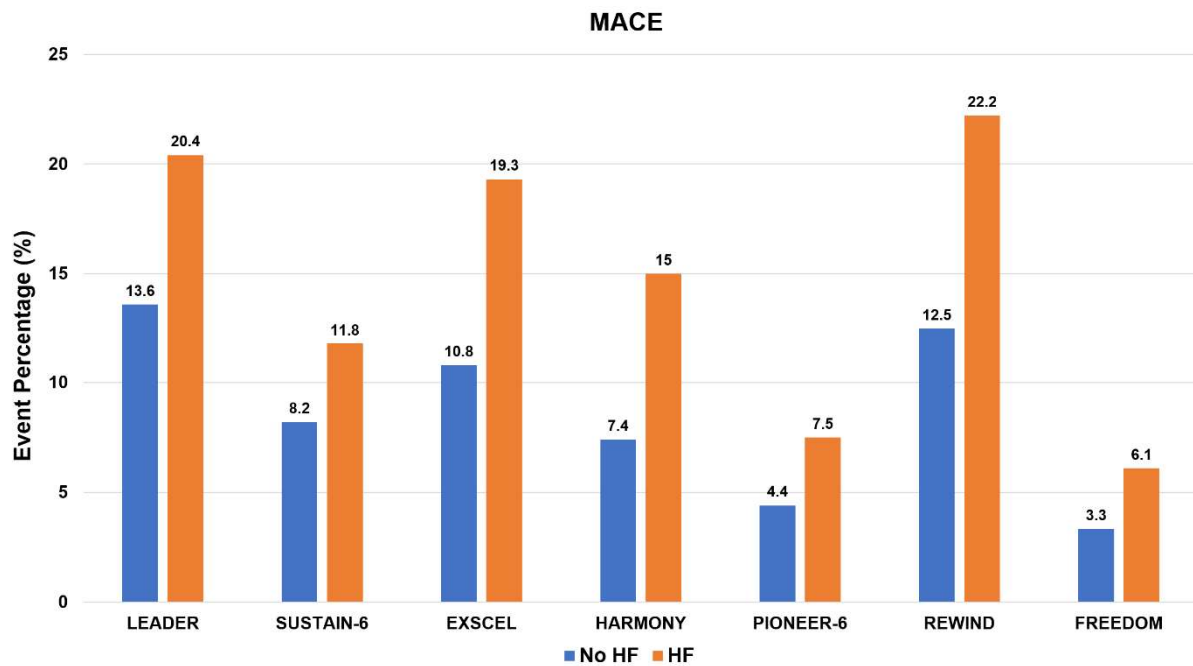
C) Cardiovascular death



D) All-cause death

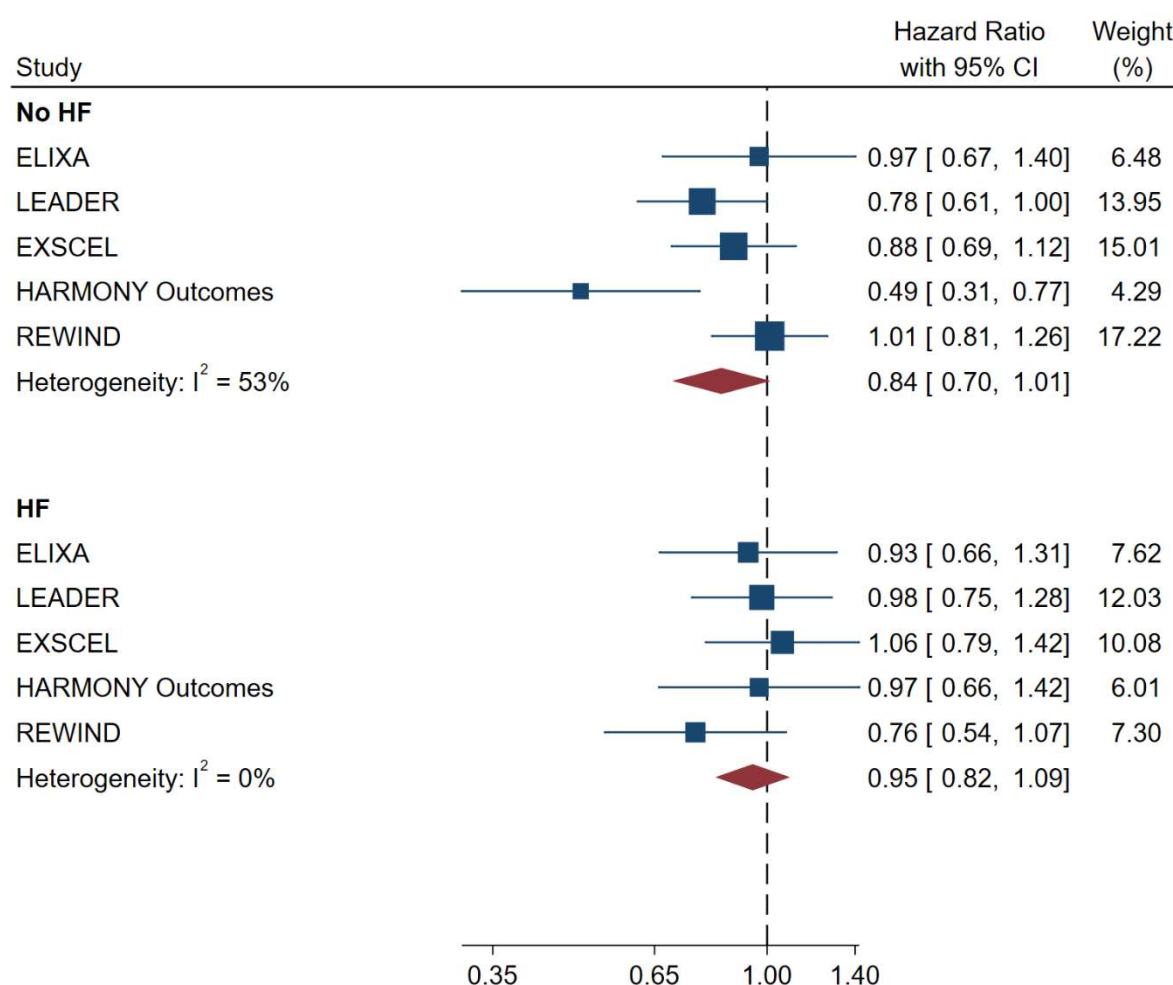


E) MACE



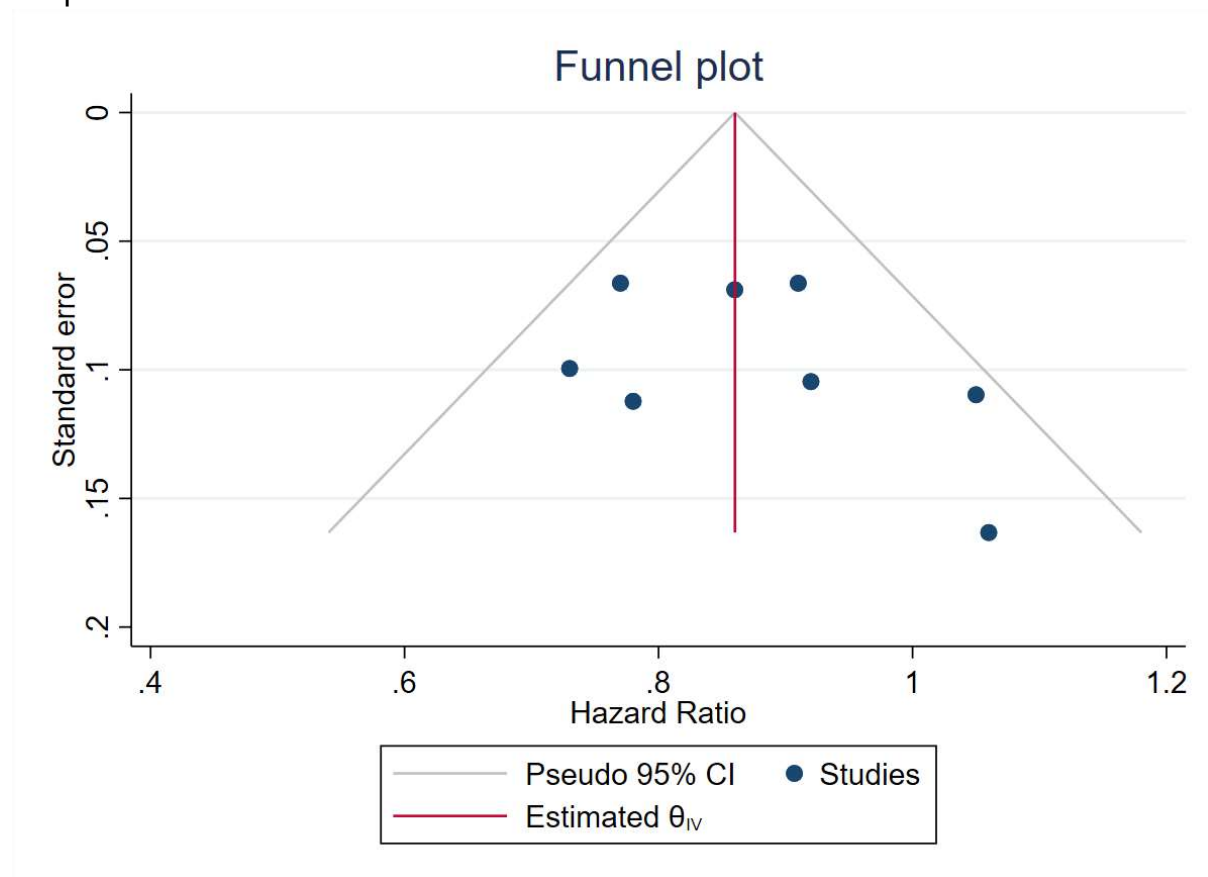
Legend: HF, heart failure; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome; SUSTAIN-6, Trial to Evaluate Cardiovascular and Other Long Term Outcomes with Semaglutide in Subjects with Type 2 Diabetes; EXSCEL, Exenatide Study of Cardiovascular Event Lowering; HARMONY, Albiglutide and Cardiovascular Outcomes in Patients With Type 2 Diabetes and Cardiovascular Disease; PIONEER-6, Peptide Innovation for Early Diabetes Treatment; REWIND, Researching Cardiovascular Events with a Weekly Incretin in Diabetes; FREEDOM, A Study to Evaluate Cardiovascular Outcomes in Patients With Type 2 Diabetes Treated With ITCA 650.

Supplementary Figure 2. Heart failure hospitalizations including the ELIXA trial



Legend: HF, heart failure; ELIXA, Evaluation of Cardiovascular Outcomes in Patients With Type 2 Diabetes After Acute Coronary Syndrome During Treatment With Lixisenatide; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome; SUSTAIN-6, Trial to Evaluate Cardiovascular and Other Long Term Outcomes with Semaglutide in Subjects with Type 2 Diabetes; EXSCEL, Exenatide Study of Cardiovascular Event Lowering; HARMONY, Albiglutide and Cardiovascular Outcomes in Patients With Type 2 Diabetes and Cardiovascular Disease; PIONEER-6, Peptide Innovation for Early Diabetes Treatment; REWIND, Researching Cardiovascular Events with a Weekly Incretin in Diabetes.

Supplementary Figure 3. Funnel plot for the composite outcome of heart failure hospitalization or cardiovascular death



Egger test P-value =0.28; Trim-and-fill observed hazard ratio =0.86 and observed plus imputed hazard ratio =0.85.

All tests support the absence of significant bias in this meta-analysis.



Article 12

Risk of adverse events with liraglutide in heart failure with reduced ejection fraction: A post hoc analysis of the FIGHT trial

Neves JS, Vasques-Nóvoa F, Borges-Canha M, Leite AR, Sharma A, Carvalho D, Packer M, Zannad F, Leite-Moreira A, Ferreira JP.

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ORIGINAL ARTICLE

Risk of adverse events with liraglutide in heart failure with reduced ejection fraction: A post hoc analysis of the FIGHT trial

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Abstract

Aim: To perform a post hoc analysis of the FIGHT trial, evaluating the effect of liraglutide (vs. placebo) on the totality of events in patients with heart failure with reduced ejection fraction (HFrEF).

Materials and Methods: FIGHT was a double-blind randomized controlled trial (RCT) that studied liraglutide versus placebo in 300 recently hospitalized patients with HFrEF followed for 180 days. The main outcome of the present analysis was total events of hospitalizations for heart failure (HF) or all-cause death. Secondary outcomes included total arrhythmic events and prespecified total events of interest (arrhythmias, sudden cardiac death, acute coronary syndrome, worsening HF, cerebrovascular event, venous thromboembolism, lightheadedness, presyncope/syncope or worsening renal function). Treatment effect was evaluated with negative binomial regression.

Results: Compared to placebo, there was a trend towards increased risk with liraglutide of total HF hospitalizations or all-cause deaths (96 vs. 143 events, incidence rate ratio [IRR] 1.41, 95% confidence interval [CI] 0.98–2.04; $P = 0.064$) and total arrhythmias (21 vs. 39, IRR 1.76, 95% CI 0.92–3.37; $P = 0.088$). Total prespecified events of

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interest were increased with liraglutide compared to placebo (196 vs. 295, IRR 1.43, 95% CI 1.06-1.92; $P = 0.018$). The risk of HF hospitalizations or all-cause deaths with liraglutide was higher among patients in New York Heart Association (NYHA) Class III to IV (IRR 1.86, 95% CI 1.21-2.85) than in those in NYHA Class I to II (IRR 0.62, 95% CI 0.31-1.23; interaction $P = 0.008$), and among patients with diabetes (interaction $P = 0.051$). The risk of arrhythmic events was higher among those without an implanted cardiac device (interaction $P = 0.047$).

Conclusions: In patients with HFrEF, liraglutide might increase the risk of cardiovascular adverse effects, an effect possibly driven by excess risk of arrhythmias and worsening HF events. As this was a post hoc analysis, these results should be interpreted as exploratory and hypothesis-generating. Further RCTs must be conducted before drawing definitive conclusions.

KEYWORDS

adverse events, arrhythmia, GLP-1 receptor agonists, heart failure hospitalizations, heart failure with reduced ejection fraction, liraglutide

1 | INTRODUCTION

Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are a drug class that promote glycaemic control and weight loss, lower blood pressure and improve lipid profile.¹ In randomized controlled trials (RCTs), GLP-1RAs decreased the risk of major adverse cardiovascular events (MACE) in patients with type 2 diabetes (T2D) with a high cardiovascular risk,² and current guidelines for treatment of T2D propose GLP-1RAs as a preferential class for those patients at high risk of atherosclerotic cardiovascular events.^{3,4}

Despite these beneficial effects, GLP-1RAs are known for increasing heart rate and activating cyclic adenosine monophosphate (cAMP)-dependent pathways,⁵ which have been associated with detrimental effects in patients with heart failure with reduced ejection fraction (HFrEF).⁶

Despite the reduction in MACE in RCTs with patients with T2D, these trials included only a small fraction of heart failure (HF) patients without measuring ejection fraction or natriuretic peptides;⁷ thus, the efficacy and safety of GLP-1RAs among patients with HF, particularly those with HFrEF, is not well established.

In two small RCTs with the GLP-1RA liraglutide in HFrEF, namely, the Heart Failure Network Functional Impact of GLP-1 for Heart Failure Treatment (FIGHT)⁸ and the Effect of Liraglutide on Left Ventricular Function in Stable Chronic Heart Failure Patients with and without Diabetes (LIVE) studies,⁹ treatment with liraglutide was not associated with beneficial effects and there was a higher number of hospitalizations for worsening HF and arrhythmia-related events in the group treated with liraglutide compared to placebo, albeit not reaching statistical significance. The analysis of both trials focused on the evaluation of first events which may have limited their power to detect potential adverse effects of GLP-1RAs. The effect of the GLP-1RA albiglutide was also evaluated in patients with stable HFrEF.¹⁰ In that small RCT (82 participants, 12 weeks follow-up), albiglutide did not

improve cardiac function or myocardial glucose use. Although albiglutide was well tolerated in the RCT, the number of participants and the short follow-up may have limited the detection of potential adverse effects (no hospitalizations for worsening HF were reported during this period).

In the present analysis, we aimed to evaluate the effect of the GLP-1RA liraglutide (vs. placebo) on the totality of events of HF hospitalization or all-cause death in patients with HFrEF enrolled in the FIGHT trial.

2 | MATERIALS AND METHODS

2.1 | FIGHT trial

FIGHT was a multicentre, double-blind RCT, designed to determine if the use of the GLP-1RA liraglutide improved clinical stability in recently hospitalized patients with HFrEF.¹¹ The trial was conducted between August 2013 and March 2015 at 24 sites in the United States, and participants were identified based on hospital admission records. Participants, aged 18 years or older, were required to have an established diagnosis of HF and a left ventricular ejection fraction (LVEF) of 40% or lower during the preceding 3 months. Additional inclusion criteria included: (i) a recent (within 14 days) hospitalization for acute HF and (ii) a preadmission oral diuretic dose of at least 40 mg of furosemide or an equivalent per day. Key exclusion criteria were: (i) a recent acute coronary syndrome or coronary intervention; (ii) known intolerance of GLP-1RA therapy; and (iii) severe renal, hepatic or pulmonary disease. Subjects with and without T2D were enrolled in the trial. In total, 300 patients were randomized. A permuted block randomization scheme, stratified by clinical site and T2D status, was performed. Of these, 154 were randomized to liraglutide and 146 to placebo. Liraglutide and placebo were packaged identically

to maintain blinding to patients and investigators. Study drug dosage was uptitrated as tolerated every 14 days from 0.6 mg/d to 1.2 mg/d to 1.8 mg/d during the first 30 days of the trial. In the primary analysis, the main outcome was a global rank score in which patients were ranked across three hierarchical tiers (with higher values indicating better health): time to death, time to rehospitalization for HF, and time-averaged proportional change in N-terminal pro-B-type natriuretic peptide (NT-proBNP) level from baseline to 180 days. There was no significant between-group difference in the global rank scores (146 for the liraglutide group vs. 156 for the placebo group; $P = 0.31$).

The FIGHT trial was sponsored by the National Heart, Lung, and Blood Institute (NHLBI). The study protocol was approved by the institutional review board and ethics committee at each participating centre. Access to the FIGHT database was provided through NHLBI/BioLINCC (https://biolincc.nhlbi.nih.gov/studies/hfn_fight/) with ethical approval from the Centro Hospitalar Universitário São João / Faculdade de Medicina da Universidade do Porto (process number #432/21).

2.2 | Study outcomes

The main outcome of this analysis was total events (first and recurrent) of HF hospitalizations or all-cause death. As secondary outcomes we evaluated: (i) total HF hospitalizations; (ii) all-cause death; (iii) total events of HF hospitalizations; urgent HF visits requiring intravenous diuretic treatment or all-cause death; (iv) total events of urgent HF visit requiring intravenous treatment; (v) total arrhythmic events (investigator reported); and (vi) prespecified total events of interest (predefined by the study investigators as any of the following: arrhythmias, sudden cardiac death, acute coronary syndrome, worsening HF, cerebrovascular event, venous thromboembolism, lightheadedness, presyncope or syncope, or worsening renal function).

2.3 | Statistical analysis

The main analyses of this study were conducted using the intention-to-treat principle and included all randomized participants during the study follow-up of approximately 180 days. The main and secondary outcomes were analysed by a negative binomial regression model with count of events as outcome and treatment group as independent variable. The results of the negative binomial test for the treatment effect of liraglutide versus placebo are described as incidence rate ratios (IRRs) with the respective 95% confidence intervals (CIs). Andersen-Gill models for recurrent events were also performed for internal consistency assessment. The introduction of anti-arrhythmic drugs during the follow-up period was assessed by mixed-effects logistic regression as an exploratory analysis.

Subgroup analyses were performed according to age (≥ 65 years vs. < 65 years), sex (female vs. male), diagnosis of T2D (yes vs. no), LVEF ($\leq 25\%$ vs. $> 25\%$), body mass index (BMI; ≥ 30 kg/m² vs. < 30 kg/m²), NT-proBNP levels (≥ 2000 pg/mL vs. < 2000 pg/mL), New York Heart Association (NYHA) class (Class III-IV vs. Class I-II), HF aetiology

(ischaemic vs. non-ischaemic), use of cardiac resynchronization therapy or implantable cardioverter-defibrillator (yes vs. no), use of digoxin (yes vs. no), and use of amiodarone or other anti-arrhythmic drugs (yes vs. no), with differences in the effect of liraglutide versus placebo assessed using subgroup analyses with interaction terms.

A two-sided P value of < 0.05 was taken to indicate statistical significance. No correction for multiple testing was performed due to the exploratory nature of this work. All analyses were performed using Stata® (StataCorp. 2021. Stata Statistical Software: Release 17; StataCorp LLC College Station, Texas).

3 | RESULTS

3.1 | Baseline patient characteristics by number of total events

The median (percentile₂₅₋₇₅) age of the patients was 61 (52-68) years, the median LVEF was 25 (19-33)%, and 64 (21%) were women. Compared to patients alive and without HF hospitalizations during the 180-day follow-up ($N = 156$), those who had only one ($N = 56$) and particularly those who had two or more HF hospitalizations or a fatal event ($N = 88$) presented more frequently with severely symptomatic HF (NYHA Class III or IV: 63.8% vs. 69.1% vs. 79.1%; $P = 0.050$), had lower LVEF (median: 23% vs. 25% vs. 20%; $P = 0.016$), had more frequent previous HF hospitalizations (two or more: 40.4% vs. 60.7% vs. 69.3%; $P < 0.001$), used cardiac devices more often (78.2% vs. 73.2% vs. 88.6%; $P = 0.048$), had lower haemoglobin levels (median: 13 g/dL vs. 12 g/dL vs. 12 g/dL; $P = 0.041$), and used angiotensin-converting enzyme inhibitors/ angiotensin II receptor blockers less frequently (76.9% vs. 75.0% vs. 61.4%; $P = 0.029$ [Table 1]). The distribution of total HF hospitalizations or death is shown in Figure 1A. The distribution of total investigator-reported events of interest is shown in Figure 1B.

3.2 | Effect of liraglutide versus placebo on total events

During the 6-month follow-up period, in participants treated with liraglutide, compared to placebo, there was a trend towards increased number of total HF hospitalizations or all-cause deaths (96 vs. 143 events, IRR 1.41, 95% CI 0.98-2.04; $P = 0.064$), total HF hospitalizations (80 vs. 124 events, IRR 1.47, 95% CI 0.98-2.20; $P = 0.061$), intravenous diuretic therapy, HF hospitalizations or all-cause deaths (102 vs. 153 events, IRR 1.48, 95% CI 1.00-2.19; $P = 0.056$) and total investigator-reported arrhythmias (21 vs. 39, IRR 1.76, 95% CI 0.92-3.37; $P = 0.088$). Total prespecified investigator-reported events of interest were increased with liraglutide compared to placebo (196 vs. 295, IRR 1.43, 95% CI 1.06-1.92; $P = 0.018$ [Table 2]). The Andersen-Gill model provided similar results: hazard ratio [HR] 1.53, 95% CI 1.02 to 2.31 ($P = 0.040$) for total HF hospitalizations or all-cause deaths and HR 1.41, 95% CI 1.01 to 1.97 ($P = 0.043$) for total prespecified investigator-reported events of interest (Figure 2).

TABLE 1 Baseline characteristics of the patients by the number of events of heart failure hospitalization or all-cause mortality

	Number of events of HF hospitalization or all-cause mortality			P value*
	None (N = 156)	1 event (N = 56)	2 or more events (N = 88)	
Age, years	61.0 (53.0, 68.5)	62.5 (50.5, 68.0)	61.0 (51.0, 66.0)	0.57
Women, n (%)	27 (17.3)	12 (21.4)	25 (28.4)	0.13
Race, n (%)				0.42
White	94 (60.3)	30 (53.6)	48 (54.5)	
Black	53 (34.0)	25 (44.6)	37 (42.0)	
Other	9 (5.8)	1 (1.8)	3 (3.4)	
BMI, kg/m ²	31.6 (26.1, 35.6)	32.1 (25.5, 36.6)	30.8 (26.0, 36.9)	0.96
NYHA Class III-IV, n (%)	97 (63.8)	38 (69.1)	68 (79.1)	0.050
Systolic BP, mmHg	107.0 (98.0, 120.0)	110.0 (96.0, 120.5)	108.0 (100.0, 117.0)	0.97
Diastolic BP, mmHg	66.0 (59.0, 75.0)	67.0 (59.5, 79.0)	66.0 (59.0, 74.0)	0.67
Heart rate, bpm	76.0 (68.0, 86.0)	76.0 (66.5, 89.5)	75.5 (69.0, 86.0)	0.61
LVEF, %	23.0 (18.0, 25.0)	25.0 (17.8, 30.0)	20.0 (15.0, 25.5)	0.016
HF of ischaemic aetiology, n (%)	125 (80.1)	47 (83.9)	74 (84.1)	0.68
Time from HF diagnosis, years	6.5 (3.3, 11.1)	5.1 (3.0, 9.6)	7.3 (3.3, 12.5)	0.63
HHF within past year, n (%)				<0.001
0	22 (14.1)	6 (10.7)	10 (11.4)	
1	71 (45.5)	16 (28.6)	17 (19.3)	
2+	63 (40.4)	34 (60.7)	61 (69.3)	
Type 2 diabetes, n (%)	93 (59.6)	29 (51.8)	56 (63.6)	0.37
Hypertension, n (%)	122 (78.2)	45 (81.8)	68 (77.3)	0.80
Atrial fibrillation / flutter, n (%)	78 (50.0)	34 (60.7)	42 (47.7)	0.28
History of sustained VT, VF or resuscitated cardiac arrest, n (%)	23 (14.7)	6 (10.7)	19 (21.6)	0.18
Pacemaker or ICD, n (%)	122 (78.2)	41 (73.2)	78 (88.6)	0.048
Stroke of TIA, n (%)	23 (14.8)	7 (12.5)	11 (12.8)	0.86
Chronic kidney disease, n (%)	54 (34.6)	22 (40.0)	42 (48.3)	0.11
Sodium, mEq/L	137.0 (135.0, 140.0)	136.5 (134.0, 139.0)	136.0 (134.0, 139.0)	0.080
Potassium, mEq/L	4.1 (3.8, 4.5)	4.0 (3.7, 4.3)	4.2 (3.8, 4.4)	0.11
eGFR, mL/min/1.73 m ²	51.1 (38.8, 69.4)	55.5 (38.4, 72.2)	49.4 (34.1, 65.9)	0.38
Cystatin C, mg/L	1.3 (1.1, 1.7)	1.5 (1.2, 1.8)	1.4 (1.1, 1.9)	0.23
Glucose, mg/dL, mmol/L	108.0 (95.0, 143.0) mg/dL = 6.0 (5.3, 7.9) mmol/L	109.0 (94.5, 137.5) mg/dL = 6.1 (5.3, 7.6) mmol/L	115.0 (96.0, 148.0) mg/dL = 6.4 (5.3, 8.2) mmol/L	0.38
HbA1c, %, mmol/mol	6.6 (6.0, 7.6) % = 49 (42, 60) mmol/mol	6.6 (5.8, 7.9) % = 49 (40, 63) mmol/mol	6.8 (6.0, 8.0) % = 51 (49, 64) mmol/mol	0.42
Haemoglobin, g/dL	13.0 (11.5, 14.5)	12.0 (11.2, 13.8)	12.3 (11.0, 13.5)	0.041
NT-proBNP, pg/mL	2150.5 (1016.5, 4271.5)	1908.5 (1153.0, 3944.0)	1982.5 (1141.0, 4754.0)	0.80
ACE inhibitors or ARBs, n (%)	120 (76.9)	42 (75.0)	54 (61.4)	0.029
Beta-blockers, n (%)	149 (95.5)	52 (92.9)	81 (92.0)	0.51
Aldosterone antagonists, n (%)	87 (56.5)	37 (66.1)	53 (60.2)	0.45
Loop diuretics, n (%)	156 (100.0)	54 (96.4)	87 (98.9)	0.070
Digoxin, n (%)	52 (33.3)	17 (30.4)	33 (37.5)	0.66
Amiodarone or other antiarrhythmic drugs, n (%)	38 (24.4)	18 (32.1)	26 (29.9)	0.44

Note: Results are presented as median (IQR) unless otherwise stated.

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; BP, blood pressure; bpm, beats per minute; eGFR, estimated glomerular filtration rate (CKD-EPI creatinine formula); HbA1c, glycated haemoglobin; HF, heart failure; HHF, heart failure hospitalizations; ICD, implantable cardioverter-defibrillator; IQR, interquartile range; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; TIA, transient ischaemic attack, VF, ventricular fibrillation; VT, ventricular tachycardia.

*P value for trend.

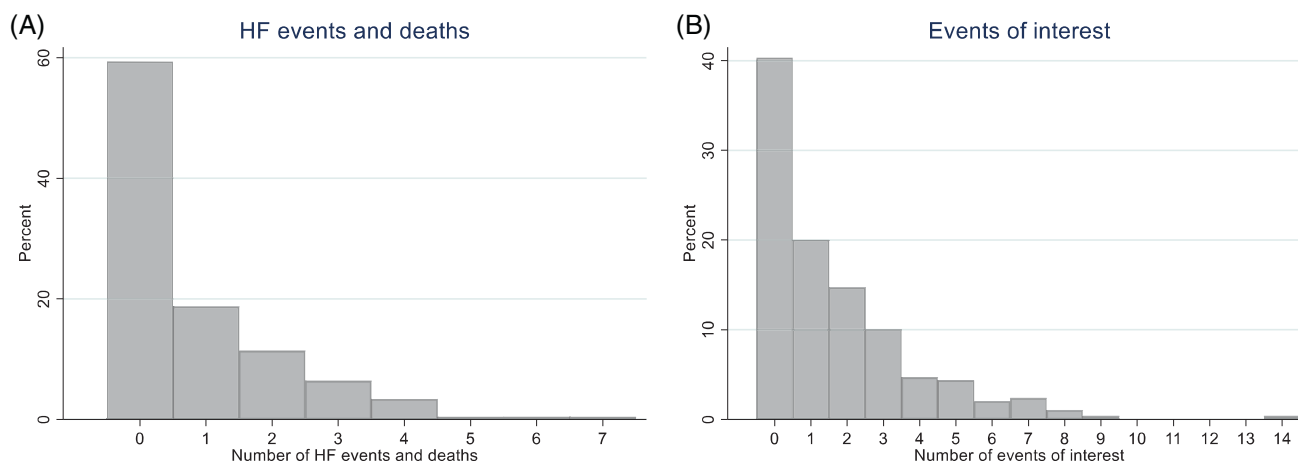


FIGURE 1 Total number of events of heart failure (HF) hospitalizations or all-cause mortality (A). Total number of events of interest, predefined by the study investigators as any of the following: arrhythmias, sudden cardiac death, acute coronary syndrome, worsening HF, cerebrovascular event, venous thromboembolism, lightheadedness, presyncope or syncope, or worsening renal function (B)

TABLE 2 Study outcomes (results are displayed as total events)

Outcome	Events placebo (N = 146)	Events liraglutide (N = 154)	Incidence rate ratio (95% CI)	P value
Total HHF or death	96	143	1.41 (0.98-2.04)	0.064
Total HHF	80	124	1.47 (0.98-2.20)	0.061
Death	16	19	1.13 (0.58-2.19)	0.72
Total HHF, urgent HF visit or death	102	153	1.48 (1.00-2.19)	0.056
Urgent HF visits	6	10	1.58 (0.52-4.81)	0.42
Total arrhythmic events ^a	21	39	1.76 (0.92-3.37)	0.088
Total events of interest ^{a,b}	196	295	1.43 (1.06-1.92)	0.018

Abbreviations: HF, heart failure; HHF, hospitalizations for heart failure.

^aInvestigator-reported.

^bEvents of interest, predefined by the study investigators as any of the following: arrhythmias, sudden cardiac death, acute coronary syndrome, worsening HF, cerebrovascular event, venous thromboembolism, lightheadedness, presyncope or syncope, or worsening renal function.

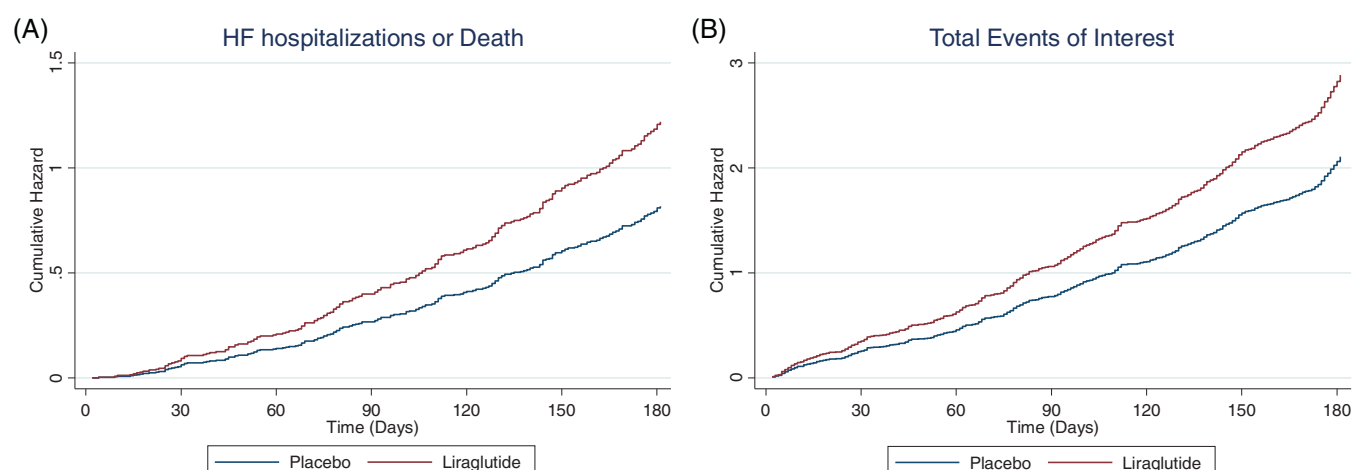


FIGURE 2 Cumulative hazard (Andersen-Gill model) of total events of heart failure (HF) hospitalization or all-cause mortality: hazard ratio [HR] 1.53, 95% confidence interval [CI] 1.02-2.31, $P = 0.040$ (A) and total events of interest: HR 1.41, 95% CI 1.01-1.97, $P = 0.043$ (B)

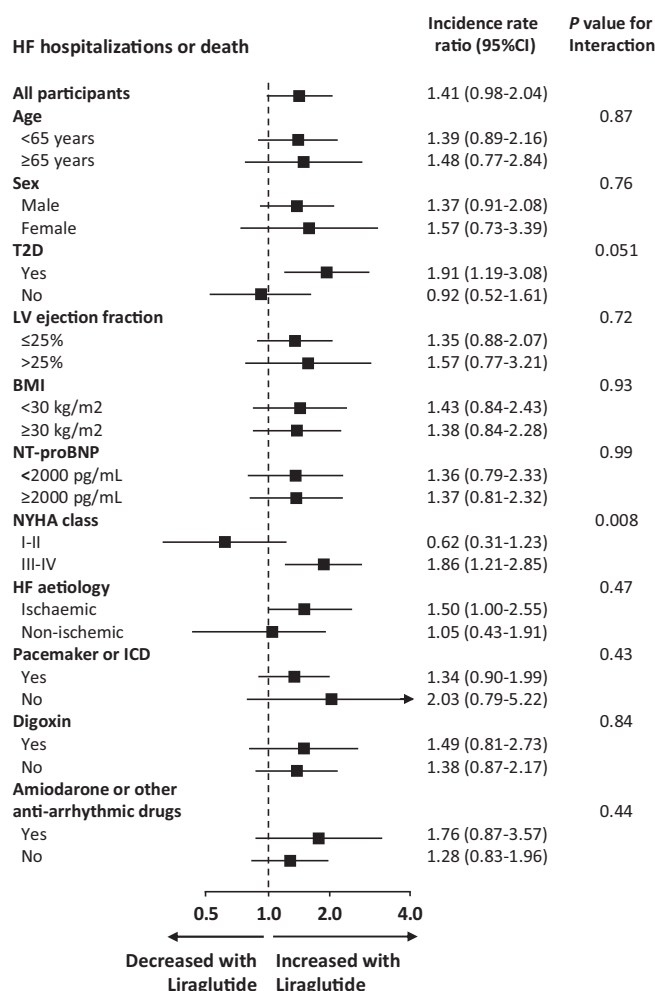


FIGURE 3 Subgroup analyses for the total events of heart failure (HF) hospitalization or all-cause mortality. CI, confidence interval; T2D, type 2 diabetes; LV, left ventricular; BMI, body mass index; NYHA New York Heart Association; ICD, implantable cardioverter-defibrillator

Liraglutide increased the new use of anti-arrhythmic drugs (amiodarone, propafenone, digoxin): 75 versus 115 (odds ratio 4.01, 95% CI 0.78-20.6; $P = 0.097$).

3.3 | Subgroup analyses

The risk of total HF hospitalizations or all-cause deaths with liraglutide was higher among patients in NYHA Class III or IV (IRR 1.86, 95% CI 1.21-2.85) than in those in NYHA Class I or II (IRR 0.62, 95% CI 0.31-1.23; interaction $P = 0.008$) and among patients with T2D (IRR 1.91, 95% CI 1.19-3.08) than in those without diabetes (IRR 0.92, 95% CI 0.52-1.61; interaction $P = 0.051$ [Figure 3]). The risk of total arrhythmic events with liraglutide was higher among those without an implantable cardioverter-defibrillator (IRR 12.57, 95% CI 1.42-111.54) than in those with an implantable cardioverter-defibrillator (IRR 1.23, 95% CI 0.61-2.48; interaction $P = 0.047$) and in those not using

digoxin (IRR 3.11, 95% CI 1.26-7.68) than in those using digoxin (IRR 0.92, 95% CI 0.34-2.50; interaction $P = 0.076$ [Figure S1]). A similar pattern was found for total investigator-reported events of interest (Figure S2).

4 | DISCUSSION

Our re-analysis of the FIGHT trial found a consistent pattern of increased risk of adverse events with liraglutide compared to placebo. Although it did not reach the statistical significance threshold of 0.05 for many of the studied endpoints, the consistency of the pattern across all outcomes strongly suggests that there is a potential risk associated with the use of liraglutide (and probably other GLP-1RAs) in patients with HFrEF. This risk is possibly driven by an excess of arrhythmias and worsening HF events with liraglutide use. A potentially increased risk of arrhythmic and worsening HF events with the use of GLP-1RAs in HFrEF is biologically plausible and has been externally replicated. In the small LIVE trial that enrolled 241 stable HFrEF patients randomized to either liraglutide or placebo (a lower-risk population compared to that in FIGHT), an excess risk of serious cardiac events was seen in the liraglutide group, driven by ventricular tachycardias (including one death) and atrial fibrillation (10% vs. 3%; $P = 0.04$).⁹

Despite its modest size and short follow-up (300 patients followed for 180 days), the FIGHT trial included a remarkably high-risk population due to the requirement for high natriuretic peptide levels and recent HF hospitalization prior to inclusion. Thus, the FIGHT trial was able to provide many “hard” events to assess the effect of liraglutide among patients with advanced HFrEF. Contrary to the original analysis, we evaluated not only time to first event, but the total number of events, which may more fully capture the total burden of disease in HF.¹² Potentially harmful effects of liraglutide could already be seen in the primary report of the FIGHT trial, with a time-to-first-event analysis, where the risk of: hospitalization for cardiovascular reasons (HR 1.33, 95% CI 0.95-1.85; $P = 0.09$); emergency department visit, hospitalization for cardiovascular reasons, or all-cause death (HR 1.34, 95% CI 1.00-1.80; $P = 0.05$); and emergency department visit, HF hospitalization, or all-cause death (HR 1.36, 95% CI 0.99-1.85; $P = 0.05$) all increased with liraglutide compared to placebo.⁸ These findings are reinforced and expanded by the present total event analysis, highlighting the risk of HF re-hospitalizations, arrhythmias (supported by the introduction of anti-arrhythmic agents), and total events of interest (including arrhythmias, sudden cardiac death, acute coronary syndrome, worsening HF, cerebrovascular event, venous thromboembolism, lightheadedness, presyncope or syncope, or worsening renal function). The heightened risk among patients with severe symptoms (NYHA III-IV), and those not treated with cardiac devices or anti-arrhythmic agents such as digoxin, suggests that liraglutide may be particularly harmful among unstable HFrEF patients who are not protected against arrhythmias, including potentially fatal ventricular arrhythmias.

The mechanisms by which liraglutide and other GLP-1RAs may increase the risk of adverse cardiovascular events in patients with HFrEF are not fully understood but might relate to altered intracellular cAMP dynamics. GLP-1 receptors are expressed in cardiomyocytes and sinoatrial node cells, signalling through a cAMP-dependent pathway.¹³ Preclinical data have already shown an increase in cardiac intracellular cAMP levels with GLP-1RA treatment.^{14–16} Furthermore, extensive mechanistic data have shown that increased intracellular cAMP raises the risk of arrhythmia and leads to myocardial dysfunction in HF.^{17–19} Importantly, the macromolecular complexes responsible for restricting cAMP action have been shown to be disrupted in HF,²⁰ extending cAMP-dependent signalling activation in time and space within the cardiomyocyte, causing calcium overload and predisposing to myocardial dysfunction and fatal arrhythmic events.^{17,21} This is in accordance with data from clinical trials, showing that drugs that increase cAMP levels (eg, milrinone) increase the risk of arrhythmic events and mortality in HFrEF,²² while drugs that decrease cAMP levels (eg, beta-blockers) are associated with decreased risk.²³ GLP-1RAs increase cAMP levels by pathways independent of beta-adrenergic receptors.^{24–26} The increased risk of cardiovascular adverse effects in the FIGHT trial, despite more than 90% of participants being treated with beta-blockers, suggests that beta-blockers do not protect from arrhythmic events and worsening HF events with GLP-1RAs in HFrEF.

While in populations without myocardial dysfunction and with low risk of arrhythmias, the myocardial effects of GLP-1RAs may not significantly increase the risk of HF events or arrhythmias,²⁷ in patients with HFrEF, the lower cardiac reserve and the higher pro-arrhythmogenic potential may make this population particularly susceptible to adverse cardiac effects with GLP-1RAs. In agreement with this hypothesis, some RCTs with GLP-1RAs in T2D showed significant interactions of the treatment arm with baseline HF status on study endpoints, with event rate reductions seen only in patients without HF at baseline. In the EXSCeL trial with exenatide, all-cause mortality was not reduced in the subgroup with HF (HR 1.05, 95% CI, 0.85–1.29) but was significantly reduced in those without HF at baseline (HR 0.79, 95% CI, 0.68–0.92; interaction $P = 0.03$).²⁸ Also, in a combined analysis of the SUSTAIN-6 and PIONEER-6, semaglutide reduced MACE among participants without HF (HR 0.79, 95% CI 0.68–0.92), but not in those with HF at baseline (HR 1.06, 95% CI 0.72–1.57; interaction $P = 0.03$).²⁹ In the LEADER trial, liraglutide reduced the composite of HF hospitalization or cardiovascular death in the subgroup without baseline HF history (HR 0.77, 95% CI 0.65–0.91) but not in the subgroup with baseline HF (HR 0.92, 95% CI, 0.74–1.15), even though the interaction was not significant (interaction $P = 0.19$).³⁰ In all these trials, HF subgroup included patients with HFrEF and patients with HF with preserved ejection fraction (HFpEF), which may explain the differences compared to the FIGHT trial. From the RCTs with GLP-1RAs in T2D, only the EXSCeL reported LVEF and, among participants with baseline HF and documented ejection fraction, only 22% had reduced ejection fraction.²⁸ Furthermore, some trials of GLP-1RAs versus placebo in patients with T2D have reported an excess of risk of ventricular fibrillation/

tachycardia or cardiovascular conduction disorders with GLP-1RAs (EXSCeL: 41/7356 vs. 26/7396; LEADER 18/4668 vs. 8/4672; REWIND: 216/4949 vs. 192/4952).^{31–33}

The findings reported here have important clinical consequences and further trials are needed to confirm the potential excess risk with GLP-1RAs in patients with HFrEF. Until such trials are conducted, GLP-1RAs should be avoided in patients with HFrEF.

Whether this increased risk is also observed in patients with HFpEF is uncertain; no RCTs have specifically evaluated GLP-1RAs in this population. Given the contribution of metabolic dysfunction and obesity to the pathophysiology of HFpEF,³⁴ the systemic effects of GLP-1RAs may counterweight, at least partially, the potential direct increased risk of arrhythmogenic effects. The Research Study to Investigate How Well Semaglutide Works in People Living With Heart Failure and Obesity (STEP-HFpEF; clinicaltrials.gov identifier: NCT04788511) and the Research Study to Look at How Well Semaglutide Works in People Living With Heart Failure, Obesity and Type 2 Diabetes (STEP HFpEF DM; clinicaltrials.gov identifier: NCT04916470) are evaluating the effect of the GLP-1RA semaglutide on the function and symptoms of patients with HFpEF.

It is important to distinguish the effects of GLP-1RAs in patients with T2D without HF from the effects in patients with established HF. While our results suggest that, in those with established HFrEF, treatment with GLP-1RAs may increase the risk of adverse effects, and in those without HF, GLP-1RAs may prevent the development of HF. A recent meta-analysis of RCTs in T2D showed that treatment with GLP-1RAs significantly reduced hospitalizations for HF.³⁵ Observational data also suggest that GLP-1RAs may decrease HF events in primary prevention.³⁶ The improvement of metabolic control and the prevention of coronary atherosclerotic disease with GLP-1RAs may explain the primary prevention of HF development in T2D. Furthermore, GLP-1RAs decrease epicardium fat,³⁷ which likely plays a role in the pathogenesis of HFpEF.³⁸ It is plausible that HF events prevented by GLP-1RAs in diabetes are predominantly HFpEF events.

Given the potential adverse effects of GLP-1RAs in HFrEF, in patients with T2D or obesity with symptoms suggestive of or clinical suspicion of HF, an echocardiogram and natriuretic peptides may be considered before starting the treatment. On the other hand, in those without HFrEF, our results should not discourage clinicians from using GLP-1RAs, given their well-established atherosclerotic cardiovascular benefit in T2D² and their potential cardiovascular benefits in obesity.³⁹

Our study has some limitations. This was a post hoc analysis of an RCT and some studied endpoints did not reach the statistical significance threshold of 0.05; however, the consistent trend for increased risk of adverse events across different outcomes, the biological plausibility, and the finding of similar results in the LIVE trial provide robustness to our analyses.⁹ This RCT had a short follow-up (6 months) and a modest size sample (300 participants); however, the remarkably high-risk population included led to a high number of “hard” outcomes during the trial. The increased risk observed in this high-risk population with a recent hospitalization may not apply to other groups of patients with HFrEF. The FIGHT trial was conducted

between August 2013 and March 2015, and new therapeutic interventions have been introduced since then in the management of HFrEF (including ARNI and SGLT2 inhibitors); although the potential detrimental effects of GLP-1RAs are not expected to be modified by the current management of HFrEF, we cannot exclude this possibility. We could not determine the cause of HF decompensation that led to hospitalization, but it is possible that arrhythmias could have contributed to some of these HF events. No correction for multiplicity of tests was made, which may increase the risk of chance findings and type I error.

Despite these limitations, this analysis is an important addition to what was already known from the original analysis. In the original analysis, the global rank score did not differ between groups, and the study was interpreted as neutral. Although there was already a trend towards worse outcomes with liraglutide in the original analysis (Table S1), the use of totality of events allowed a clearer assessment of the effects of liraglutide in advanced HFrEF. The identification that the risk of HF hospitalizations or death with liraglutide was higher in patients in NYHA Class III to IV is clinically relevant. Furthermore, the higher risk of HF hospitalizations or death with liraglutide in patients with T2D is also relevant as this is the population most commonly treated with GLP-1RAs. Arrhythmic events were not specifically evaluated in the original analysis and our analysis showed a trend towards an increased risk of arrhythmias, particularly among those without an implanted cardiac device. As with any post hoc analysis, our study must be interpreted as exploratory and hypothesis-generating, and further RCTs must be conducted before drawing definitive conclusions.

In conclusion, in patients with HFrEF, treatment with liraglutide might increase the risk of cardiovascular adverse effects, an effect possibly driven by an excess risk of arrhythmias and worsening HF events. Further trials with GLP-1RAs should be performed to better assess the risks versus benefit of GLP-1RAs in patients with HFrEF; until then, the use of liraglutide, and possibly other GLP-1RAs, should be avoided in patients with advanced HFrEF.

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CONFLICT OF INTERESTS

Dr Neves has received consulting or speaker fees from AstraZeneca, BIAL, Boehringer Ingelheim, Lilly, Merck and Novo Nordisk. Dr Sharma is supported by the McGill University Health Centre (MUHC) Foundation, Montreal General Hospital (MGH) Foundation, Sarah Louise King Award, Marjorie Cadham Award, Inez and Willena Beaton Award, *Fonds de Recherche Santé Québec* (FRSQ) Junior 1 clinician scholars' program, and Canada Institute for Health Research grant – 175 095. Dr Sharma reports receiving support from the European Society of Cardiology young investigator grant, Roche Diagnostics, Boehringer-Ingelheim, Novartis and Takeda. Dr Carvalho has received consultancy fees from Novo-Nordisk and Eli-Lilly, and has held

lectures Novo-Nordisk, Eli-Lilly and Astra-Zeneca. Dr Packer has received personal fees from Abbvie, Actavis, Amarin, Amgen, AstraZeneca, Boehringer Ingelheim, Caladrius, Casana, CSL Behring, Cytokinetics, Imara, Lilly, Moderna, Novartis, Reata, Relypsa and Salamandras. Dr Zannad reports personal fees from Boehringer Ingelheim, Janssen, Novartis, Boston Scientific, Amgen, CVRx, AstraZeneca, Vifor Fresenius, Cardior, Cereno Pharmaceutical, Applied Therapeutics, Merck, Bayer and Cellprothera, outside the submitted work, and other support from CVCT and Cardiorenal, outside the submitted work. Dr Ferreira is a consultant for Boehringer-Ingelheim, and receives research support from AstraZeneca and Novartis. All other authors have no potential conflicts of interest to disclose.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/dom.14862>.

DATA AVAILABILITY STATEMENT

The FIGHT database can be fully available from NHLBI/BioLINCC upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

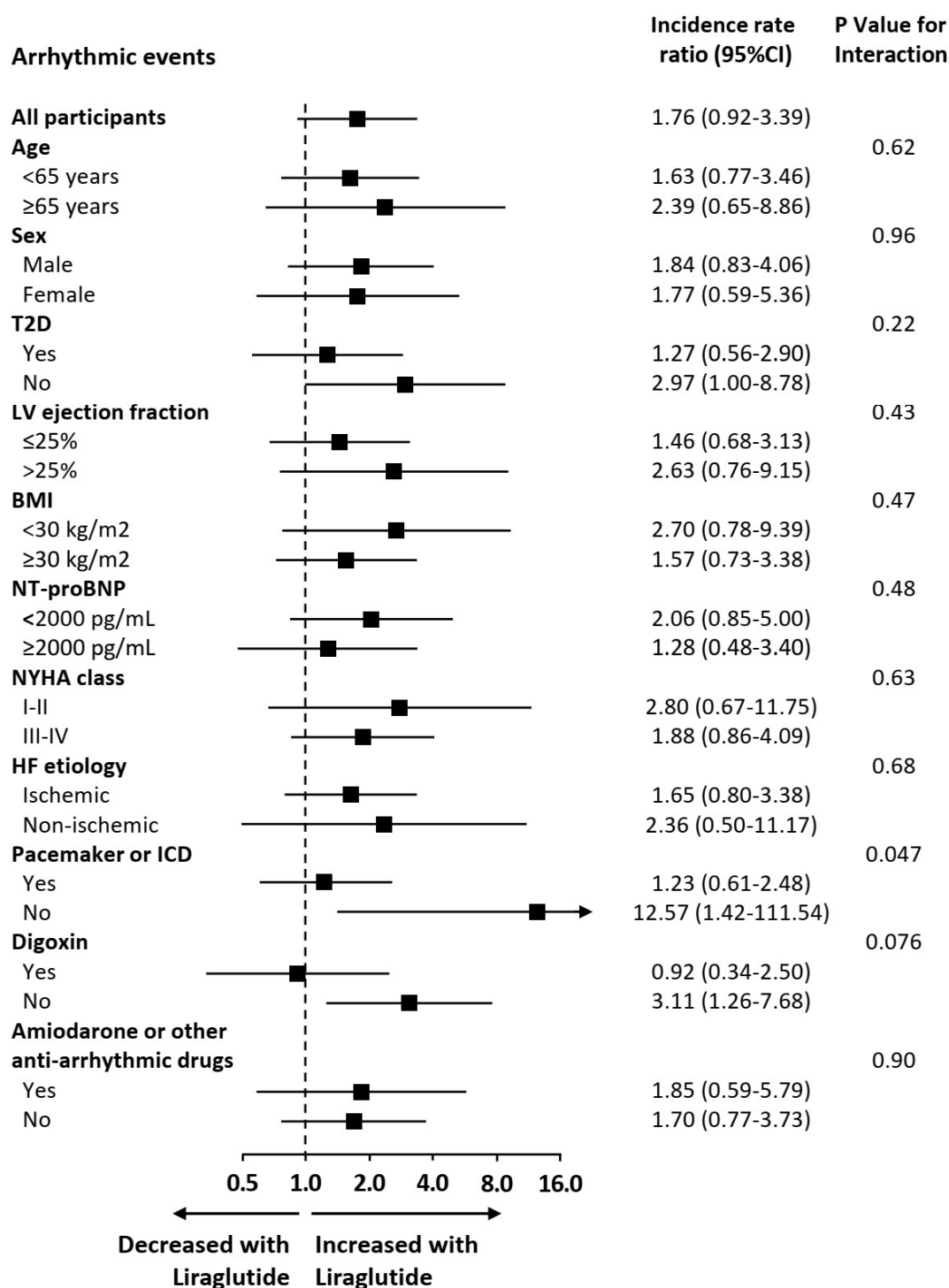
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Supplemental Material

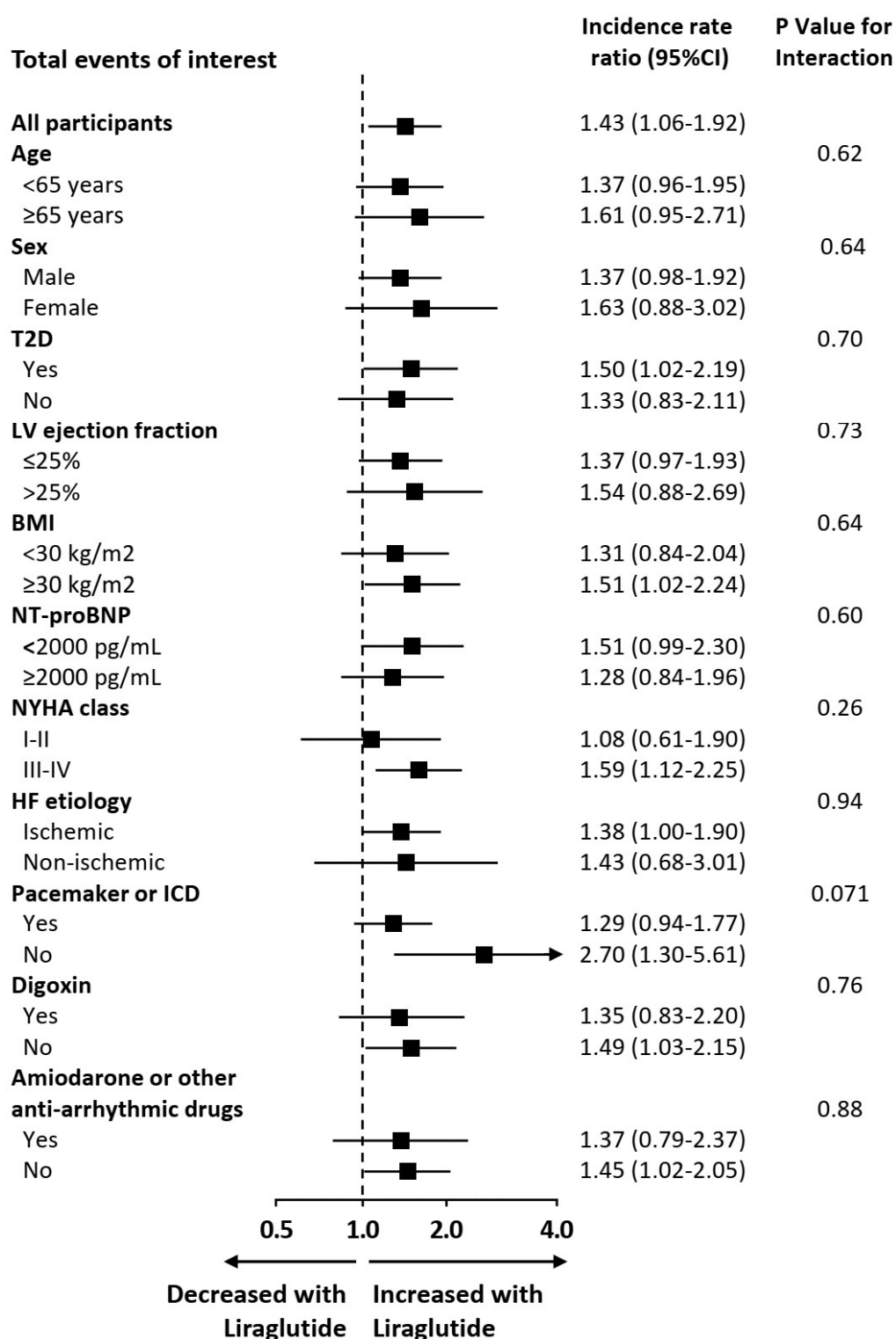
Risk of Adverse Events with Liraglutide in HFrEF: A Total Event Re-analysis of the FIGHT

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Supplemental Figure 1. Subgroup analyses for the total arrhythmic events. HF, heart failure; CI, confidence interval; T2D, type 2 diabetes; LV, left ventricle; BMI, body mass index; NYHA New York Heart Association; ICD, implantable cardioverter-defibrillator.



Supplemental Figure 2. Subgroup analyses for the total events of interest - predefined by the study investigators as any of the following: arrhythmias, sudden cardiac death, acute coronary syndrome, worsening heart failure, cerebrovascular event, venous

thromboembolism, lightheadedness, presyncope or syncope, or worsening renal function.

HF, heart failure; CI, confidence interval; T2D, type 2 diabetes; LV, left ventricle; BMI, body mass index; NYHA New York Heart Association; ICD, implantable cardioverter-defibrillator.

Outcome	Original analysis (time-to-first-event analysis) <i>Cox regression</i>				Post-hoc analysis (based on totality of events) <i>Negative binomial regression</i>			
	Placebo (N =146)	Liraglutide (N =154)	Hazard ratio (95%CI)	P-value	Placebo (N =146)	Liraglutide (N =154)	Incidence rate ratio (95%CI)	P-value
Mean Global Rank Score ^a	156	146	-	0.31	-	-	-	-
HFH or death	57	72	1.30 (0.92-1.84)	0.14	96	143	1.41 (0.98-2.04)	0.064
HFH	50	63	1.30 (0.89-1.88)	0.17	80	124	1.47 (0.98-2.20)	0.061
Death	16	19	1.10 (0.57-2.14)	0.78	16	19	1.13 (0.58-2.19)	0.72
HFH, ED visit or death ^b	72	89	1.36 (0.99-1.85)	0.05	-	-	-	-
HFH, urgent HF visit or death ^b	-	-	-	-	102	153	1.48 (1.00-2.19)	0.056
ED visit ^b	28	39	1.41 (0.87-2.30)	0.16	-	-	-	-
Urgent HF visits ^b	-	-	-	-	6	10	1.58 (0.52-4.81)	0.42
Arrhythmic events ^c	16	26	-	-	21	39	1.76 (0.92-3.37)	0.088
Events of interest ^d	82	97	-	-	196	295	1.43 (1.06-1.92)	0.018

Supplemental Table 1. Comparison of the results of the original analysis (based on time-to-event analysis) and this post-hoc analysis (based on totality of events). ED, emergency department; HF, heart failure; HHF, heart failure hospitalizations. a. Ranked across 3 hierarchical tiers: time to death, time to rehospitalization for heart failure, and time-averaged proportional change in N-terminal pro-B-type natriuretic peptide level from baseline to 180 days. Higher values indicate better health (range, 1 [an early death] to 300 [a patient who survived free from rehospitalization and had an improvement in N-terminal pro-B-type natriuretic peptide level]). Analysis of the global rank end point was based

on the Wilcoxon test statistic. b. In the original analysis all ED visits were included, while in the current post-hoc analysis only urgent HF visits requiring intravenous diuretic treatment were counted. c. Investigator-reported; hazard ratio and p-value were not calculated in the original analysis. d. Events of interest, predefined by the study investigators as any of the following: arrhythmias, sudden cardiac death, acute coronary syndrome, worsening heart failure, cerebrovascular event, venous thromboembolism, lightheadedness, presyncope or syncope, or worsening renal function; hazard ratio and p-value were not calculated in the original analysis.



Article 13

Increased risk of heart failure hospitalization with GLP-1 receptor agonists in patients with reduced ejection fraction: a meta-analysis of the EXSCEL and FIGHT trials

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Increased Risk of Heart Failure Hospitalization With GLP-1 Receptor Agonists in Patients With Reduced Ejection Fraction: A Meta-Analysis of the EXSCEL and FIGHT Trials

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GLP-1 receptor agonists (GLP-1 RA) are usually described as having beneficial effects on atherosclerotic cardiovascular disease (ASCVD) and neutral effects on heart failure (HF) events. However, recent data suggest that the effect of GLP-1 RA may differ according to baseline HF status, with decreased risk of HF events in those without established HF and increased risk of HF events among those with HF with reduced ejection fraction (HFrEF).^{1–4} Treatment with GLP-1 RA liraglutide did not improve clinical stability in the FIGHT (Functional Impact of GLP-1 for Heart Failure Treatment) trial and did not improve left ventricle ejection fraction (LVEF) in the LIVE (Effect of Liraglutide on Left Ventricular Function in Stable Chronic Heart Failure Patients with and without Diabetes) trial.^{2,3} In both randomized clinical trials (RCTs), all patients had HFrEF, and there was a trend to increased risk of adverse outcomes. Whether this risk is limited to those with severe HFrEF or is also observed in stable ambulatory

patients with type 2 diabetes and with reduced LVEF is unknown. At this moment, the only RCT testing GLP-1 RA in type 2 diabetes that presented data concerning LVEF was the EXSCEL (Exenatide Study of Cardiovascular Event Lowering) trial. However, in the EXSCEL trial, the influence of baseline LVEF on the effects of GLP-1 RA on heart failure hospitalization (HFH) has not been evaluated previously.

In this report, we aimed to evaluate the interaction of GLP-1 RA effects with the risk of HFH according to baseline LVEF in the EXSCEL trial and to perform a meta-analysis of the effect of GLP-1 RA on HFH among patients with LVEF < 40%.

Data for HFH in the EXSCEL trial according to LVEF were extracted from Tables 2 and S4 of the secondary analysis assessing the effects of exenatide by baseline HF status. (It is to be noted that in this publication, there was an error in Table S4, where the headers of groups were switched).⁵ Data for HFH in the FIGHT trial were extracted from the primary analysis of the trial.² The odds ratio (OR) of HFH was calculated using logistic regression models. Estimates from the FIGHT trial and the subgroup with LVEF < 40% in the EXSCEL trial were combined by the use of inverse variance-weighted averages of logarithmic or in random-effects analysis. In the EXSCEL trial, the effect modification of baseline LVEF (< 40%, 40%–55% or ≥ 55%) on the effect of exenatide on HFH was evaluated by using a treatment-by-LVEF interaction term. A 2-sided *P* value of < 0.05 was considered statistically significant. All analyses were performed using Stata 17 (StataCorp, College Station, TX).

In the EXSCEL trial, there were 249 first HFHs during a median follow-up of 3.2 years among the 4892 participants (with or without HF) with baseline LVEF available (33.2% of the EXSCEL population). There was a significant interaction of LVEF with the effect of exenatide on HFH: LVEF > 55% OR 0.73, 95%CI 0.48–1.13; LVEF 40%–55% OR 0.77, 95%CI 0.50–1.20; LVEF < 40% OR 1.70, 95%CI 1.02–2.83 (*P*-

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See page 3 for disclosure information.

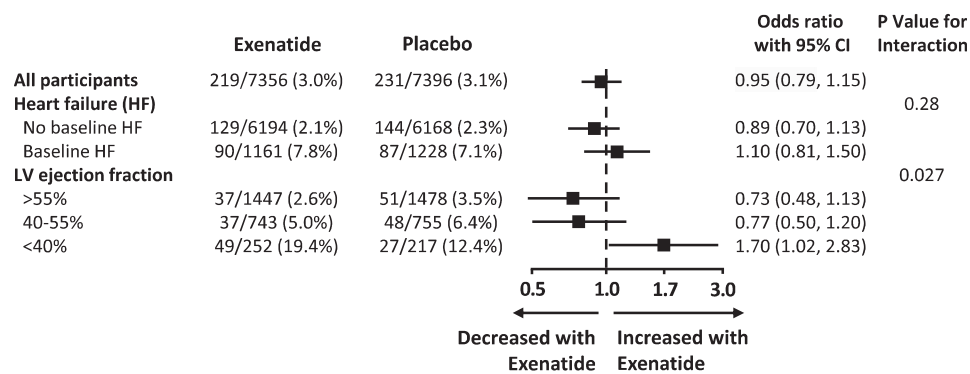
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A. Heart Failure Hospitalization in the EXSCEL trial



B. Meta-analysis of Heart Failure Hospitalization with GLP-1 RA (FIGHT trial and subgroup with LVEF <40% in the EXSCEL trial)

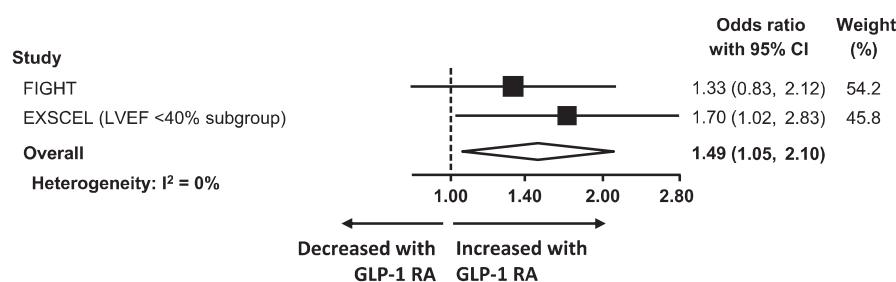


Fig. 1. A, Heart failure hospitalization in the EXSCEL trial according to HF status and LVEF. B, Meta-analysis of heart failure hospitalization with GLP-1 RA in patients with LVEF < 40% (FIGHT trial and subgroup with LVEF < 40% in the EXSCEL trial). EXSCEL, Exenatide Study of Cardiovascular Event Lowering; FIGHT, Functional Impact of GLP-1 for Heart Failure Treatment; GLP-1 RA, GLP-1 receptor agonists; HF, heart failure; LV, left ventricle; LVEF, left ventricle ejection fraction.

interaction = 0.027) (Fig. 1A). When restricted to participants diagnosed with HF, a similar pattern was observed: LVEF $\geq 40\%$ OR 0.71, 95%CI 0.44–1.15, LVEF < 40% OR 1.49, 95%CI 0.82–2.72 (P -interaction = 0.059).

In the FIGHT trial, there were 113 first HFH events during a follow-up of 180 days. The OR for HFH with liraglutide vs placebo was 1.33, 95% CI 0.83–2.12.

The meta-analyzed GLP-1 RA effect on the risk of HFH in patients with LVEF < 40% gave an OR of 1.49, 95%CI 1.05–2.10 (P value = 0.02; $I^2 = 0\%$) (Fig. 1B).

The results of the LIVE study also support an increased risk of adverse events with GLP-1 RA in HFrEF.³ In the LIVE study, there was a significant increase in the risk of serious cardiac events with liraglutide, including 1 death, caused by nonfatal ventricular tachycardia, atrial fibrillation and aggravation of ischemic heart disease, and 1 case of worsening of HF over a period of 24 weeks. LIVE was not included in our meta-analysis because only 1 case of worsening of HF was reported.

From a biological perspective, GLP-1 RAs are known to increase heart rate and intracellular cyclic adenosine 3',5'-cyclic monophosphate (cAMP), and both are known to affect outcomes in HFrEF adversely.⁴ Although drugs that decrease cAMP improve the prognosis in HFrEF (eg, beta-blockers), drugs that increase cAMP (eg, milrinone) increase the risk of HFH, ventricular arrhythmias and death in those with HFrEF.

In conclusion, GLP-1 RA may increase the risk of HFH in patients with LVEF < 40%. These results must be interpreted as exploratory and hypothesis generating because they are based on a subgroup of the EXSCEL trial with LVEF available ($n = 4892$, 33.2% of the study population, which may not be missing at random) and on a small trial with a short follow-up (the FIGHT trial). More dedicated RCTs evaluating the effects of GLP-1 RA in patients with HF, particularly HFrEF, are needed. Until then, the use of GLP-1 RA should be avoided in patients with reduced LVEF.



João Sérgio Neves

Disclosures

JSN has received consulting or speaker fees from AstraZeneca, BIAL, Boehringer Ingelheim, Lilly, Merck, and Novo Nordisk. MP has received personal fees from Abbvie, Actavis, Amarin, Amgen, AstraZeneca, Boehringer Ingelheim, Caladrius, Casana, CSL Behring, Cytokinetics, Imara, Lilly, Moderna, Novartis, Reata, Relypsa, and Salamandras. JPF is a consultant for Boehringer Ingelheim and AstraZeneca and has received research support from Novartis, Boehringer Ingelheim, AstraZeneca, and Bayer through his institution.

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Chapter 4

DISCUSSION



Thyroid function within the euthyroid range has an important impact on cardiovascular risk factors and on cardiac function

In our analysis of the EPIPorto study, we showed that variations of thyroid function within the euthyroid range were associated with risk factors for cardiovascular disease and with cardiac function.

From a metabolic perspective, markers of decreased conversion of T4 to T3 were associated with a higher prevalence of diabetes, a more adverse lipid profile and a lower estimated glomerular filtration rate (eGFR). This is concordant with the known biological effects of thyroid hormones^{43,105} and with the metabolic consequences of hypothyroidism.¹⁰⁶ This supports the hypothesis that thyroid hormones' effects on metabolism are a continuum that extends into the euthyroid range. Furthermore, we showed that this translates into an increased 10-year risk of cardiovascular events, suggesting that the magnitude of the variations of cardiovascular risk factors may be of clinical relevance. On the other hand, participants with higher BMI and larger waist circumference had higher FT3 and higher FT3/FT4 ratio; this is compatible with a central activation of the hypothalamus-pituitary-thyroid axis in overweight and obesity,^{107,108} as we also characterized in the evaluation of thyroid function in patients submitted to bariatric surgery (detailed in the next topic).

Regarding the effect of thyroid function on cardiac function, increasing thyroid function was associated with a higher heart rate, lower diastolic blood pressure, and larger LV volumes. LV wall thickness and ejection fraction had a U-shaped association with thyroid hormones. This data suggests that both low-normal and high-normal thyroid hormone levels may be detrimental to cardiac function and that normalization of thyroid function is a potential therapeutic target to improve cardiac function.

Thyroid function and body weight regulation have a bidirectional relationship

Obesity is one of the main drivers of metabolic and cardiovascular dysfunction.¹⁰⁹ Thyroid hormones directly modulate adipose tissue function and energy intake.^{110,111} Despite the known effect of thyroid hormones on body weight, it was not clear whether variations of thyroid function within the euthyroid range were associated with the efficacy of weight loss interventions. Bariatric surgery is a therapeutic approach that is used to treat the more severe forms of obesity and, as such, a unique opportunity to evaluate the effects of thyroid function on a weight loss intervention. Our results showed that, in patients with morbid obesity submitted to bariatric surgery, those with higher levels of FT3 have the greatest weight loss. These results are reinforced by similar results in patients submitted to lifestyle interventions for weight loss.^{112,113} This suggests that thyroid function may help to predict weight loss after bariatric surgery and that modulation of thyroid function is a potential approach to improve the results of weight loss interventions.

While the effects of thyroid hormones on body weight regulation are well-recognized, the effects of obesity on the hypothalamus-pituitary-thyroid axis are frequently overlooked. Previous studies had already shown that people with obesity have higher levels of TSH and plasma thyroid hormone levels.¹¹⁴⁻¹²¹ This pattern is consistent with a central activation of the hypothalamus-pituitary-thyroid axis, which has been proposed to be mediated by leptin.^{122,123} Our results contribute to a better understanding of this relationship by: 1) showing that there is a positive association between leptin and TSH levels in the EPIPorto cohort, and 2) showing that TSH levels decrease after bariatric surgery and that this decrease is proportional to the weight loss observed. Our results, taken together with the previously available evidence, highlight a bidirectional association of thyroid function with body weight: 1) obesity promotes activation of the thyroid hormones axis, and 2) higher thyroid hormone levels improve weight loss after a weight loss intervention.

Supplementation of thyroid hormones is a potential approach for treatment of HFpEF

Beyond the evaluation of the association of thyroid function with cardiovascular risk factors and cardiac function, one of the main objectives of this project was to evaluate the effects of thyroid hormone supplementation on HFpEF. We decided to evaluate the effects of thyroid hormones on this specific clinical syndrome because 1) changes in thyroid function are common in heart failure (due to the nonthyroidal illness syndrome);³⁸ 2) diastolic dysfunction and systemic metabolic dysfunction are central in the pathophysiology of HFpEF;^{43,124} 3) thyroid hormones improve both diastolic dysfunction and systemic metabolic function.⁴⁷ We opted to evaluate the effects of thyroid hormones on an animal model of HFpEF due to the lack of previous robust preclinical data to support the implementation of a clinical trial in humans.

Our results showed that thyroid hormones can improve myocardial and metabolic function and that their use must be regarded as a potential therapeutic target in HFpEF. Supplementation with T3 increased the myocardial expression of the calcium transporter ryanodine receptor 2 (RyR2) and of the α -myosin heavy chain (MHC), with a lower expression of the β -MHC. Thyroid hormones were able to decrease body weight, improve glucose metabolism, cardiomyocyte Ca^{2+} transients and sarcomere shortening and relaxation. Thyroid hormones reduced cardiomyocytes size and myocardial fibrosis, and improved both diastolic and systolic function. The functional benefits of T3 supplementation were offset by an increased risk of sudden death with the high dose supplementation (further discussed in the next section).

The detailed mechanisms by which thyroid hormones may improve the prognosis of HFpEF are described in the following review article.



Article 14

Thyroid hormones and modulation of diastolic function: a promising target for heart failure with preserved ejection fraction

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Thyroid hormones and modulation of diastolic function: a promising target for heart failure with preserved ejection fraction

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Abstract: Heart failure with preserved ejection fraction (HFpEF) is a clinical syndrome with high mortality for which there is no proven therapy to improve its prognosis. Thyroid dysfunction is common in heart failure (HF) and is associated with worse prognosis. In this review, we discuss the cardiovascular effects of thyroid hormones, the pathophysiology of HFpEF, the prognostic impact of thyroid function, and the potential of thyroid hormones for treatment of HFpEF. Thyroid hormones have a central role in cardiovascular homeostasis, improving cardiac function through genomic and non-genomic mechanisms. Both overt and subclinical hypothyroidism are associated with increased risk of HF. Even when plasmatic thyroid hormones levels are normal, patients with HF may have local cardiac hypothyroidism due to upregulation of type 3 iodothyronine deiodinase. Thyroid hormones improve several pathophysiological mechanisms of HFpEF, including diastolic dysfunction and extra-cardiac abnormalities. Supplementation with thyroid hormones (levothyroxine and/or liothyronine), modulation of deiodinase activity, and heart-specific thyroid receptor agonists are potential therapeutic approaches for the treatment of HFpEF. Further preclinical and clinical studies are needed to clarify the role of thyroid hormones in the treatment of HFpEF.

Keywords: diastolic function, heart failure, hypothyroidism, non-thyroidal illness syndrome, thyroid hormones

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Introduction

Thyroid hormones have a central role in cardiovascular system development and homeostasis. Both hypothyroidism and hyperthyroidism are associated with characteristic cardiovascular changes and even subclinical dysfunction is known to increase cardiovascular risk.¹

Heart failure (HF) is the final stage of several cardiovascular conditions, affecting over 23 million people worldwide.² HF can be divided into two major entities according to the ejection fraction (EF): HF with reduced EF (HFrEF) and HF with preserved EF (HFpEF). The latter is

responsible for over 50% of all cases. Like HFrEF, HFpEF is associated with decreased functional capacity, decreased quality of life, and high mortality. However, the pathophysiology of HFpEF is less well understood and there is as yet no proven therapy to improve its prognosis. Although its core feature was long held to be diastolic dysfunction, systemic disturbances that jeopardize cardiovascular reserve may also constitute essential pathophysiological mechanisms.³

In this review, we discuss the cardiovascular effects of thyroid hormones, the pathophysiology of HFpEF, the prognostic impact of thyroid

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function, and the potential of thyroid hormones for treatment of HFpEF.

Cardiovascular effects of thyroid hormones

Thyroid hormones modulate the cardiovascular system by genomic and non-genomic mechanisms.⁴ The thyroid gland produces thyroxine (T4) hormone in greater quantity than triiodothyronine (T3), at a ratio of 10:1. T3 is biologically more active than T4 and is considered the active form of thyroid hormones.⁵ The primary mechanism of action of T3 is the interaction with thyroid hormone receptors (TR) – a process that can either enhance or repress the transcription of specific target genes.⁴ There are two TR genes (TR α and TR β) with specific patterns of expression in different tissues. Both genes produce different isoforms as a result of alternative splicing.⁶ TR α 1 is expressed predominantly in brain and heart. TR β 1 is expressed in liver, kidney, and skeletal muscles, and, at lower levels, in most tissues including the heart. On the other hand, TR β 2 is expressed predominantly in brain, pituitary gland, retina, and inner ear, and appears to be important for regulating the negative-feedback loop of the hypothalamus-pituitary-thyroid axis.^{4,6} About 80% of the circulating T3 is produced in peripheral tissues by conversion of T4. This conversion is mediated by tissue deiodinases. Type 1 and type 2 deiodinases (D1 and 2, respectively) mainly convert T4 into T3, while type 3 deiodinase (D3) converts T4 and T3 into the functionally inactive reverse T3 (rT3) and 3,3-diiodothyronine (T2), respectively.^{7,8} D3 has higher affinity in inactivating T3 and plays a critical role in regulating T3 availability.⁹ Deiodinases regulate both serum and intracellular tissue levels of thyroid hormones. Several conditions, including chronic inflammation, neoplastic diseases, chronic kidney disease, myocardial ischemia, and HF, alter the pattern of deiodinase activity, increasing the conversion of T4 into rT3 and decreasing the availability of T3.^{8,10,11–13} T3 improves systolic and diastolic myocardial function and increases heart rate. Thyroid hormones enhance the expression of genes encoding sarco/endoplasmic reticulum calcium-ATPase (SERCA2a), fast α -isoform of myosin heavy chain (α -MHC), Na⁺/K⁺ ATPase, and voltage-gated K⁺ channels (Kv1.5 and Kv4.2), and negatively regulates the transcription of phospholamban (PLN) and slow β -isoform of myosin heavy chain (β -MHC).¹⁴ Both myosin

heavy chains are components of the cardiac contractile apparatus, and this change in expression pattern results in an increased velocity of contraction.¹⁵ The increase of SERCA2a and the inhibition of PLN increase the calcium available for systolic contraction, and improve the reuptake of calcium into the sarcoplasmic reticulum during relaxation of the heart.¹⁵ Efficient kinetics of calcium is indispensable for energetically optimal cardiac myocyte relaxation and contraction. Furthermore, thyroid hormones increase the gene expression of the β -adrenergic receptors, enhancing the response to catecholamines, which act in synergy with thyroid hormones.¹⁶ Thyroid hormones also protect the heart from ischemic lesion by decreasing coronary resistance, reducing the activation of the pro-apoptotic p38 MAPK signaling pathway and increasing the activity of myocardial PKC δ and the expression of heat shock proteins 27 and 70.¹⁷ In addition, thyroid hormones stimulate cell growth and neo-angiogenesis, and decrease cardiac fibrosis by enhancing metalloproteinase and antifibrotic effects.¹⁷

The effects of thyroid hormones on the vasculature include genomic and non-genomic mechanisms. Non-genomic effects include ion channel modulation and regulation of specific transduction pathways. In vessels, thyroid hormones activate phosphatidylinositol 3-kinase (PI3K)/serine/threonine-protein kinase (AKT) signaling pathways enhancing nitric oxide production by endothelial cells and activate non-genomic pathways that induce smooth muscle relaxation, thereby decreasing vascular resistance and left ventricular (LV) afterload.¹⁸ The decrease in systemic vascular resistance, coupled with the inotropic effects, leads to an increase in cardiac output.¹⁹

Thyroid hormones also have favorable effects on plasma lipid profile, which may decrease the risk of atherosclerosis development and progression.¹ This beneficial effect on the lipid profile is due to the increase of sterol regulatory element-binding protein-2 (SREBP-2), which regulates the expression of the LDL receptors.²⁰

Cardiovascular manifestations in thyroid dysfunction

Given the known effects of thyroid hormones on the cardiovascular system, the association of thyroid dysfunction with cardiovascular changes has

Table 1. Cardiovascular changes, comorbidities and mortality in thyroid dysfunction.

	Overt hypothyroidism	Subclinical hypothyroidism	Subclinical hyperthyroidism	Overt hyperthyroidism
Systolic dysfunction	↑↑	↑	↓/↑	↓/↑
Diastolic dysfunction	↑↑	↑↑	↓/↑	↓/↑
Heart rate	↓↓	↓	↑	↑↑
Hypertension	↑ (diastolic)	↑ (diastolic)	↑ (systolic)	↑ (systolic)
Dyslipidemia	↑↑	↑	↓	↓
Heart failure	↑↑	↑	-/↑	↑↑
Coronary artery disease	↑↑	↑	-/↑	-/↑
Atrial fibrillation	-/↓	-/↓	↑	↑↑
Atherosclerosis	↑↑	↑	-/↑	-/↑
Pulmonary hypertension	-	-	-	↑
Cardiovascular mortality	↑	-/↑	-/↑	↑
All-cause mortality	↑	-/↑	-/↑	↑

↑↑: markedly increased; ↑: increased; -/↑: possibly increased; -: no effect; -/↓: possibly decreased; ↓/↑: possibly decreased or increased; ↓: decreased; ↓↓: markedly decreased. See text for details.

been evaluated by many studies. These associations are better established in overt thyroid dysfunction than in subclinical dysfunction. Table 1 summarizes the cardiovascular changes in thyroid dysfunction.

Subclinical hypothyroidism is defined as elevated TSH with normal levels of free T4. The results of studies evaluating the effects of subclinical hypothyroidism on the cardiovascular system are inconsistent. Some, but not all, have shown increased all-cause and cardiovascular mortality, higher risk of coronary heart disease and HF.^{21–24} Most studies suggest that the risk of adverse cardiovascular outcomes is higher when TSH ≥ 10 mIU/l.²² In the Penn Heart Failure Study, a prospective cohort of patients with HFrEF and HFpEF, TSH ≥ 7 mIU/l was associated with an increased risk of a composite end point of ventricular assist device placement, heart transplantation, or death in patients.²⁵ Subclinical hypothyroidism has been associated with impaired systolic and diastolic cardiac function, increased carotid artery intima-media thickness, vascular dysfunction, and higher blood pressure.^{26–28} On the contrary, subclinical hypothyroidism may be associated with a lower risk of atrial fibrillation.²⁹

Overt hypothyroidism is defined as high TSH with low free T4.¹ In most studies, it has been associated with increased risk of HF, coronary artery disease, and all-cause and cardiovascular mortality.^{30,31} Overt hypothyroidism is associated with decreased cardiac output and contractility, lower heart rate, and higher systemic vascular resistance.³⁰ Diastolic dysfunction is a characteristic feature in most studies.^{32,33} Cardiovascular risk factors are amplified in patients with overt hypothyroidism, particularly diastolic hypertension and dyslipidemia. Most studies have also shown increased carotid artery intima-media thickness in overt hypothyroidism.^{34,35}

Subclinical hyperthyroidism is defined by low TSH with normal free T4.¹ It has been associated with a higher risk of cardiovascular disease, including coronary events, HF, and atrial fibrillation.^{36,37} Some studies showed an increased risk of all-cause and cardiovascular mortality in patients with subclinical hyperthyroidism, but others have shown no association.^{36,38–40} The strongest association of subclinical hyperthyroidism appears to be with atrial fibrillation. However, some studies suggest that this association may only be seen when TSH < 0.1 mIU/l.^{36,40}

Subclinical hyperthyroidism is also associated with a higher heart rate, higher frequency of premature atrial, and ventricular beats and ventricular hypertrophy,^{41,42} although the latter is not seen in all studies.^{43,44} Interestingly, as seen in subclinical hypothyroidism, subclinical hyperthyroidism is also associated with increased carotid artery intima-media thickness.⁴⁵ Regarding cardiac function, the possible association of subclinical hyperthyroidism with systolic and diastolic dysfunction is yet to be clarified, as there is evidence both for and against it.^{41,46}

Overt hyperthyroidism is defined as low TSH with high free T4. It is associated with a hyperdynamic state, characterized by tachycardia, increased cardiac preload and contractility, and diminished systemic vascular resistance. In the short term, it may improve cardiovascular function, improving both systolic function and left ventricular relaxation. However, when sustained, it may induce high-output HF, even in the absence of underlying heart disease.⁴⁷ Furthermore, overt hyperthyroidism is also strongly associated with atrial fibrillation.⁴⁸ Overt hyperthyroidism has also been associated with pulmonary hypertension.⁴⁹ Finally, untreated overt hyperthyroidism has consistently been associated with a higher risk of adverse cardiovascular events, as well as a higher risk of cardiovascular and all-cause mortality.^{21,50}

Modulation of diastolic function by thyroid hormones

Low thyroid hormone levels are associated with both systolic and diastolic dysfunction. However, both basic and clinical studies highlight that in hypothyroidism the diastolic abnormalities predominate.⁵¹ In a study of patients with subclinical hypothyroidism and matched controls, patients with subclinical hypothyroidism showed significant prolongation of the isovolumic relaxation time, increased A wave, and reduced E/A ratio (early to late ventricular filling velocities ratio).²⁷ Furthermore, in a subgroup of patients that were reevaluated after thyroid hormone profile normalization, diastolic abnormalities were reversed and comparable with controls.²⁷ Interestingly, the alterations in cardiac gene expression in HF is similar to the alterations observed in hypothyroidism.⁵²

Thyroid hormones also enhance relaxation through improving bioenergetics. Treating subclinical

hypothyroidism with levothyroxine improves cardiac phosphocreatine to ATP ratio,⁵³ which may be related to the effects of thyroid hormones in cardiac mitochondrial function, including stimulation of cardiac mitochondrial biogenesis and improvement in oxidative phosphorylation. Moreover, vascular effects of thyroid hormones may contribute to enhance diastolic function as well.³ Experimental data also suggest that it may decrease myocardial stiffness as a rat model of propylthiouracil-induced hypothyroidism showed increased LV stiffness due to increased collagen deposition, despite overexpression of the larger and more compliant (N2BA) isoform of titin.⁵⁴ Nevertheless, the effects on titin are not settled. Although thyroid hormones promote an increase in N2B/N2BA isoform ratio, it is possible that a higher titin phosphorylation mediated by PKG (secondary to improved endothelial function) and PKA (increased sensitivity to β -adrenergic stimulation) may outweigh the isoform shift effects on titin passive tension.

Pathophysiology of HFpEF

HFpEF is a clinical syndrome consisting of symptoms and signs of HF that cannot be attributed to other causes, despite normal LV EF on echocardiographic evaluation. From a pathophysiological point of view, it is characterized by diastolic dysfunction with abnormal relaxation and/or increased passive stiffness that manifests as prolonged isometric relaxation, slow left ventricle filling and increased diastolic stiffness.^{3,55} The myocardial stiffening in HFpEF can be ascribed to the giant cytoskeletal protein titin at physiological sarcomere lengths or to the extracellular matrix at higher sarcomere lengths. HFpEF patients show both increased collagen content and titin-dependent stiffness, which is related to isoform shifts or decreased phosphorylation by PKA, PKG, and CAMKII δ , though the latter seems to dominate.^{56,57} Changes in calcium kinetics, including increased diastolic calcium levels,⁵⁸ are important contributors to abnormal relaxation in HFpEF. Impaired myocardial bioenergetics has also been proposed as a key mechanism for development of HFpEF, as it impairs an effective relaxation.³

Recently, the focus has shifted from cardiac mechanisms to extra-cardiac disturbances. Arterial stiffness, poor ventricular-arterial coupling, increased central volume, impaired vasodilation, pulmonary

hypertension, endothelial dysfunction, and dysfunction of other tissues, including the lungs, skeletal muscle, adipose tissue, and kidneys, contribute to impaired cardiovascular reserve.^{3,59} Indeed, systemic involvement seems crucial in HFpEF. Patients are typically elderly, obese, with hypertension and diabetes, showing increased mortality due to non-cardiac causes when compared with HFrEF, and, therefore, warrant a strict control of the underlying comorbidities to improve cardiovascular reserve.

Abnormal thyroid function in HF

Hypothyroidism is one of the most frequent endocrine abnormalities in the general population. A prevalence of 4–20% has been reported for the spectrum of hypothyroidism (subclinical or overt) in the general population.¹ In HFpEF, the prevalence of hypothyroidism may be even higher as it is more common in women and the elderly – a group of individuals frequently diagnosed with HFpEF. In patients with HF (both HFrEF and HFpEF), non-thyroidal illness syndrome or low T3 syndrome is also common.²⁵ Upregulation of D3 is one of the main mechanisms of low T3 levels in these patients. D3 overexpression is a common inflammatory response seen in non-thyroidal illness syndrome. Recent studies evidence that D3 expression is enhanced in certain pathological contexts in a cell-specific manner.⁶⁰ Therefore, D3 upregulation in cardiomyocytes may contribute to the exacerbation of local cardiac hypothyroidism in association with decreased peripheral conversion of T4 to T3.⁶¹ This impaired peripheral conversion may be explained by the decreased activity of D2, seen in advanced heart disease.⁶² The exact mechanism by which D3 is enhanced is not fully understood; some studies show this may be mediated by inflammatory cytokines and catecholamines, both increased in HF.⁶³ This cell-specific regulation is important to take into account because it may be masked due to the maintenance of constant circulating thyroid hormones concentration.⁹ Lower T3 levels have been associated with increased cardiovascular mortality in HF, in patients with cardiovascular disease, and in the general population.^{25,64,65} Low T3 levels have also been associated with higher in-hospital and 1-year mortality in patients hospitalized for acute decompensated HF.⁶⁶ In a group of 89 consecutive patients with HFpEF, 22% had low T3 levels and 10% had elevated TSH. Low T3 was associated

with markers of severity, including BNP and echocardiographic parameters of diastolic dysfunction.⁶⁷ Changes in the gene expression associated with HF are similar to the fetal gene program and resembles that observed in hypothyroidism.⁶⁸ Therefore, local cardiac hypothyroidism may reduce Ca^{2+} transients and induce an α -MHC to β -MHC shift.⁶⁸ In an animal model of low T3 syndrome induced by chronic caloric deprivation, there was a significant decrease of SERCA2a and α -MHC with impairment of cardiac contraction and relaxation. T3 supplementation reverted these changes, highlighting the potential contribution of the low T3 syndrome to cardiac dysfunction.⁶⁹

In patients with normal TSH, T3, and T4 serum levels – normal systemic thyroid function – important changes in thyroid hormone effects may still be present. Several animal studies suggest that HF is associated with local tissue hypothyroidism. Different animal models in recent years have shown that HFrEF and several important risk factors for HFpEF, including ischemia, hypertension, and diabetes mellitus, induce an increase in the expression of cardiac D3, and, consequently, a decrease in local cardiac T3 levels – locally impaired thyroid function.⁷⁰ Most importantly, correction of cardiac hypothyroidism in animal models attenuated cardiac remodeling and myocardial dysfunction.⁷⁰ As shown by Trivieri *et al.*, enhanced D2 activity in a rodent model increases cardiac T3 levels, improves cardiac inotropism and prevents deterioration of cardiac function after pressure overload.⁷¹ In addition, D2 upregulation also reverses the expression of genes associated with pathological remodeling.⁷¹

Thyroid hormones as a therapeutic target in HFpEF

Given their cardiovascular effects, particularly concerning diastolic function, and the prognostic impact of thyroid function, modulation of thyroid hormone levels may constitute a promising therapeutic target in HFpEF (Figure 1). Indeed, diastolic dysfunction in hypothyroidism or subclinical hypothyroidism is reversible with thyroid hormone supplementation.²⁷ A randomized clinical trial of patients with advanced HFrEF and low T3 levels showed improved neuroendocrine profile and ventricular performance after short-term intravenous T3.⁷² In an animal model of myocardial infarction-induced HF, T3 replacement to

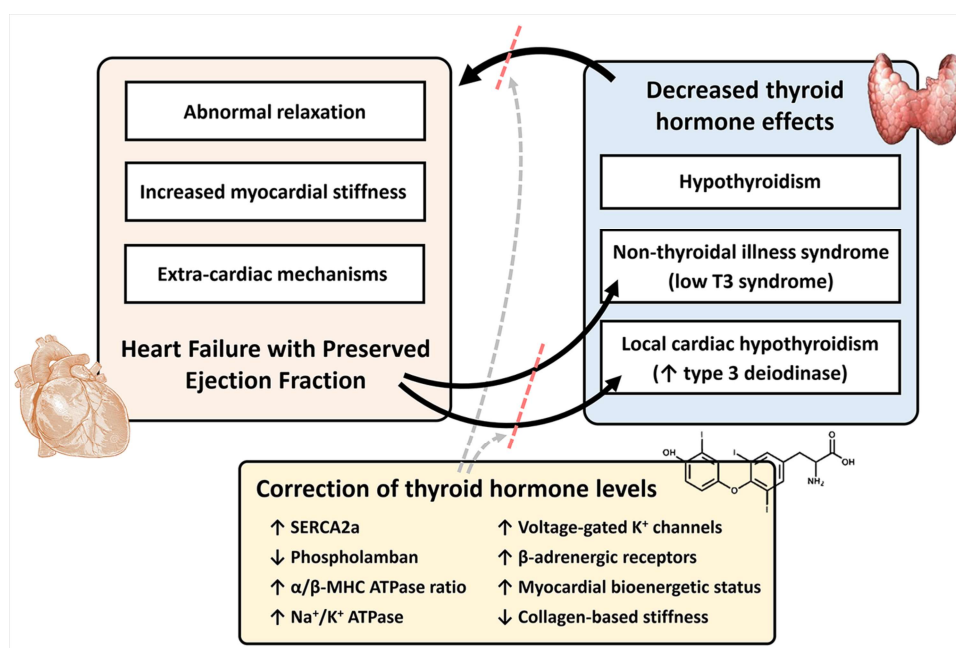


Figure 1. Decreased thyroid hormone effects worsen pathophysiologic changes of HFpEF. HFpEF is itself associated with low T3 syndrome and local cardiac hypothyroidism. Correction of tissue thyroid hormone levels has several effects that improve diastolic function and break the vicious cycle between cardiac dysfunction and decreased thyroid hormone effects, representing a promising therapeutic target in HFpEF. HFpEF, heart failure with preserved ejection fraction; MHC, myosin heavy chain; SERCA2a, sarco/endoplasmic reticulum calcium-ATPase; T3, triiodothyronine.

euthyroid levels improved both systolic and diastolic functions.⁷³ Even without primary thyroid disease or abnormal hormone plasma levels, thyroid hormone supplementation may have beneficial effects. Correction of local tissue hypothyroidism with thyroid hormone supplementation improved diastolic function in animal models of HF.^{74,75} It is important to highlight that treatment with thyroid hormones may improve symptoms and morbidity in HFpEF, not only due to cardiac actions but also to extra-cardiac effects, including decreased adiposity and improved endothelial function, arterial compliance, and skeletal muscle function.⁷⁶ Epicardial fat tissue has also been proposed as a cardiovascular risk factor, and it has been shown to be increased in hypothyroidism and in patients with HFpEF.⁷⁷ Thus, the decrease of the epicardial fat tissue, and, possibly, the modulation of the profile of adipocytokines secreted by adipose tissue may contribute to the benefits of thyroid hormone supplementation.⁷⁷

Thyroid hormone supplementation in HF has been studied mostly using HFrEF animal models.⁵¹ Furthermore, to this date, all clinical trials supplementing HF patients with thyroid hormones or their analogues refer to HFrEF (recently reviewed

by Razvi *et al.*).⁷⁸ Evidence from trials in HFrEF,⁷² and from trials in patients without HF, suggests a positive impact of thyroid hormone supplementation in diastolic function.^{72,79,80} However, clinical trials focused in HFpEF patients are necessary to fully understand the role of thyroid hormones as a potential therapeutic target for HFpEF.

The type of thyroid hormone to be used for the treatment of individuals with HF is an unsettled question. In patients with primary thyroid dysfunction, treatment with levothyroxine is the standard of care.⁸¹ The fact that patients with HF have decreased conversion of T4 into T3 suggests that a combination of levothyroxine and liothyronine could be associated with improvement of cardiac T3 levels. However, at the present time, there are no clinical studies to confirm this hypothesis. In patients with HF and low T3 syndrome, liothyronine may be the most appropriate approach from a pathophysiological perspective. Comparisons of liothyronine with levothyroxine or combined levothyroxine and liothyronine therapy in low T3 syndrome are also lacking.

The potential benefits of thyroid hormone supplementation should be weighed against the risks

of overtreatment. Subclinical hyperthyroidism has been associated with myocardial hypertrophy and dysfunction, and increased risk of arrhythmias, mainly atrial fibrillation.¹ It is also associated with increased risk of non-cardiovascular adverse consequences, including osteoporosis, anxiety, disturbances of sleep, and possibly cognitive dysfunction.¹ Patients treated with thyroid hormones should be monitored regularly, and dosage must be adjusted according to plasma hormone levels to avoid overtreatment.

The minimization of potential adverse effects may be a key factor for successful use of thyroid hormones in HFpEF. A significant part of cardiovascular adverse effects from thyroid hormones supplementation is related to an increase in sympathetic activity. In order to minimize cardiovascular risk, an interesting approach may be the co-administration of a beta blocker. This would decrease the risk of arrhythmias, myocardial hypertrophy, and tachycardia-mediated myocardial dysfunction, without affecting the direct inotropic effects of thyroid hormones.⁸²

An alternative approach to enhance thyroid hormone effects in patients with HFpEF, particularly in those with normal plasma thyroid hormones levels, would be the use of heart-specific TR agonists. This would avoid the possible extra-cardiac negative impact of thyroid hormone overtreatment, and would avoid the interference with the hypothalamus-pituitary-thyroid axis regulation. Although various thyromimetics that specifically target TR β have been developed, no effective TR α -specific or heart-specific thyromimetic is known at this moment. DITPA (3,5-diiodothyropropionic acid) was also proposed as a potential thyromimetic with beneficial cardiac effects. DITPA has inotropic selectivity, without significant tachycardic effect.⁸³ However, a multicenter clinical trial did not show improvement of clinical outcomes with DITPA in HFrEF.⁸³

The modulation of the local cardiac deiodinase system is also an interesting target to increase the myocardial concentration of T3 without undesirable extra-cardiac effects. As stated earlier, recent evidence shows that D2 and D3 are expressed in a dynamic balance to control intracellular T3 levels and upregulation of D3 is involved in the genesis of a local cardiac hypothyroid state in HFpEF.^{82,84,85} Changes in redox balance may be central to the upregulation of D3. Reactive

oxygen species (ROS) are known to disrupt peripheral deiodinase function, increasing D3 expression and activity, through mechanisms not yet fully understood.^{86,87} In addition, ROS production is also implicated in the pathophysiology of cardiac hypertrophy and remodeling, including in HFpEF.⁸⁴ Thus, when redox imbalance is corrected, improvements in cardiac structure and function are expected. This was demonstrated in several studies using N-acetylcysteine, a precursor of glutathione, in different experimental models of HF.^{88,89} A significant part of these effects may be mediated by modulation of metabolism of thyroid hormones. Indeed, a recent study in a male rat model of myocardial infarction showed that N-acetylcysteine is able to revert the cardiac hypothyroid state and improve cardiac performance.⁸⁷ Moreover, as N-acetylcysteine's effects are not heart-specific, it may also interfere with deiodinase action, particularly D3, in other tissues, contributing to the prevention or resolution of the non-thyroidal illness syndrome.^{87,90}

Conclusion

Thyroid hormones have an important role in cardiac and vascular function through genomic and non-genomic mechanisms. HFpEF is a clinical syndrome characterized by diastolic dysfunction and extra-cardiac disturbances, for which there is no proven therapy to improve its prognosis. Thyroid hormone axis modulation holds potential for improving the prognosis in patients with HFpEF. Although different therapeutic approaches may allow the optimization of thyroid hormone effects in HFpEF, it is still not clear which have more potential for clinical use. Furthermore, a more comprehensive characterization of the thyroid system in HFpEF patient cohorts and further pre-clinical tests in animal models of HFpEF are needed to hasten translation to clinical trials in a disease that has so far eluded conventional therapeutic approaches.

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Conflict of interest statement

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Treatment with thyroid hormones in heart failure has a narrow therapeutic window

One of the main findings from the HFpEF animal model was the absence of a linear dose-dependency of the benefits of supplementing with thyroid hormones. In fact, we observed that both low-dose and high-dose were associated with improvement of cardiac function, but only the high dose was associated with an increased risk of sudden death. Interestingly, while some effects of thyroid hormones were more marked with the high-dose than the lower-dose (high-dose had greater improvements in glycemic control and weight loss), others were more marked with the lower-dose (low-dose T3 decreased more the myocardial hypertrophy and fibrosis than the high-dose), and some adverse effects were observed only in the high-dose (increase in proinflammatory markers).

From a clinical perspective, our results in this animal model highlight that treatment with thyroid hormones in heart failure has a narrow therapeutic window. At this time, more studies are needed to understand how to offset the increased risk of adverse events with higher doses. There are several potential approaches that may contribute to increased safety of supplementation of thyroid hormones in patients with cardiac disease: 1) starting with lower doses and slowly titrating the thyroid hormones to narrow targets; 2) using markers of tissue effects of thyroid hormones (currently, good surrogate markers for the cardiovascular effects of thyroid hormones are not known^{125,126}); 3) using devices to monitor changes in heart rhythm and/or devices to revert arrhythmic events (e.g. implantable cardioverter-defibrillator); 4) combining supplementation of thyroid hormones with other cardioprotective agents (e.g. simultaneous treatment with beta-blockers to reduce the risk of arrhythmias).

While our results support thyroid hormones as a potential target for the treatment of heart failure, they also highlight the need to consider other approaches that may lower the risk of adverse events with thyroid hormones in cardiovascular diseases.

Prediabetes defined by HbA1c is a risk factor for kidney dysfunction and cardiovascular adverse events

The effect of prediabetes on the risk of kidney dysfunction and cardiovascular events has been controversial with different studies suggesting the presence or the absence of an increased risk of cardiorenal dysfunction. To answer this question, we evaluated two different populations: 1) participants included in the SPRINT (The Systolic Blood Pressure Intervention Trial) – at high risk of cardiovascular events, mostly without baseline kidney dysfunction, and with prediabetes evaluated by fasting plasma glucose; 2) participants included in the CRIC (Chronic Renal Insufficiency Cohort Study) – with baseline kidney dysfunction, and with prediabetes evaluated both by fasting plasma glucose and HbA1c.

Our results help to better understand the inconsistencies between previous studies. In the SPRINT study, where prediabetes was assessed only by fasting plasma glucose, there was no association between the presence of prediabetes and the progression of renal dysfunction. On the other hand, in the CRIC cohort, prediabetes was not associated with the decline of eGFR but was associated with proteinuria progression. Prediabetes was also associated with an increased risk of cardiovascular events and a trend to increased all-cause mortality. Interestingly, all these associations were observed when prediabetes was defined by HbA1c, but not when it was defined by fasting plasma glucose. These results suggest that 1) HbA1c is a better predictor of the risk of adverse cardiovascular events and progression of albuminuria than fasting plasma glucose; 2) the main component of kidney dysfunction observed with prediabetes is the progression of proteinuria. This is pathophysiologically plausible because proteinuria due to glomerular hyperfiltration is also the first clinical manifestation of kidney dysfunction in diabetes.¹²⁷

From a clinical standpoint, our results highlight the need for clinicians to assess HbA1c in patients at increased risk of cardiorenal dysfunction and the need to intensify interventions to reduce cardiorenal risk among those with increased HbA1c in the prediabetes range.

Clinical approach to patients with prediabetes must include interventions to reduce the cardiovascular risk

In prediabetes, most of the focus of clinical research has been on the risk of progression from the state of prediabetes to diabetes.¹²⁸ Despite the growing evidence of an increased risk of adverse cardiovascular events in those with prediabetes, as supported by the results of this thesis, most clinical guidelines have only recommended specific interventions to reduce the progression of prediabetes to diabetes.¹²⁹ The increased risk of cardiovascular events has not been adequately addressed by current recommendations.

A recent meta-analysis that analyzed 106 prospective studies, including our analysis of the CRIC cohort, showed that prediabetes is associated with increased risk for all-cause mortality, cardiovascular mortality, incident cardiovascular events, incident heart disease and incident stroke.¹³⁰ These associations varied according to the definition of prediabetes used. The associations were stronger with impaired glucose tolerance or HbA1c definition (particularly HbA1c 6.0-6.4%) than with impaired fasting glucose, which is also what we observed in our analysis of the CRIC cohort.¹³¹ This is relevant because it helps to stratify the risk of adverse cardiovascular events among patients with prediabetes.

From a clinical perspective, it is essential to implement strategies that reduce cardiovascular risk in patients with prediabetes. While a great success has been observed among patients with diabetes, where the risk of cardiovascular events has significantly decreased over the last decades due to intensive treatment of cardiovascular risk factors,²⁵ patients with prediabetes are still undertreated regarding cardiovascular risk factors.^{31,32} Given the need to improve the implementation of interventions that modify cardiovascular risk in prediabetes, we reviewed the epidemiology of dyslipidemia, cardiovascular disease and cardiovascular mortality in prediabetes and provided recommendations for reduction of cardiovascular risk in the following review article.



Article 15

Management of dyslipidemia and atherosclerotic cardiovascular risk in prediabetes

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Management of dyslipidemia and atherosclerotic cardiovascular risk in prediabetes

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ABSTRACT

Prediabetes affects at least 1 in 3 adults in the U.S. and 1 in 5 in Europe. Although guidelines advocate aggressive management of lipid parameters in diabetes, most guidelines do not address treatment of dyslipidemia in prediabetes despite the increased atherosclerotic cardiovascular disease (ASCVD) risk. Several criteria are used to diagnose prediabetes: impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and HbA1c of 5.7–6.4%. Individuals with prediabetes have a greater risk of diabetes, a higher prevalence of dyslipidemia with a more atherogenic lipid profile and an increased risk of ASCVD. In addition to calculating ASCVD risk using traditional methods, an OGTT may further stratify risk. Those with 1-hour plasma glucose ≥ 8.6 mmol/L (155 mg/dL) and/or 2-hour ≥ 7.8 mmol/L (140 mg/dL) (IGT) have a greater risk of ASCVD. Diet and lifestyle modification are fundamental in prediabetes. Statins, ezetimibe and PCSK9 inhibitors are recommended in people requiring pharmacotherapy. Although high-intensity statins may increase risk of diabetes, this is acceptable because of the greater reduction of ASCVD. The LDL-C goal in prediabetes should be individualized. In those with IGT and/or elevated 1-hour plasma glucose, the same intensive approach to dyslipidemia as recommended for diabetes should be considered, particularly if other ASCVD risk factors are present.

1. Introduction

As in type 2 diabetes (T2D), people with prediabetes have greater atherosclerotic cardiovascular disease (ASCVD) risk. Prediabetes is defined by impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or HbA1C between 5.7% and 6.4% (39–46 mmol/mol) (Table 1) [1,2]. Many people also have metabolic syndrome due to either dyslipidemia, hypertension, or obesity [1]. In some studies, people with metabolic syndrome experienced ASCVD events at levels almost equivalent to those with T2D [3].

Although global guidelines advocate aggressive lipid management in T2D, most have not asserted a similar position for prediabetes [1,4].

Literature describing the relationship between hyperlipidemia, prediabetes and ASCVD largely recommends diabetes preventive measures rather than specifically addressing the management of hyperlipidemia in prediabetes *per se* [1,4,5].

On a global level, prediabetes remains largely unrecognized; in one study 36% of people with acute coronary syndrome were newly diagnosed with prediabetes and 22% with T2D at the time of admission [6]. The US Preventive Services Task Force (USPTF) recommends that adults 35–70 years who are overweight or obese should be screened for both T2D and prediabetes [7].

The lack of recognition that prediabetes is a cardiovascular risk factor leads to a significant undertreatment of this condition. In a recent

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Table 1
Definition of prediabetes.

	ADA [1]	WHO [2]
Impaired Fasting Glucose (IFG)	5.6–6.9 mmol/L (100–125 mg/dL)	6.1–6.9 mmol/L (110–125 mg/dL)
Impaired Glucose Tolerance (IGT)	7.8–11.0 mmol/L (140–199 mg/dL) ^a	7.8–11.0 mmol/L (140–199 mg/dL) ^a
Elevated HbA1c	5.7–6.4% (39–46 mmol/mol)	–

^a 2-hour plasma glucose value during a 75-g oral glucose tolerance test.

Danish study, that included 326,305 Danish participants with a first HbA1c between 5.8% and 6.8% (40–51 mmol/mol), the highest risk of cardiovascular events and all-cause mortality was found in people with HbA1c below the diagnostic threshold for diabetes [8]. They were also less likely to receive prescriptions for glucose lowering drugs, statins, and renin-angiotensin system inhibitors, compared with others with newly diagnosed T2D.

This review will discuss the epidemiology of dyslipidemia, ASCVD and cardiovascular mortality in prediabetes. Furthermore, recommendations for lipid-lowering therapies are considered in light of the potential for the development of diabetes associated with statin therapy.

2. Epidemiology of dyslipidemia in prediabetes

Individuals with prediabetes have a higher prevalence of

dyslipidemia than those with normoglycemia. In NHANES 2011–2014, the prevalence of dyslipidemia defined as use of lipid-lowering drugs or non-HDL cholesterol ≥ 4.1 mmol/L (160 mg/dl) in prediabetes was 51.2% vs 31.0% in normoglycemia [9].

The typical pattern of dyslipidemia in prediabetes, similar to T2D, includes hypertriglyceridemia, reduced HDL cholesterol (HDL-C), and increased small dense atherogenic LDL particles [10], the prevalence varying depending on whether prediabetes is defined by IFG, IGT or HbA1C. In the Baltimore Longitudinal Study on Aging, individuals with isolated IGT had higher triglyceride and lower HDL-C levels than age-matched subjects with normal glucose tolerance or isolated IFG [11]. In the Insulin Resistance Atherosclerosis Study (IRAS), subjects with isolated IFG had higher apoB levels and total LDL particles; participants with isolated IGT had higher triglycerides, increased VLDL particles and small LDL; and participants with combined IFG/IGT had higher apoB, more LDL particles, higher triglycerides and lower HDL cholesterol [12]. In an unselected cohort of adults from Finland, participants with HbA1c in the prediabetes range had higher triglycerides and lower HDL-C than those with IFG. These people were likely similar to those with IGT in other studies as increased postprandial glucose with normal fasting glucose likely explains the elevation in HbA1c [13].

In summary, people with IGT have smaller LDL particles, higher levels of triglycerides and lower levels of HDL-C in comparison with prediabetes defined by either IFG or HbA1c. Whether this lipid pattern or other associated changes drive the higher ASCVD risk associated with IGT compared with IFG is unknown.

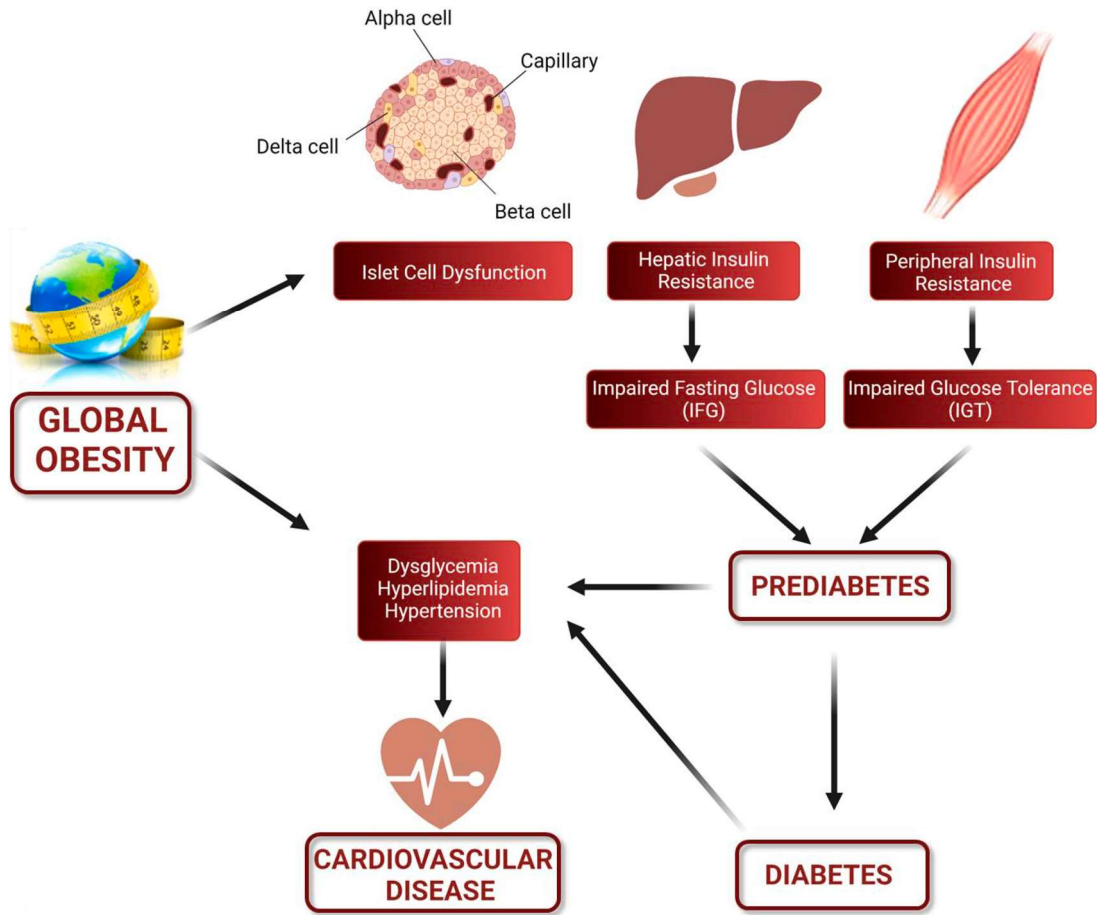


Fig. 1. Mechanisms of cardiovascular risk in prediabetes. The global obesity epidemic is associated with islet cell dysfunction, hepatic insulin resistance and peripheral insulin resistance, and is a main contributor to the high prevalence of prediabetes globally. In individuals with predominant hepatic insulin resistance, the phenotype of impaired fasting glucose (IFG) predominates, whereas in those with predominant peripheral insulin resistance, impaired glucose tolerance (IGT) is observed. Prediabetes is a risk factor for development of diabetes but, even in those who do not develop diabetes, dysglycemia, hyperlipidemia and hypertension associated with prediabetes lead to an increased risk of cardiovascular disease.

3. Mechanisms of atherosclerotic cardiovascular risk in prediabetes

Although the pathophysiology responsible for the increased risk of ASCVD in prediabetes and diabetes is an investigative area, clinical markers include hyperglycemia and related risk factors [14,15]. Obesity leads to insulin resistance and development of both T2D and prediabetes [15]. Individuals with prediabetes have a higher prevalence of hypertension, more atherogenic lipid profile, greater endothelial dysfunction and increased evidence for a prothrombotic state, each representing an independent CVD risk factor [14] (Fig. 1). Thus, dysglycemia *per se* causes or converges with processes that accelerate atherosclerosis.

Obesity, mainly with central (visceral) fat distribution, is a source of inflammatory cytokines associated directly and indirectly with vascular damage [16]. Insulin resistance leads to impaired signaling via the PI3-kinase pathway in endothelial cells reducing NO production with attenuation of the beneficial vascular effects of insulin (i.e., vasodilation, regulation of vascular smooth muscle proliferation and expression of adhesion molecules) [14,16]. In addition, insulin signaling through the mitogen-activated protein-kinase pathway (MAP-kinase) increases the growth-promoting effects of compensatory hyperinsulinemia, thereby favoring progression of atherosclerosis via mitogenesis and vasoconstriction [16] (Fig. 2).

Hyperglycemia also promotes oxidative stress, formation of advanced glycation end products and endothelial dysfunction. Although mean glucose levels in individuals with prediabetes assessed by HbA1c may be near-normal, glycemic excursion and variability may explain the increased risk of CVD. In a study of individuals with normal glucose regulation, prediabetes or newly diagnosed T2D, postprandial glucose excursions were associated with increased oxidative stress [17]. Several *in-vitro* studies have also shown that glucose oscillations promote endothelial dysfunction, oxidative stress and tissue damage in different organs [18]. Although the role of glycemic variability in prediabetes is not as well characterized as in T2D, the probable association of glycemic

excursions with CVD risk may explain the greater risk for CVD with IGT than IFG.

Hypertension is also a major risk factor for ASCVD in prediabetes [19]. Insulin resistance and hyperinsulinemia play pivotal roles in the pathogenesis of high blood pressure via reduced atrial natriuretic peptide and enhancement of renin-angiotensin-aldosterone activity as well as increased sympathetic tone [20]. Furthermore, both insulin resistance and dysglycemia are associated with a hypercoagulable state [21]. Alterations in the levels of coagulating proteins and impaired fibrinolytic activity, in conjunction with hyper-reactive platelets (adhesion, activation and aggregation), promote a prothrombotic state that with other pathophysiological defects contribute to ASCVD [22,23].

In summary, ASCVD in prediabetes is the consequence of multiple glycemic and non-glycemic risk factors including atherogenic dyslipidemia and hypertension, embedded in a background of insulin resistance and hyperinsulinemia.

4. Stratification of atherosclerotic cardiovascular risk in prediabetes

Prediabetes is associated with increased risk of ASCVD, and vascular and all-cause mortality [24]. However, the three categories (IFG, IGT and elevated HbA1c) that define prediabetes do not predict ASCVD equally. Cai et al. [25], in a *meta-analysis* of observational studies adjusted for potential confounders including participants with and without baseline ASCVD (129 studies, 10,069,955 participants) found that when compared to normoglycemia, prediabetes was associated with increased risk of all-cause mortality (relative risk [RR] 1.13, 95% CI 1.10–1.17), coronary heart disease (RR 1.16, 95% CI 1.11–1.21), and stroke (RR 1.14, 95% CI 1.08–1.20). In this *meta-analysis*, IGT was associated with a higher risk of ASCVD (RR 1.23, 95% CI 1.13–1.32 vs 1.09, 95% CI 1.03–1.15) and all-cause mortality (RR 1.25, 95% CI 1.17–1.32 vs 1.07, 95% CI 1.03–1.12) than IFG. In IFG, the risk of all-cause mortality was mainly associated with a fasting plasma glucose

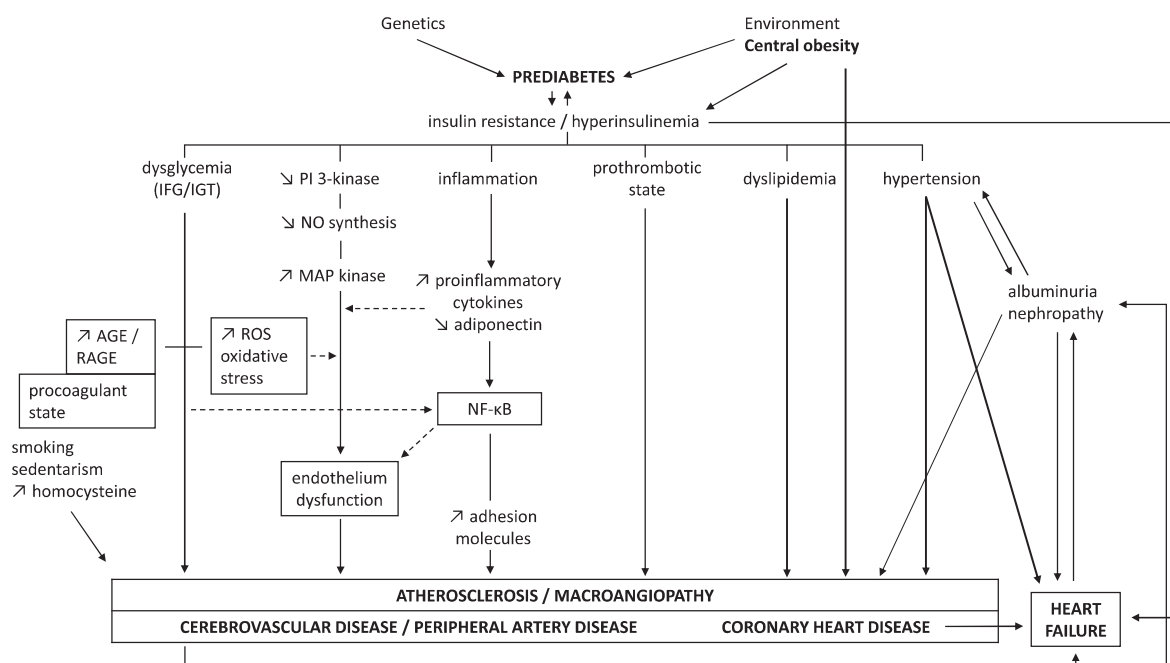


Fig. 2. Potential mechanisms for ASCVD risk in prediabetes. Prediabetes, in the context of insulin resistance/hyperinsulinemia is associated with atherosclerosis via glycemic and non-glycemic dysregulated metabolic pathways together with the presence of conventional cardiovascular risk factors (including obesity, dyslipidemia, hypertension, nephropathy and others). Coronary artery disease, hypertension, kidney dysfunction, as well as insulin resistance and dysglycemia are main pathophysiological conditions accounting for the development of heart failure in prediabetes. IFG = impaired fasting glucose; IGT = impaired glucose tolerance; PI3-kinase = phosphatidylinositol 3-kinase; NO = nitric oxide; ROS = reactive oxygen species; MAP-kinase = mitogen-activated protein-kinase; NF-κB = transcription factor nuclear factor-κB; AGE = advanced glycation end products; RAGE = receptor for advanced glycation end products; CMO = cardiomyopathy.

(FPG) between 6.1 and 6.9 mmol/L (110–125 mg/dL), consistent with the WHO definition of prediabetes [25].

An elevated 1-hour plasma glucose is associated with biologic markers demonstrating increased risk for ASCVD [26] and is more sensitive than an elevated 2-hour plasma glucose (IGT) for predicting microvascular and macrovascular complications, as well as mortality in long-term follow-up studies [27,28]. Individuals with both a 1-hour ≥ 8.6 mmol/L (155 mg/dL) and 2-hour ≥ 7.8 mmol/L (140 mg/dL) (IGT) have an even greater risk of these complications. Determination of the 1-hour plasma glucose may allow for the identification of high-risk individuals even if they do not meet the 2-hour plasma glucose criteria for IGT [28].

Whether the risks associated with prediabetes are similar in older, compared to younger, people is uncertain given the lack of robust comparative data. In the Atherosclerosis Risk in Communities Study (ARIC) study, the prevalence of prediabetes (defined by HbA1c and FPG) was high among older adults (71 to 90 years), however, the progression from prediabetes to diabetes was uncommon, and the regression to normoglycemia or death was more frequent than progression to T2D [29]. A relevant limitation of this study is the absence of OGTT data. HbA1c may be particularly inaccurate in older people because of an increase of HbA1c with aging that is independent of glucose levels, and a higher prevalence of comorbidities that can affect the lifespan of red blood cells [30].

The assessment of ASCVD risk in adults (irrespective of glycemic status) should focus on traditional risk factors that can be used to calculate the 10-year ASCVD risk [31]. The 2019 ACC/AHA guidelines on primary prevention of ASCVD considers diabetes a major risk factor for ASCVD and recommends the use of at least a moderate-intensity statin in adults between 40 and 75 years with T2D, regardless of the calculated 10-year ASCVD risk [31]. Prediabetes *per se* is not considered in the ACC/AHA risk assessment. However, the metabolic syndrome

which includes IFG is considered an ASCVD “risk-enhancer” [31]. The 2021 ESC guidelines on cardiovascular disease prevention [32] use the risk estimation system SCORE2 (or SCORE2-OP for people aged ≥ 70 years) to calculate the 10-year risk of fatal and non-fatal ASCVD events. Certain individuals are at high or very high ASCVD risk without the need for risk scoring, including those with T2D. The ESC guidelines do not make specific recommendations for people with prediabetes. On the other hand, AACE/ACE guidelines recommend that people with prediabetes should be considered at increased risk for ASCVD and advocates the same lipid treatment goals as for diabetes (Table 2) [33].

In summary, prediabetes is associated with increased risk of ASCVD. This association is stronger among those with IGT. The determination of a 1-hour plasma glucose may further help stratify the risk of ASCVD as this is a more sensitive marker than the 2-hour plasma glucose. People having both 1-hour ≥ 8.6 mmol/L (155 mg/dL) and 2-hour ≥ 7.8 mmol/L (140 mg/dL) are at even greater ASCVD risk. The stratification of ASCVD risk in people with prediabetes should also include a careful assessment of common associated risk factors.

5. Lipid-lowering therapies in prediabetes: Atherosclerotic cardiovascular outcomes and risk of progression to diabetes

Several lipid-lowering drugs were found to reduce ASCVD in randomized clinical trials (RCTs). Current guidelines recommend statins as first line pharmacological therapy, and either ezetimibe or PCSK9 inhibitors for additional LDL-C reduction [32]. Statins and PCSK9 inhibitors demonstrated their protective effects in analyses of subgroups with prediabetes as detailed below. There is evidence that ezetimibe can reduce ASCVD when combined with statins [34] although no data is available on the subgroup with prediabetes. While many ASCVD outcome trials focused on secondary prevention, almost all therapies that reduce recurrent events provide benefit for primary prevention. In

Table 2

Society guidelines for stratification of ASCVD risk and treatment of dyslipidemia.

ACC/AHA 2019 [31]	ESC 2021 [32]	AACE/ACE 2017 [33] (2020 update)	Endocrine Society 2020 [61]
<i>Summary of main recommendations</i>			
- In clinical ASCVD initiate high-intensity statin treatment to reduce LDL-C by $\geq 50\%$. - If LDL-C level ≥ 4.9 mmol/L (190 mg/dL), initiate high-intensity statin therapy. - In diabetes with 40–75 years of age and LDL-C ≥ 1.8 mmol/L (70 mg/dL), start moderate-intensity statin therapy (if multiple ASCVD risk factors consider high-intensity statin to reduce LDL-C levels by 50% or more). - In adults 40–75 years of age without diabetes and with LDL-C levels ≥ 1.8 mmol/L (70 mg/dL), calculate the 10-year ASCVD risk and consider statin treatment (if risk $\geq 7.5\%$, start a moderate-intensity statin after discussion of treatment options). Risk enhancing factors favor statin treatment if borderline risk ($5 < 7.5\%$) or intermediate risk ($\geq 7.5 < 20\%$). If a decision about therapy is uncertain, consider measuring CAC. Reduce LDL-C by $\geq 30\%$ (if 10-year risk is $\geq 20\%$, reduce by $\geq 50\%$).	LDL-C goals according to risk: - Very-high risk (goal of <1.4 mmol/L [<55 mg/dL] and $\geq 50\%$ reduction): documented ASCVD (clinical or unequivocal on imaging), diabetes with severe target organ damage; FH with major risk factor; severe CKD; very high ASCVD risk according to SCORE2 or SCORE2-OP. - High risk (goal of <1.8 mmol/L [<70 mg/dL] and $\geq 50\%$ reduction): FH without major risk factors; diabetes without ASCVD or severe target organ damage and not fulfilling the moderate risk criteria; moderate CKD; high ASCVD risk according to SCORE2 or SCORE2-OP. - Low-to-moderate risk (LDL-C goal of <2.6 mmol/L [<100 mg/dL]): Well controlled short-standing diabetes (<10 years) without target organ damage or ASCVD risk factors; low-to-moderate ASCVD risk according to SCORE2 or SCORE2-OP.	LDL-C goals according to risk: - Extreme risk (goal of <1.4 mmol/L [<55 mg/dL]): progressive ASCVD including unstable angina; clinical ASCVD plus diabetes or CKD stage 3 + or FH; or history of premature ASCVD. - Very high risk (goal of <1.8 mmol/L [<70 mg/dL]): Clinical ASCVD; 10-year risk $> 20\%$; diabetes with ≥ 1 risk factors; CKD stage 3 + with albuminuria; FH. - High risk (LDL-C goal of <2.6 mmol/L [<100 mg/dL]): ≥ 2 risk factors and 10-year risk of 10–20%; CKD stage 3 + or diabetes without risk factors. - Moderate risk (LDL-C goal of <2.6 mmol/L [<100 mg/dL]): < 2 risk factors and 10-year risk of $<10\%$. - Low risk (goal of <3.4 mmol/L [<130 mg/dL]): no risk factors.	- In T2D with CV risk factors, statin should be initiated if LDL-C > 1.8 mmol/L (70 mg/dL). LDL-C should be <1.4 mmol/L (55 mg/dL) in patients with ASCVD or multiple risk factors. - In T1D with ≥ 40 years of age or with > 20 years of disease or microvascular complications, initiate statin. - In persistent endogenous Cushing syndrome, initiate statin therapy irrespective of the cardiovascular risk score. - In patients without established ASCVD or long-standing diabetes, calculate the 10-year ASCVD risk. Assess for risk-enhancing factors, including persistent Cushing syndrome, high-dose chronic glucocorticoids, and possibly adult GHD, acromegaly, and hypothyroidism. In patients with borderline-to-intermediate risk, consider a measuring CAC, particularly when risk enhancing factors are present.
<i>Recommendations for prediabetes</i>			
No specific recommendation for prediabetes (metabolic syndrome is considered a risk enhancer).	No specific recommendation for prediabetes.	Individuals with prediabetes, are considered at increased risk for ASCVD. Lipid treatment goals should be the same in individuals with prediabetes as in individuals with diabetes.	No specific recommendation for prediabetes (metabolic syndrome is considered a risk enhancer).

ASCVD: atherosclerotic cardiovascular disease; CAC: coronary artery calcium; FH: familial hypercholesterolemia; SCORE: Systematic Coronary Risk Estimation 2; SCORE2-OP Systematic Coronary Risk Estimation 2-Older Persons; CKD: chronic kidney disease.

RCTs and *meta*-analyses, treatment with statins increased newly diagnosed diabetes, particularly in those with risk factors for T2D. As individuals with prediabetes are at particularly high risk of progressing to T2D, the benefit of LDL-C lowering in reducing ASCVD events must be weighed against the potential for development of diabetes [35].

The first prospective study of newly diagnosed diabetes was the Justification for the Use of Statins in Prevention (JUPITER) [36]. During a median follow-up period of 1.9 years, LDL-C was reduced by 50% in the rosuvastatin group and the hazard ratio (HR) for major cardiovascular events was 0.56 (95% CI 0.46–0.69; $p < 0.00001$). Physician-reported diabetes was significantly greater with rosuvastatin compared to placebo (270 vs 216 reports, $p = 0.01$). In addition, median HbA1c was significantly higher in the rosuvastatin treatment group compared to the placebo group (5.9% [41 mmol/mol] vs 5.7% [39 mmol/mol]) [36]. Among participants with risk factors for diabetes at study entry (metabolic syndrome, ADA-IFG, BMI ≥ 30 kg/m² or HbA1c $> 6.0\%$ [>42 mmol/mol]), rosuvastatin prevented cardiovascular events (HR 0.61, 95% CI 0.47–0.79) but increased risk for diabetes (HR 1.28, 95% CI 1.07–1.54); for every 54 cases of new diabetes, 134 vascular events or death were avoided [37]. Post-hoc analyses of other trials with statins demonstrated a significant reduction of major coronary events in secondary prevention in those with IFG [38]. Low-intensity statins have a small adverse effect on glycemic control in patients with diabetes. A pooled analysis of 9 RCTs in 9969 patients with T2D found an average 0.12% increase in HbA1C with low-intensity statin therapy [39].

Table 3 summarizes the results of *meta*-analyses of 16 RCTs comparing statins to placebo or no treatment and 5 RCTs comparing intensive to moderate dose statin therapy. There was a 10% relative increased odds of newly diagnosed diabetes in statin-treated patients (vs placebo) followed for a median of 4.8 years (absolute risk 4.9% vs 4.4%), and a 12% relative increased odds of diabetes in those taking intensive doses (vs moderate) over a median of 4.9 years (absolute risk 8.8% vs 8.0%) [40].

Alternative strategies for intensive lipid-lowering treatment using lower intensity statin therapy (e.g., low-intensity statin combined with ezetimibe) might allow LDL-C reduction with a lower risk of new onset diabetes. This approach has not been evaluated in RCTs and assumes that LDL-C reduction by any means should have similar benefits, i.e., that statins in high dose do not provide additional benefits.

In a pre-specified analysis of the ODYSSEY OUTCOMES trial, the relative risk reduction of the primary composite ASCVD outcome with the PCSK9 inhibitor alirocumab in participants with prediabetes (defined by HbA1c and ADA-IFG) was similar to the reduction in participants with normoglycemia or diabetes (HR of 0.85 for normoglycemia, 0.86 from prediabetes and 0.84 for diabetes) [41]. In the FOURIER trial, evolocumab consistently reduced cardiovascular outcomes in patients with and without diabetes [42], which suggests that the benefit may be similar among patients with prediabetes. In both trials, PCSK9 inhibitors did not increase the incidence of new onset diabetes nor increased HbA1c [41,42].

In contrast to statins, bile acid sequestrants (BAS) improve glycemic control. In a *meta*-analysis of 17 trials, randomization to BAS was associated with a reduction in HbA1c by 0.55% (95% CI -0.64 to -0.46) [43]. The clinical utility of BAS is limited by the absence of

ASCVD outcome data when used in combination with statins and by the observed increase in triglyceride levels.

Bempedoic acid was recently approved by the FDA for adults with heterozygous familial hypercholesterolemia or established ASCVD who require additional lowering of LDL-C. The CLEAR OUTCOMES trial is an on-going RCT evaluating the reduction in the risk of ASCVD with bempedoic acid in those with statin intolerance. In a *meta*-analysis of 5 RCTs, bempedoic acid reduced new onset or worsening of diabetes by 34% (95% CI 10–52%) [44]. However, there were many limitations of this *meta*-analysis, including very short follow-up time, lack of a separate analysis of new onset diabetes, and analysis of group level rather than participant level data. Furthermore, bempedoic acid is associated with increased uric acid levels and gout attacks [44].

The impact of intensive LDL cholesterol-lowering treatment for prevention of major vascular events seems to be independent of glycaemic status [45]. In a *meta*-analysis of 52 RCTs of statins, ezetimibe, and/or PCSK9 inhibitors, each 1 mmol/L (39 mg/dL) reduction in LDL-C was associated with a 19% relative risk reduction for major vascular events; there were no differences in the relative risk reduction between participants with and without diabetes [46].

In summary, current evidence suggests that the benefits of lipid-lowering therapies on the reduction of ASCVD in prediabetes are similar to those observed in diabetes. While the relative risk reduction of ASCVD with lipid-lowering therapies appear to be similar in individuals with normoglycemia, prediabetes and diabetes, the absolute risk reduction is greater in prediabetes or diabetes due to the greater absolute risk of ASCVD. In people with prediabetes, high-intensity statins increased risk of new onset diabetes, but markedly reduced ASCVD events.

6. Treatment of dyslipidemia and reduction of atherosclerotic cardiovascular risk in prediabetes

6.1. Lifestyle modification and weight loss

Diet and lifestyle modification are fundamental for improved glucose control although their benefit for reduction of ASCVD risk has not been established. In the Diabetes Prevention Program (DPP), weight loss of 7–10%, achieved by a low-fat diet and increased physical activity, delayed progression from prediabetes to diabetes [47]. In this study, 5 kg weight loss was associated with a 55% reduction in diabetes over a mean of 3.2 years. Participants randomized to intensive lifestyle group in the DPP also experienced a reduction in triglycerides, an increase in HDL-C and a modest reduction in systolic blood pressure compared to placebo [47]. However, there was no reduction in cardiovascular events with follow-up over 21 years in the Diabetes Prevention Program and Its Outcome Study (DPPOS) perhaps related to the availability of lifestyle intervention in all participants, utilization of statin and antihypertensive agents, and reduction in metformin use during the study with administration of out-of-study metformin [48]. Furthermore, neither lifestyle intervention nor metformin reduced all-cause or cardiovascular mortality [49].

The effect of weight loss on dyslipidemia is variable with reduction in triglycerides greater than LDL-C. In a *meta*-analysis of 30 RCTs with 2434 subjects who were overweight or obese, lifestyle interventions

Table 3
New Diagnosis of Diabetes in Statin Trials: Group Level Meta-analyses.

Trials	New diabetes active arm (%)	New diabetes control arm (%)	Odds ratio (95% CI)
<i>Statin vs placebo or standard of care</i> 16 trials	2641/54 137 (4.9%)	2407/54255 (4.4%)	1.10 (1.03–1.18)
<i>Intensive vs moderate dose statin</i> 5 trials	1449/16 408 (8.8%)	1300/16344 (8.0%)	1.12 (1.04–1.22)

Ref. [40].

reduced triglycerides by 0.045 mmol/L (4 mg/dL) per kg weight loss, and LDL-C by 0.033 mmol/L (1.28 mg/dL) per kg weight loss at 12 months [50]. These data demonstrate that weight loss alone is unlikely to achieve the reduction in LDL-C needed for cardiovascular prevention.

6.2. Pharmacotherapy for prediabetes and associated reduction in ASCVD risk and risk factors

ADA recommends metformin for prevention of T2D in prediabetes, especially in those 25–59 years with BMI ≥ 35 kg/m², higher FPG (≥ 110 mg/dL; 6.1 mmol/L), and higher HbA1c ($\geq 6.0\%$; 42 mmol/mol), and in women with prior gestational diabetes mellitus [11]. Individuals with IGT should also be a priority group for treatment with metformin, since all participants included in the DPP trial had IGT. The effect of metformin on cardiovascular outcomes in patients with prediabetes and ASCVD is currently being studied in a secondary prevention trial, Investigation of Metformin in Pre-Diabetes on Atherosclerotic Cardiovascular Outcomes (VA-IMPACT; clinicaltrials.gov identifier: NCT02915198). Sodium glucose co-transporter 2 (SGLT2) inhibitors reduced ASCVD events in people with diabetes and established ASCVD (but not in primary prevention), and the glucagon-like peptide receptor agonists (GLP-1ras) reduced ASCVD in people with diabetes and previous ASCVD or cardiovascular risk factors [51]. Because SGLT2 inhibitors do not cause hypoglycemia and benefit those with heart failure even without T2D, their use in prediabetes (which increases the risk of heart failure [52]) would appear to be sensible. In the DAPA-HF trial, that included participants with heart failure and reduced ejection fraction, dapagliflozin reduced the incidence of diabetes by 32% among those

without diabetes [53].

GLP-1ras have become first-line therapy for weight reduction. In the STEP 1 trial that included adults with overweight/obesity but not diabetes, after 68 weeks of treatment, participants randomized to semaglutide 2.4 mg/week plus lifestyle intervention had a significantly lower risk of progression to diabetes (0.5% vs 3.0%) and a greater probability of reversion from prediabetes to normoglycemia (84.1% vs 47.8%) [54]. Thus, as a global approach, GLP1ras seem ideal for the management of prediabetes.

6.3. Treatment of hypertension

The combination of hypertension and prediabetes act synergistically to increase ASCVD. The ACC/AHA guidelines and the ESC guidelines recommend a blood pressure treatment goal $<130/80$ mmHg for most people with hypertension and diabetes [32,55]. ADA recommends a blood pressure $<130/80$ mmHg for individuals with diabetes at higher cardiovascular risk and a blood pressure target of $<140/90$ mmHg in those at lower risk. While there are no definitive recommendations regarding those with prediabetes, the beneficial effects of intensive blood pressure among those at higher cardiovascular risk may also apply to this population [56].

Angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) should be preferred in people with prediabetes. In a meta-analysis of 19 RCTs, treatment with ACE inhibitors or ARBs decreased risk of new-onset diabetes by 16% [57] whereas thiazide diuretics or β -blockers increased this risk by 20% and 48%, respectively.

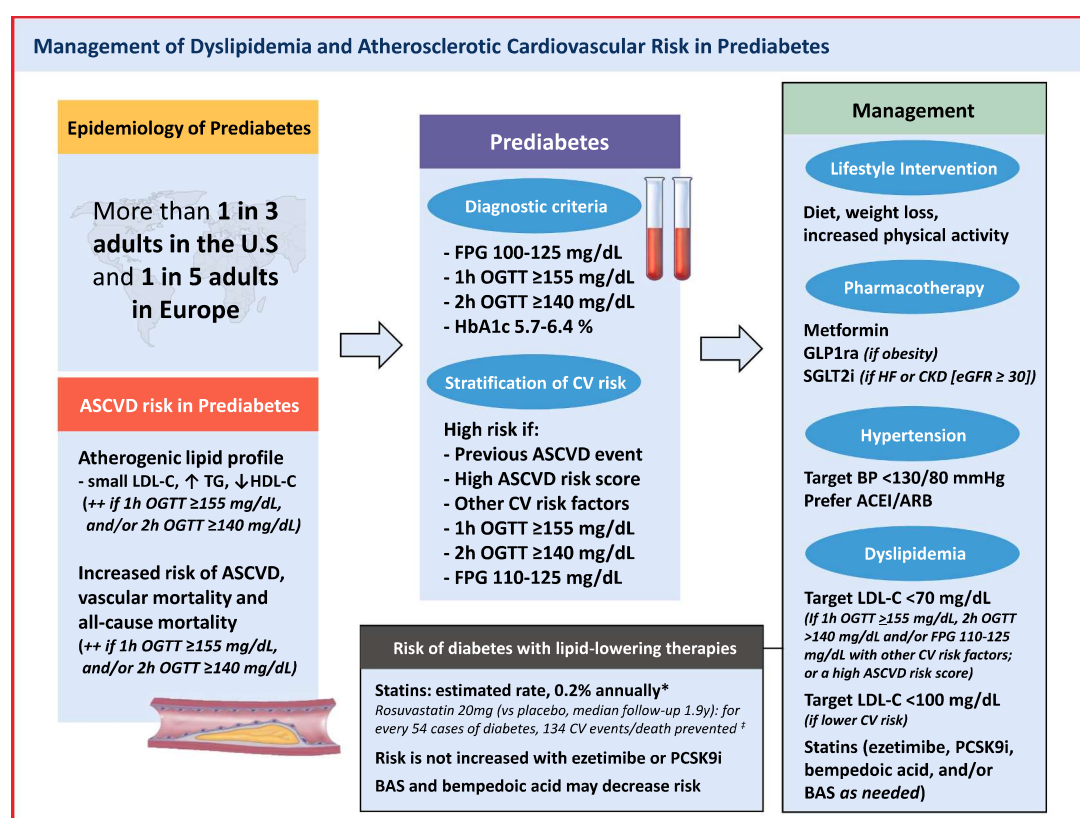


Fig. 3. Overview of the epidemiology, diagnostic criteria, ASCVD risk and management of prediabetes. * Rate is dependent upon the baseline risk of the population. † Data from the Justification for the Use of Statins in Prevention (JUPITER) trial. SI units: FPG 100–125 mg/dL = 5.6–6.9 mmol/L; FPG 110–125 mg/dL = 6.1–6.9 mmol/L; 1 h OGTT 155 mg/dL = 8.6 mmol/L; 2 h OGTT 140 mg/dL = 7.8 mmol/L; HbA1c 5.7–6.4 % = 39–46 mmol/mol; LDL-C 70 mg/dL = 1.8 mmol/L; LDL-C 100 mg/dL = 2.6 mmol/L. ACEi = Angiotensin-converting enzyme inhibitors; ARBs = angiotensin II receptor blockers; ASCVD = atherosclerotic cardiovascular disease; BAS = bile acid sequestrants; BP = blood pressure; CKD = chronic kidney disease; CV = cardiovascular; eGFR = estimated glomerular filtration rate (in mL/min/1.73 m²); FPG = fasting plasma glucose; GLP1ra = GLP1 receptor agonists; HDL-C = HDL cholesterol; HF = heart failure; LDL-C = LDL cholesterol; OGTT = oral glucose tolerance test; PCSK9i = PCSK9 inhibitors; SGLT2i = SGLT2 inhibitors; TG = triglycerides.

6.4. Treatment of hypercholesterolemia and ASCVD risk

LDL-C reduction with moderate-intensity statin therapy should be recommended as the initial step in those with hypercholesterolemia and risk of ASCVD. Ezetimibe could be added if additional LDL-C reduction is required. PCSK9 inhibitors could be added if a statin and ezetimibe do not provide sufficient reduction in LDL-C or are not tolerated. Alternatively, BAS or bempedoic acid may be used if cost of a PCSK9 inhibitor is prohibitive (Fig. 3).

The LDL-C goal should be customized based on the risk of ASCVD. In individuals with prediabetes, in addition to calculation of ASCVD risk using traditional methods, an OGTT with 1-hour and 2-hour time points may further stratify risk. An isolated FPG between 5.6 and 6.9 mmol/L (100–110 mg/dL) probably does not significantly increase the risk of ASCVD and therefore does not require pharmacotherapy. However, the presence of risk factors may justify a LDL-C target of <5.6 mmol/L (100 mg/dL). Those with a FPG between 6.1 and 6.9 mmol/L (110–125 mg/dL) and/or 1-hour $\geq$ 8.6 mmol/L (155 mg/dL) and/or 2-hour $\geq$ 7.8 mmol/L (140 mg/dL) (IGT), without other risk factors and with a low ASCVD score, a target LDL-C < 5.6 mmol/L (100 mg/dL) is reasonable. Otherwise, those with other risk factors or a high ASCVD risk score, the target LDL-C should be <3.9 mmol/L (70 mg/dL).

6.5. Hypertriglyceridemia

While LDL-C is the principal target of therapy to reduce ASCVD, those with prediabetes commonly have hypertriglyceridemia. Subgroup analysis of RCTs with fenofibrate in T2D have suggested ASCVD benefit in those with triglyceride levels > 2.3 mmol/L (200 mg/dL) and HDL-C levels < 1.0 mmol/L (40 mg/dL) [58]. However, most subgroup analyses were not pre-specified, and fenofibrate did not reduce ASCVD in the main analysis. In the REDUCE-IT trial that included high-risk individuals treated with statins, eicosapentaenoic acid (EPA) ethyl ester 4 g daily reduced the risk of ASCVD events although this was not related to baseline or on-treatment triglyceride levels [59]. More recently, the STRENGTH trial using a combination of EPA and docosahexaenoic acid (DHA) failed to show benefit in reducing ASCVD [60]. The difference in outcome may have been related to different control treatments (mineral oil versus corn oil) [60]. Nonetheless, many physicians may opt to use EPA until additional data are available, although this may increase the incidence of atrial fibrillation [59].

The primary treatment for elevated triglycerides is lifestyle therapy: avoidance of high glycemic index foods, weight loss, physical activity, and abstinence from alcoholic beverages [61]. Medications that increase triglycerides (e.g., glucocorticoids, oral estrogens, thiazide diuretics, β blockers, or second-generation antipsychotic agents) should be avoided [61]. In those with persistent elevations in triglycerides approximating 5.6 mmol/L (500 mg/dL), statins, which reduce triglycerides by 20–40%, may be used. If triglycerides $\geq$ 5.6 mmol/L (500 mg/dL), omega-3-fatty acids and fibrates may be used alone or added to statins [61].

7. Conclusions

Prediabetes is associated with an atherogenic lipid profile and increased risk of ASCVD. Intensive lifestyle modification should be offered to all individuals with prediabetes to decrease the risk of progression to diabetes. Treatment with statins reduces cardiovascular risk and provides greater benefit in higher risk populations as in those with multiple-risk factors or diabetes. The use of statins for secondary prevention in all individuals including those with prediabetes is supported by large RCTs and meta-analyses.

Due to the considerable time and substantial cost investment, a randomized controlled cardiovascular outcomes trial in prediabetes without other risk factors is unlikely. Therefore, in the absence of data how should the primary care physician or specialist approach this

situation? The association of IGT and an elevated 1-hour plasma glucose with macrovascular disease and mortality confers risk approximating that seen with T2D. In those with IGT and/or an elevated 1-hour plasma glucose, the intensive approach to lipid management recommended for T2D should be considered (LDL goal < 1.8 mmol/L [70 mg/dL]), particularly if other ASCVD risk factors are present. Given the prevalence of prediabetes estimated to occur in at least 1 in 3 adults in the U.S. and >1 in 5 adults in Europe, [7,62] this therapeutic approach is anticipated to improve cardiovascular disease and quality of life thus having a substantial impact on global public health.

Conflict of Interest

IJG has received consulting fees from Akcea and Arrowhead Pharma. All other authors have no conflict of interest to declare.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contribution

JSN and MB developed the original idea for the manuscript. All authors contributed to the design of the manuscript and to the first draft. All authors critically revised the manuscript and approved the final version.

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GLP-1 receptor agonists are an effective approach to reduce cardiovascular complications in type 2 diabetes

Cardiovascular diseases are among the leading causes of death and morbidity in patients with type 2 diabetes.¹³² In recent years, guidelines for the treatment of type 2 diabetes have moved from a glucocentric approach, where the main priority was to control HbA1c, to a more holistic approach where reduction of cardiovascular complications is one of the main priorities.¹³³ The 2022 consensus report of ADA/EASD (American Diabetes Association and the European Association for the Study of Diabetes) for the Management of Hyperglycemia in type 2 diabetes recommends that individuals with (or at high risk of) cardiovascular disease or chronic kidney disease should be treated with GLP-1 receptor agonists or SGLT2 inhibitors provided that these drugs have high-grade of evidence of cardiovascular benefits.¹⁴ Regarding the GLP-1 receptor agonists, a meta-analysis of 8 randomized clinical comprising 60080 patients showed that GLP-1 receptor agonists reduced major atherosclerotic cardiovascular events by 14% (HR 0.86 [95% CI 0.80-0.93]; $p<0.0001$), reduced all-cause mortality by 12% (HR 0.88 [95% CI 0.82-0.94]; $p=0.0001$), hospital admission for heart failure by 11% (HR 0.89 [95% CI 0.82-0.98]; $p=0.013$), and the composite kidney outcome by 21% (HR 0.79 [95% CI 0.73-0.87]; $p<0.0001$).⁸⁹ This meta-analysis showed no heterogeneity of effect according to structural homology, previous cardiovascular atherosclerotic disease, baseline HbA1c, body mass index, age or baseline eGFR.

However, two main questions remain unanswered from this meta-analysis: 1) are the benefits of GLP-1 receptor agonists maintained when combined with SGLT2 inhibitors? 2) are the effects of GLP-1 receptor agonists modified by the presence of heart failure?

In patients with high risk of atherosclerotic cardiovascular events, GLP-1 receptor agonists should be used even in patients already treated with SGLT2 inhibitors

Patients with type 2 diabetes are at increased risk of adverse cardiovascular events.¹³⁴ The risk of new cardiovascular events in patients with type 2 diabetes who have already had a previous atherosclerotic event is even greater, with a 2- to 3-fold increased risk compared to patients with type 2 diabetes without established cardiovascular disease and a 4- to 6-fold increased risk compared to subjects without diabetes or cardiovascular disease.¹³⁵

In patients with type 2 diabetes and previous cardiovascular events, a significant residual risk of subsequent adverse events is still observed among those treated only with SGLT2 inhibitors or GLP-1 receptor agonists. In the Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) trial, despite the 14% decrease in the risk of major atherosclerotic cardiovascular events (MACE) with empagliflozin, the risk of MACE during a median follow-up of 3.1 years was still 10.5% among those in the empagliflozin arm.¹³⁶ In the Dapagliflozin Effect on Cardiovascular Events (DECLARE-TIMI), the risk of MACE was 8.8% at 4.2 years in the group treated with dapagliflozin,¹³⁷ and in the Canagliflozin Cardiovascular Assessment Study (CANVAS) the rate of events was still 2.7 events per 100 patient-years in the group treated with canagliflozin.¹³⁸ The initiation of a GLP-1 receptor agonist in patients already treated with SGLT2 inhibitors may be an important intervention to further reduce the risk of adverse cardiovascular and renal events. However, before our analysis of the Harmony Outcomes trial and the meta-analysis with the AMPLITUDE-O trial, the evidence for such combination was very limited.

In our article analyzing the effects of GLP-1 receptor agonists with and without SGLT2 inhibitor in type 2 diabetes, we showed that the reduction in the risk of major adverse cardiovascular events and of heart failure hospitalization was similar among those with and without SGLT2 inhibitors. These findings support the use of GLP-1 receptor agonists in addition to SGLT2 inhibitors to reduce cardiovascular risk in type 2 diabetes with established

atherosclerotic cardiovascular disease. Interestingly, in the HARMONY Outcomes trial, the incidence rate of cardiovascular death, myocardial infarction, or stroke in participants without SGLT2 inhibitor and without GLP-1 receptor agonists (placebo arm) was 9.1 events per 100 patients-year, while in the group with SGLT2 inhibitor and albiglutide the incidence rate was 5.5 events per 100 patients-year. Similarly, comparing the same subgroups, the incidence rate of cardiovascular death was decreased from 2.7 to 1.9 events per 100 patients-year, the incidence rate of heart failure hospitalization was reduced from 2.4 to 1.0 events per 100 patients-year and all-cause mortality was reduced from 4.4 to 2.9 events per 100 patients-year. This represents a 30 to 60% lower risk of risk of these events in participants with combination therapy compared to those without SGLT2 inhibitors and without GLP-1 receptor agonists.

Several lines of evidence corroborate our findings that the benefits of SGLT2 inhibitors and GLP-1 receptor agonists may be additive. First, SGLT2 inhibitors and GLP-1 receptor agonists act through different mechanisms. While the protection from cardiovascular events with SGLT2 inhibitors may be predominantly related to renal and hemodynamic effects, the protection with GLP-1 receptor agonists appears to be mediated by anti-atherosclerotic effects.¹³⁹ Second, the effects of SGLT2 inhibitors and GLP-1 receptor agonists on cardiovascular risk factors are additive: the combination of SGLT2 inhibitors with GLP-1 receptor agonists is associated with a greater decrease of HbA1c, body weight and blood pressure than each drug alone.¹⁴⁰ Finally, observational data, with all their inherent limitations, also suggest an additional cardiovascular protection of combining SGLT2 inhibitors with GLP-1 receptor agonists compared with only one of these drug classes.^{141,142}

Our results support the need of revising current guidelines for the treatment of type 2 diabetes. At this moment, guidelines for the treatment of type 2 diabetes differ from guidelines in other fields concerning the use of multiple drugs with different mechanisms of action simultaneously.¹⁴ In other conditions with a high risk of adverse cardiovascular

outcomes, guidelines recommend the concomitant use of all interventions with clear evidence of benefit on major outcomes (e.g. treatment of acute myocardial infarction or treatment of HFrEF).^{23,143,144} The number of patients that were simultaneously treated with SGLT2 inhibitors and GLP-1 receptor agonists in our meta-analysis of Harmony Outcomes with AMPLITUDE-O (n=1174) is similar to those evaluated in trials of HFrEF that were treated simultaneously with SGLT2 inhibitors and angiotensin receptor-neprilysin inhibitors (ARNI) (n=1235).¹⁴⁵ It is consensual that SGLT2 inhibitors and ARNI should be used in patients with HFrEF, and we believe that a similar approach should be applied for patients with type 2 diabetes and established cardiovascular disease; i.e. GLP-1 receptor agonists should be used on top of SGLT2 inhibitors in type 2 diabetes with established cardiovascular disease, independently of HbA1c values, to further reduced the cardiovascular risk.¹⁴⁵

Cost-efficacy considerations must be taken before recommending GLP-1 receptor agonists and SGLT2 inhibitors to all patients with type 2 diabetes.¹⁴⁶ However, in those with a high risk of cardiovascular events, each of these drug classes has been shown to be cost-effective individually due to the reduction of the cost associated with the development of cardiorenal complications.^{147,148} Given the high residual risk with the use of only one of these drugs, it is expected that combination therapy remains cost-effective when the cardiovascular benefits are considered among those with previous cardiovascular events. Also, in a few years, some drugs in the SGLT2 inhibitor and GLP-1 receptor agonist classes will lose patent protection and cheaper generic drugs will likely be available. Our results are also relevant to this moment in time, where knowing that these drugs have additive benefits is likely to make this combination cost-effective for a broader group of patients.

In summary, our findings show that the cardiovascular benefits of GLP-1 receptor agonists are also observed in those treated with SGLT2 inhibitors. As such, in patients with type 2 diabetes and a high risk of atherosclerotic events, combination of SGLT2 inhibitors with GLP-1 receptor agonists should be used to reduce the cardiovascular risk.

GLP-1 receptor agonists must be used to prevent new-onset heart failure, are probably safe in HFpEF, but must be avoided in HFrEF

While GLP-1 receptor agonists are associated with clear benefits on atherosclerotic cardiovascular outcomes, their effects on heart failure outcomes are very heterogeneous across randomized controlled trials. As an example, in the GRADE (A Comparative Effectiveness Study of Major Glycemia-lowering Medications for Treatment of Type 2 Diabetes) the hazard ratio for heart failure hospitalization with liraglutide was 0.49 (95%CI 0.28 to 0.86),¹⁴⁹ while in the FIGHT trial the hazard ratio for the composite endpoint of emergency department visit, heart failure hospitalization, or all-cause death with liraglutide was 1.36 (95%CI 0.99 to 1.85).¹⁰¹ Heart failure status appears to be essential to understand the effects of GLP-1 receptor agonists on heart failure events. Our results suggest that GLP-1 receptor agonists may prevent new-onset heart failure in those without previous heart failure (most patients in the GRADE had a low risk of heart failure), while in those with HFrEF, GLP-1 receptor agonists increase the risk of adverse events (as shown by our analysis of the totality of events in the FIGHT trial).¹⁵⁰

The results of our analyses highlight that 1) in those without previous heart failure, GLP-1 receptor agonists should be used not only to prevent atherosclerotic cardiovascular events but also to prevent new-onset heart failure (in our meta-analysis stratified by heart failure status, among patients without baseline heart failure, the effect of GLP-1 receptor agonists on the reduction of heart failure events was similar to the reduction of atherosclerotic events); 2) in those with stable heart failure, GLP-1 receptor agonists have a neutral effect on heart failure events (it is plausible that GLP-1 receptor agonists decrease heart failure events in HFpEF and increase the risk in HFrEF); 3) in those with HFrEF, treatment with GLP-1 receptor agonists potentially increases the risk of arrhythmias and worsening heart failure events.

The mechanism underlying these effects and guidance for the use of GLP-1 receptor agonists in clinical practice are presented in the following review article.



Article 16

Glucagon-like peptide-1 receptor agonists across the spectrum of heart failure

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Glucagon-like peptide-1 receptor agonists across the spectrum of heart failure

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Abstract

Glucagon-like peptide-1 receptor agonists (GLP-1 RA) have been used to reduce body weight in overweight or people with obesity and to improve glycemic control and cardiovascular outcomes among people with type 2 diabetes (T2D) and a high cardiovascular risk. However, the effects of GLP-1 RA may be modified by the presence of heart failure (HF). In this review, we summarize the evidence for the use of GLP-1 RA across patient's risk with a particular focus in HF. After a careful review of the literature, we challenge the current views about the use of GLP-1 RA and suggest performing an active HF screening (with directed clinical history, physical examination, an echocardiogram, and natriuretic peptides) before initiating a GLP-1 RA. After HF screening, we suggest GLP-1 RA treatment decisions as follows: 1) in people with T2D without HF, GLP-1 RA should be used for reducing the risk of myocardial infarction and stroke, with a possible effect to reduce the risk of heart failure hospitalizations; 2) in patients with a heart failure and preserved ejection fraction (HFpEF), GLP-1 RA do not reduce HF hospitalizations but may reduce atherosclerotic events, and their use may be considered in an individualized manner; and 3) in patients with heart failure and reduced ejection fraction (HFrEF) and, until further randomized studies are available, GLP-1 RA should not be used due to potential increase in the risk of worsening HF events and arrhythmias.

Key-words: Glucagon-like peptide-1 receptor agonists; Heart failure; Risk modification.

Introduction

Glucagon-like peptide-1 receptor agonists (GLP-1 RA) have been used to reduce body weight in overweight or people with obesity and to improve glycemic control and cardiovascular outcomes among people with type 2 diabetes (T2D) and a high cardiovascular risk.¹⁻³

Despite the well-documented benefits of GLP-1 RA, these may not be generalized to all patient-populations. For example, in patients with heart failure (HF), particularly heart failure with reduced ejection fraction (HFrEF), some studies suggested that GLP-1 RA may produce harmful effects, increasing the risk of HF hospitalizations and ventricular arrhythmias.⁴⁻⁶ Despite these data, current recommendations do not provide guidance on GLP-1 RA prescription to patients with HF nor they differentiate between the prevention of HF in those at risk versus the treatment of patients with overt HF. Furthermore, these documents do not differentiate among those with different HF phenotypes.⁷

Based on the available data, we postulate that the decision to use a GLP-1 RA should be weighted versus the risk of each individual patient, particularly according to HF status, symptoms, and ejection fraction (EF). In this review, we provide a practical guidance for the use of GLP-1 RA according to patient's risk and HF status, backed by data from randomized controlled trials (RCTs).

Overweight or obese patients without diabetes or heart failure

Obesity is a major public health issue affecting approximately 40% of the adult population worldwide, and is associated with an increased risk of cardiovascular events¹. GLP-1 RA reduce body weight, as adjuncts to lifestyle intervention.

The potential cardiovascular benefits of GLP-1 RA in overweight or people with obesity without T2D are not well-established, mainly because GLP-1 RA trials in overweight or obese populations were relatively small or with a short follow-up time. Moreover, overweight or people with obesity without T2D or additional cardiovascular risk factors have low rate of cardiovascular events.

Still, a meta-analysis of RCTs including 11430 people overweight or people with obesity without T2D randomized to either a GLP-1 RA or placebo, suggested that GLP-1 RA might reduce the risk of any adverse cardiovascular event.³ The occurrence of HF events or cardiac arrhythmias was rare, in concordance with the low cardiovascular risk of this population.

The ongoing Semaglutide Effects on Heart Disease and Stroke in Patients With Overweight or Obesity trial (SELECT; NCT03574597) and Study of Tirzepatide on the Reduction on Morbidity and Mortality in Adults With Obesity (SURMOUNT-MMO; NCT05556512) will provide more robust evidence on the effect of the GLP-1 RA on cardiovascular outcomes in overweight or people with obesity without T2D. However, a recent meta-analysis suggested that GLP-1 RA potentially reduce cardiovascular events in overweight or people with obesity without HF.³

Type 2 diabetes without heart failure

Patients with T2D with high cardiovascular risk but without HF, benefit from GLP-1 RA for the reduction of atherosclerotic cardiovascular events (i.e., myocardial infarction, ischemic stroke, or cardiovascular death: 3P-MACE).

Several trials comparing GLP-1 RA vs. placebo performed analysis stratified on HF status. However, it is important to highlight that in these trials 80% or more of the enrolled patients did not have HF diagnosis, although an echocardiogram or natriuretic peptides were rarely available in these trials.⁸⁻¹⁵

Overall, the findings suggest that GLP-1 RA may be useful to prevent HF hospitalizations among patients without HF.^{9-14,16-21} Specifically, the effect of GLP-1 RA vs. placebo on the composite of HF hospitalizations or cardiovascular death in patients without HF history, gave a meta-analyzed pooled hazard ratio (HR) of 0.85, with a 95% confidence interval (95%CI) ranging from 0.76 to 0.94. More specifically, the effect of GLP-1 RA vs. placebo on HF hospitalizations alone yielded a borderline result, with a HR of 0.81, with 95%CI ranging from 0.63 to 1.03. The effect of GLP-1 RA vs. placebo on cardiovascular mortality gave a meta-analyzed HR of 0.85, with 95%CI ranging from 0.76 to 0.94. GLP-1 RA were also superior to placebo in reducing 3P-MACE, with a meta-analyzed HR of 0.88, 95%CI 0.83 to 0.93.²²

The GRADE (A Comparative Effectiveness Study of Major Glycemia-lowering Medications for Treatment of Type 2 Diabetes; NCT01794143) trial compared the effectiveness of four commonly used glucose-lowering medications (insulin glargine, glimepiride, liraglutide, and sitagliptin) on top of metformin.²³ Compared to all other treatments, assignment to liraglutide resulted in lower rate of HF hospitalizations with an HR of 0.49, 95%CI 0.28 to 0.86, but the number of heart failure events was small. and lower rate of death from cardiovascular causes with an HR of 0.47, 95%CI 0.23 to 0.93. Liraglutide also tended to reduce 3P-MACE with an HR of 0.75, 95%CI 0.54 to 1.03. Results according to HF subgroups were not presented in this analysis; however, few patients were expected to have symptomatic HF. The GRADE

population had a low cardiovascular risk, with overall HF hospitalization rates inferior to 0.4 events per 100 patient-years and cardiovascular death rates inferior to 0.3 events per 100 patient-years due to the exclusion of patients with a history of a major cardiovascular event in the year before randomization, HF with a New York Heart Association (NYHA) functional classification of III or higher, and an estimated glomerular filtration rate (eGFR) of less than 30 ml/min/1.73m².

Together, these results support the use of GLP-1 RA for reducing atherosclerotic events in patients with T2D without HF, with a possible effect to reduce heart failure hospitalizations.

Type 2 diabetes with heart failure and preserved ejection fraction

In contrast to patients with T2D without HF, those with HF (representing 15-20% of patients in trials) did not seem to benefit from GLP-1 RA for reducing HF hospitalizations or cardiovascular death. Still, GLP-1 RA may reduce atherosclerotic events in patients with T2D and HF.⁸⁻¹⁵

The effect of GLP-1 RA vs. placebo on the composite of HF hospitalizations or cardiovascular death in patients with HF gave a meta-analyzed HR of 0.96, 95%CI 0.84 to 1.08. The meta-analyzed effect on HF hospitalizations alone gave an HR of 0.95, 95%CI 0.81 to 1.11. The meta-analyzed effect on cardiovascular death gave an HR of 0.96, 95%CI 0.82 to 1.12.²²

Despite the neutral effect on HF hospitalizations and cardiovascular death, GLP-1 RA may be superior to placebo for reducing major atherosclerotic events in patients with T2D and HF, with a pooled 3P-MACE HR of 0.85, 95%CI 0.75 to 0.97.²²

The characterization of HF in T2D trials with GLP-1 RA was poor, mostly lacking detailed description on signs and symptoms, as well as natriuretic peptides and echocardiogram. Still, it is likely that most HF patients enrolled in T2D trials were stable outpatients with heart failure with preserved ejection fraction (HFpEF). This statement is supported with data from the EXSCEL (Exenatide Study of Cardiovascular Event Lowering Trial; NCT01144338) trial where ejection fraction (EF) data was available for 33.2% (n =4892) of the patients with 9.6% (n =469) having an ejection fraction lower than 40% i.e., >90% of the patients with echocardiographic data available had an ejection fraction of 40% or greater.¹⁷

Dedicated HFpEF trials with GLP-1 RA are ongoing (e.g., NCT04847557 and NCT04788511) and will provide more evidence about the effect of these agents in patients with HFpEF.

Based on available data, GLP1-RA did not reduce HF-related events in patients with T2D and HF, most of whom presumably had HFpEF. However, GLP-1 RA may be considered for reducing ischemic events in HFpEF patients with a high atherosclerotic risk. Notwithstanding, adequately powered trials should be conducted to test the effect of GLP-1 RA on cardiovascular (including HF) outcomes.

Heart failure with reduced ejection fraction

The effect of the GLP-1 RA liraglutide was studied in three small trials enrolling patients with heart failure with reduced ejection fraction (HFrEF).

Patients with severely symptomatic HFrEF who had been recently hospitalized for worsening HF were included in the FIGHT trial (the Heart Failure Network Functional

Impact of GLP-1 for Heart Failure Treatment; NCT01800968) and randomly assigned to either liraglutide (n =154) or placebo (n =146), and followed for 180 days.⁵ Despite no differences between liraglutide and placebo on the global rank score including time to death, time to HF rehospitalization, and time-averaged change in NT-pro BNP (primary outcome), and no differences in echocardiographic parameters or health status, liraglutide treatment showed a tendency for increasing the risk of rehospitalization for cardiovascular reasons (HR 1.33, 95%CI 0.95 to 1.85), HF re-hospitalizations (HR 1.33, 95%CI 0.83-2.12), and the risk of emergency department visit, HF hospitalization, or all-cause death (HR 1.36, 95%CI 0.99 to 1.85).⁵ In a post-hoc analysis of the FIGHT trial, reassessing the trial outcomes using total events (first and recurrent), a tendency towards increased risk of total HF hospitalizations or all-cause deaths (incidence rate ratio [IRR] 1.41, 95%CI 0.98-2.04) and total arrhythmias (IRR 1.76, 95%CI 0.92-3.37) was found with liraglutide. Of note, the risk of HF hospitalizations or all-cause deaths was higher among patients in NYHA class III-IV (interactionP=0.008), and the risk of arrhythmic events was higher among those without an implanted cardiac device (interactionP=0.047).⁴

Another relatively small (N =241) trial with a short follow-up (180 days) studying the effect of liraglutide vs. placebo in stable HFrEF was the LIVE trial (Effect of Liraglutide on Left Ventricular Function in Stable Chronic Heart Failure Patients with and without Diabetes). Despite patients in LIVE were more stable than in FIGHT, the results from LIVE also suggested an increased risk of adverse cardiac events with liraglutide, particularly ventricular tachycardias and atrial fibrillation.⁶

Another small trial (A Multi-center, Placebo-controlled Study to Evaluate the Safety of GSK716155 and Its Effects on Myocardial Metabolism, Myocardial Function, and Exercise Capacity in Patients With NYHA Class II/III Congestive Heart Failure;

NCT01357850) compared weekly placebo (n =30) to albiglutide 30 mg (n =27) during 12 weeks. No detectable effect of albiglutide on cardiac function or myocardial glucose use was found, but a modest increase in peak oxygen consumption was observed with albiglutide, which the authors attributed to noncardiac effects of albiglutide.²⁴

A post-hoc analysis of published EXSCEL data,¹⁷ suggested that patients with an EF <40% had an increased risk of HF hospitalizations with exenatide vs. placebo. In EXSCEL, there were 249 first HFH during a median follow-up of 3.2 years among the 4892 participants with baseline LVEF available (33.2% of EXSCEL population). A significant interaction of LVEF with the effect of exenatide on HFH was found with an increased risk of HF hospitalizations observed in patients with a LVEF <40%: LVEF >55% OR 0.73, 95%CI 0.48-1.13; LVEF 40-55% OR 0.77, 95% CI 0.50-1.20; LVEF <40% OR 1.70, 95%CI 1.02-2.83; interaction P=0.027. A meta-analysis of ESCEL and FIGHT trials suggested an increased risk of HF hospitalizations with GLP-1 RA in patients with LVEF <40% with little heterogeneity across studies: meta-analyzed OR 1.49, 95%CI 1.05-2.10 (overall treatment effect p-value =0.02 and heterogeneity $I^2=0\%$).²⁵

Together, these results suggest that GLP-1 RA may be associated with an increased risk of HF hospitalizations in patients with HFrEF and, until further randomized studies are available, GLP-1 RA should not be used in patients with HFrEF.

Integrated view of the role of GLP-1 RA across the spectrum of heart function

The evidence summarized above supports that the effects of GLP-1 RA are modified by HF status (*Figure 1*). Among people with T2D or obesity without HF treatment

with GLP-1 RA may be used to improve metabolic status and reduce atherosclerotic events, with a possible effect to reduce HF hospitalizations. In patients with stable HFpEF (15-20% of people with T2D) GLP-1 RA have not reduced HF hospitalizations or cardiovascular mortality but reduced atherosclerotic events and may be used in selected high-risk patients. In patients with HFrEF, GLP-1 RA may increase the risk of adverse outcomes, particularly worsening HF and ventricular arrhythmias, and the use of these agents should not be recommended (*Figure 1*).

Current guidelines support the use of GLP-1 RA or SGLT2 inhibitors (with or without metformin) for patients with T2D and cardiovascular disease, including HF.¹ The evidence here summarized challenges this “conventional” view by providing an “updated” view whereby ruling out the presence of HF, particularly HFrEF, is relevant for treatment decisions.

Practical guidance for the use of GLP-1 RA

The decision to use GLP-1 RA must be individualized. Before starting GLP-1 RA, all patients should be evaluated for symptoms and signs of HF and have natriuretic peptides (either BNP or NT-pro BNP) measured. If natriuretic peptides are elevated (>35 pg/mL for BNP and >125 pg/mL for NT-pro BNP) an echocardiogram should be performed, as recommended by international guidelines.^{26,27} This active HF screening allows the categorization of the patients in 3 categories:

- 1) No HF: GLP-1 RA are recommended for reducing atherosclerotic cardiovascular events in high-risk patients, with a possible effect to reduce HF hospitalizations.

- 2) HFpEF: After a SGLT2 inhibitor (first line agent), GLP-1 RA may be used in selected patients to reduce atherosclerotic cardiovascular events if the atherosclerotic risk is high.
- 3) HFrEF: GLP-1 RA should not be prescribed in patients with HFrEF, until further evidence is produced. SGLT2 inhibitor (first line) and metformin (second line) should be preferred. Given that other antidiabetic drugs may also not be appropriate in this setting (e.g., DDP4 inhibitors and thiazolidinediones may increase the risk of HF hospitalization, and sulfonylureas and insulin increase the risk of hypoglycemic events),²⁸ a higher HbA1c level may be acceptable in patients with HFrEF already on SGLT2 inhibitor and metformin. The risk of adverse events with GLP-1 RA in HFrEF may be particularly increased in those in NYHA class III-IV and in patients without an implanted cardiac device.⁴

These bullet-points are illustrated in *Figure 2* which may serve as a tool to support routine clinical decisions.

Mechanisms supporting the use of GLP-1 RA according to HF status

The mechanisms underlying the GLP-1 RA beneficial effects on HF prevention (among patients without HF), the neutral (HFpEF) or potentially harmful (HFrEF) effect in patients with HF are probably multifactorial. GLP-1 RA can reduce epicardial fat,^{28,29} an effect (together with the reduction of atherosclerotic cardiovascular disease) may explain why GLP-1 RA may possibly reduce HF hospitalizations (*Figure 1*). On the other hand, GLP-1 RA are known to increase heart rate which may be deleterious to HF patients.³⁰ Furthermore, the GLP-1 receptor, expressed in

cardiomyocytes and sinoatrial node cells, signals through a cyclic AMP (cAMP)-dependent pathway which may induce intracellular calcium overload and increase the risk of ventricular ectopy in high-risk patients, such as those with severely depressed LVEF.^{7,17,31-33}

Future perspectives

Notwithstanding the current evidence about the relevance of HF status for GLP-1 RA cardiovascular effects, further research in this field is required. Namely, more research is needed on the combined use of GLP-1 RA and SGLT2 inhibitors and on the efficacy and safety of GLP-1 RA (and dual glucose-dependent insulinotropic polypeptide [GIP] and GLP-1 RA agonists) among patients with HFpEF. In this regard, the effect of semaglutide in HFpEF will be studied in the STEP-HFpEF (Research Study to Investigate How Well Semaglutide Works in People Living With Heart Failure and Obesity; NCT04788511) trial, enrolling 516 patients with HFpEF and obesity, and in the STEP-HFpEF-DM (Research Study to Look at How Well Semaglutide Works in People Living With Heart Failure, Obesity and Type 2 Diabetes) trial, enrolling 610 patients with HFpEF and T2D. The ongoing SUMMIT (A Study of Tirzepatide in Participants With Heart Failure With Preserved Ejection Fraction and Obesity; NCT04847557) trial will assess the effect of tirzepatide in 700 patients with HFpEF and obesity. Despite the clinical importance of these trials, they will likely be underpowered to assess the impact of GLP-1 RA (or dual GIP/GLP-1 RA) on HF hospitalizations or mortality.

Ideally, further research on the use of GLP-1 RA in patients with HF (including HFrEF) should be incentivized by regulatory agencies, with further and larger

outcome RCTs testing the efficacy and safety of GLP-1 RA in HF patients across a wide range of ejection fractions. Nonetheless, based on the available data, enrolling patients with EF <40% possibly raises ethical issues. Still, based on the available evidence, these agents should not be prescribed in patients with HFrEF, until further evidence is produced.

Conclusion

This paper provides evidence for an “updated” view of the use of GLP-1 RA in clinical practice based on HF status. After an active HF screening, if the patient has T2D and atherosclerotic risk without HF, then GLP-1 RA should be considered for improving cardiovascular outcomes. If the patient has T2D and HFpEF a GLP-1 RA may be considered, after a SGLT2 inhibitor (first line agent) if the atherosclerotic risk is high. However, if the patient has HFrEF GLP-1 RA should not be prescribed, until further evidence is produced.

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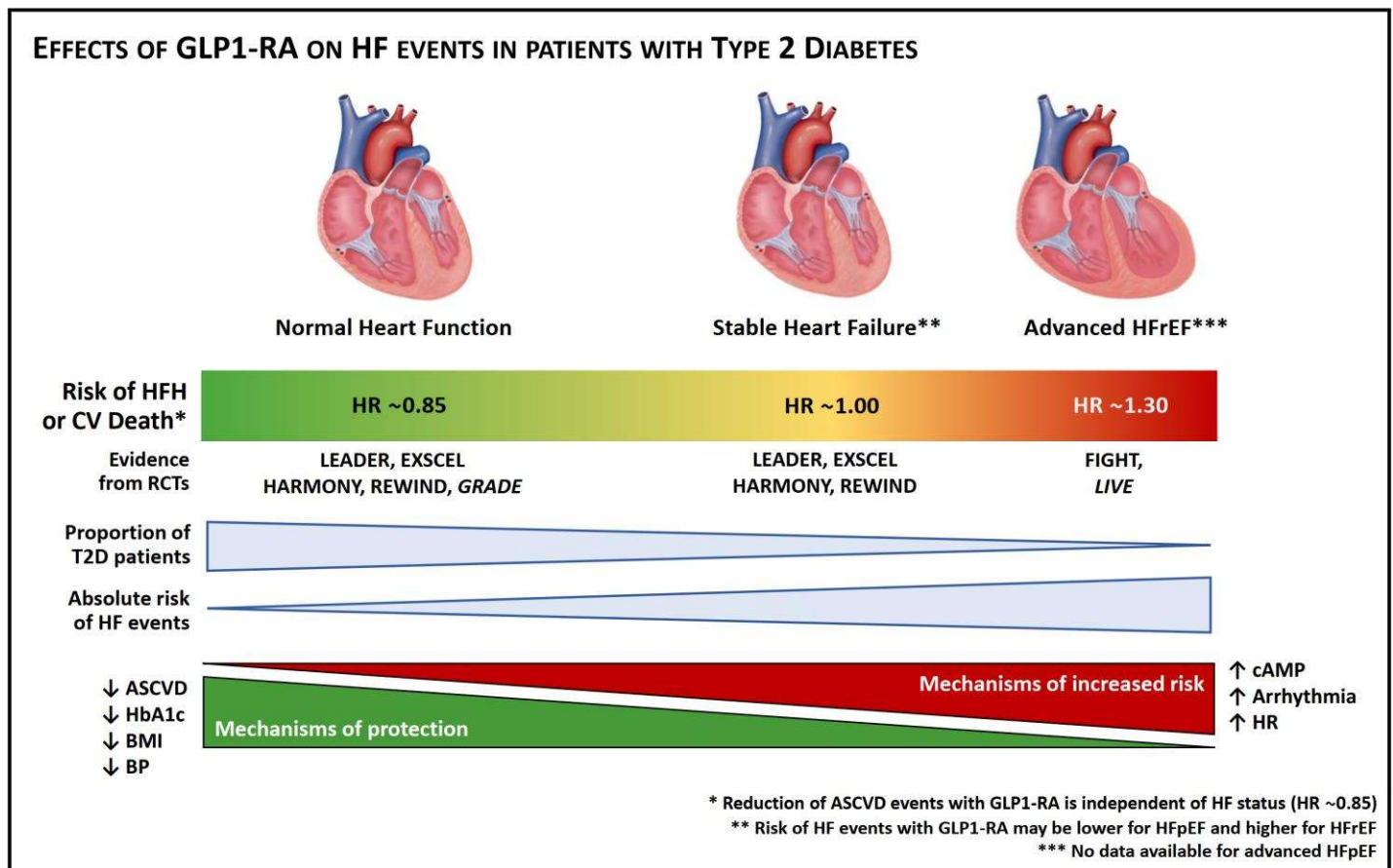
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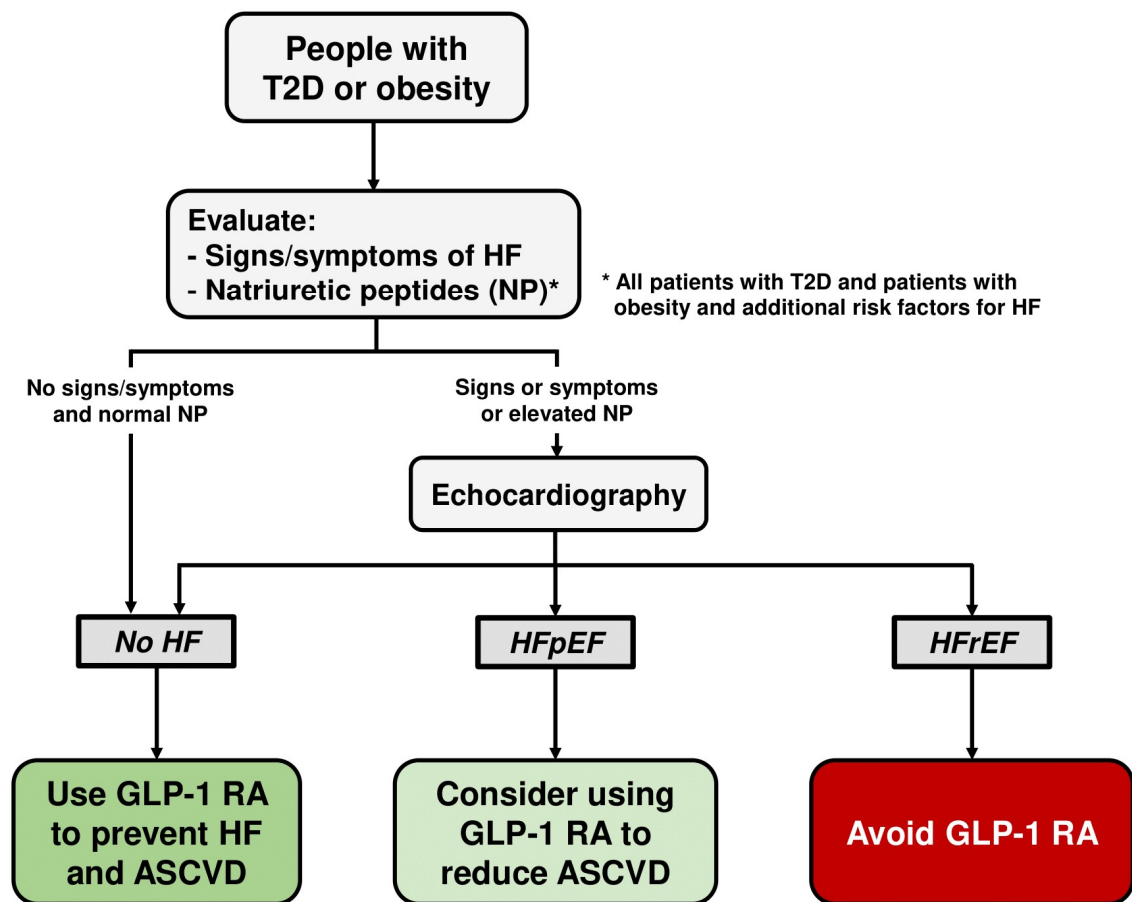
Figure 1. GLP-1RA and heart failure outcomes across patient's risk.



Legend: GLP-1RA, glucagon-like peptide-1 receptor agonists; HFH, heart failure hospitalization; CV, cardiovascular; RCT, randomized controlled trial; T2D, type 2 diabetes; ASCVD, atherosclerotic cardiovascular disease; HbA1c, glycated hemoglobin; BMI, body mass index; BP, blood pressure; HR, heart rate; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction.

Caption: GLP-1 RA reduce HF hospitalizations or cardiovascular death in patients with T2D without HF, are neutral regarding HF-related events in patients with T2D with HFpEF and may be harmful to patients with HFrEF.

Figure 2. Updated guidance for GLP-1 RA use



Legend: ASCVD, atherosclerotic cardiovascular disease; GLP-1 RA, glucagon-like peptide-1 receptor agonists; HF, heart failure; T2D, type 2 diabetes; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction, NP, natriuretic peptides.

Caption: Patients should be screened for heart failure before starting GLP-1 RA. Signs and symptoms of HF must be evaluated in all patients, and natriuretic peptides should be evaluated in all patients with T2D and in patients with obesity and additional risk factors for HF. Echocardiography should be performed in patients with signs/symptoms of HF or with elevated natriuretic peptides. GLP-1 RA should be used in patients without HF to reduce atherosclerotic events and prevent new-onset HF. GLP-1 RA may be used in selected patients with HFpEF to reduce ASCVD but not to reduce HF hospitalizations or cardiovascular death. GLP-1 RA should not be used in patients with HFrEF, where the potential harms outweigh the potential benefits.

Clinical implications: modulation of thyroid function, glucose homeostasis and incretin system

The results of this thesis have several clinical implications. From an overall perspective, this project shows that subclinical endocrine dysfunction influences cardiac function and the risk cardiovascular diseases. Our results also highlight that the modulation of thyroid function and glucose homeostasis is a relevant approach to improve cardiovascular health. More than identifying a one-fit-all approach, our work highlights the relevance of individualizing treatment approaches to reduce the risk of cardiovascular events. Three main clinical messages arise from our results: 1) modulation of thyroid function may be used to improve cardiovascular outcomes; 2) prediabetes is associated with an increased risk of cardiovascular and renal dysfunction; 3) GLP-1 receptor agonists have a major role in the modulation of cardiovascular risk. However, these messages need to be tailored to the individual characteristics of patients. Our results highlight a relevant “*dose-dependency*” of the supplementation with T3 in HFpEF, a “*definition-dependency*” of the association of prediabetes with cardiorenal outcomes, and a “*heart failure status-dependency*” of the effects of GLP-1 receptor agonists on heart failure events. Specifically, supplementation with low dose T3 may be a potential therapeutic approach to reduce adverse outcomes in patients with HFpEF, but high dose T3 may be associated with an increased risk of sudden death according to our results in the animal model of HFpEF. Prediabetes defined by HbA1c is a marker of increased risk of adverse cardiorenal outcomes, but prediabetes defined by fasting plasma glucose does not appear to be associated with a significantly increased risk. Regarding the effects of GLP-1 receptor agonists, while the protective effects on atherosclerotic events appear to be independent of heart failure status, the effects of GLP-1 receptor agonists on heart failure events differs according to baseline heart failure status.

In patients without previous heart failure, GLP-1 receptor agonists decrease the risk of heart failure events; in patients with HFpEF, GLP-1 receptor agonists are probably safe; and in patients with HFrEF, GLP-1 receptor agonists increase the risk of adverse events and heart failure hospitalization.

Figure 3 illustrates how the results from this project can be used to improve clinical practice. First, in all patients with cardiovascular diseases and in those at increased risk of cardiovascular diseases, clinicians must evaluate not only the glycemic status (which is common practice)¹⁰⁴ but also the thyroid function (which is frequently neglected).¹⁵¹ Among those with prediabetes (particularly if defined by HbA1c and/or impaired glucose tolerance) clinicians must implement measures to decrease not only the risk of type 2 diabetes but also the risk of cardiovascular complications. In patients with type 2 diabetes, the decision to use GLP-1 receptor agonists must be based not only on the atherosclerotic cardiovascular disease risk but also on the heart failure status. In patients at high risk of cardiovascular events without HFrEF, GLP-1 receptor agonists and SGLT2 inhibitors must be combined to further reduce the risk of adverse cardiovascular events. In patients with HFrEF, GLP-1 receptor agonists must be avoided to prevent adverse outcomes. Regarding thyroid function, our results highlight that the treatment target for those hypothyroidism and hyperthyroidism must consider that, even within the euthyroid range, thyroid hormone levels are associated with cardiac function and cardiovascular risk factors. Finally, in those with heart failure and nonthyroidal illness syndrome, our results do not support the use of T3 treatment aimed at normalizing the plasma and tissue levels of T3. On the other hand, clinical trials evaluating the effects of low dose T3 in HFpEF are needed to evaluate its potential role in this population.

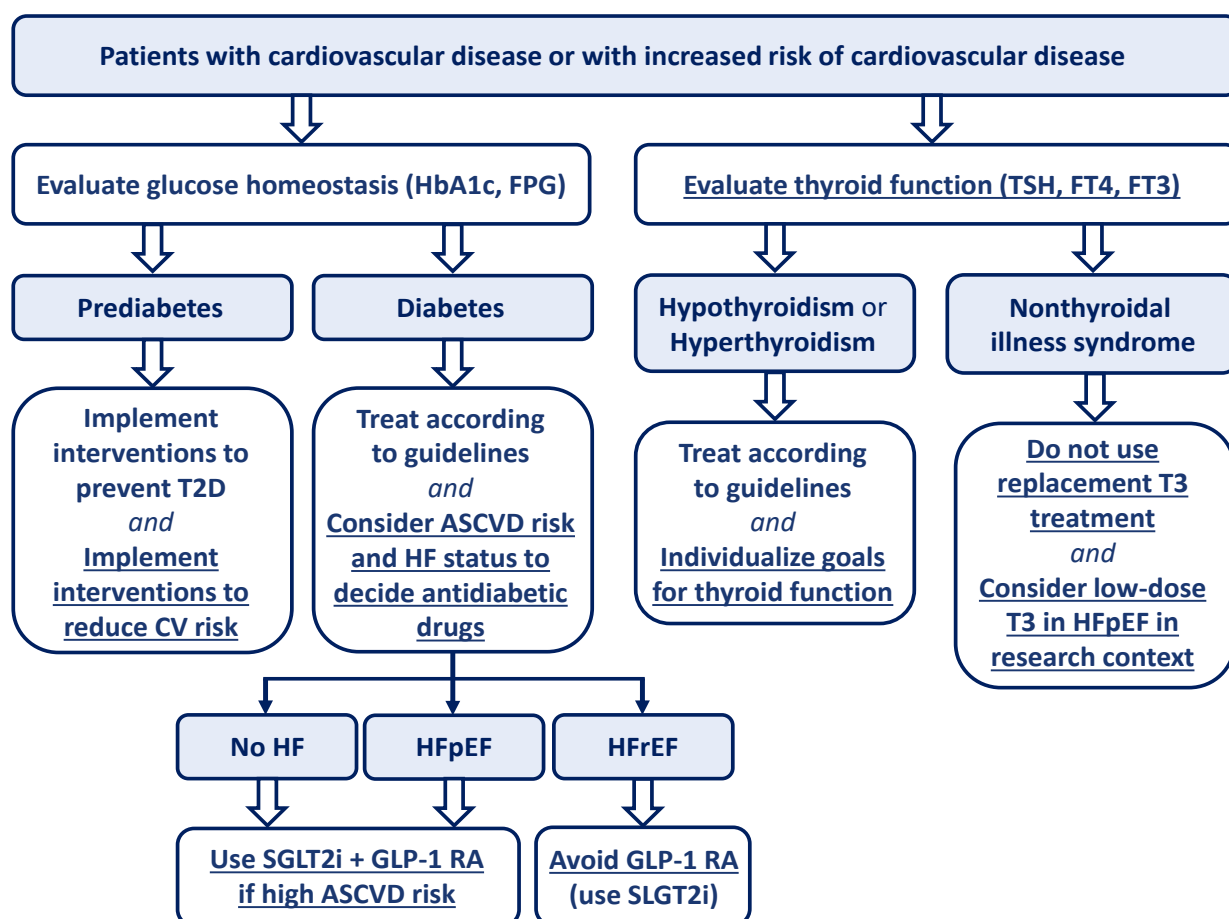


Figure 3. Approach to patients with cardiovascular disease or increased risk of cardiovascular disease. Recommendations that are based on the results of this project are highlighted in underlined text. FPG, fasting plasma glucose; SGLT2i, sodium-glucose cotransporter 2 inhibitors; GLP-1 RA, glucagon-like peptide 1 receptor agonists; TSH, thyroid-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine.

Future perspectives: areas for future research in subclinical endocrine dysfunction and cardiovascular risk

The results of this project open several pathways for future research in the field of subclinical endocrine dysfunction and cardiovascular risk. In the field of thyroid function and cardiovascular risk, our results highlight the need for testing in humans the effects of different targets of thyroid function adjusted to comorbidities. Ideally, randomized clinical trials testing individualized targets adjusted to the cardiovascular status and comorbidities compared to the usual care (i.e., aiming to TSH within the euthyroid range without further adjustment) should be used to assess the benefits of such an approach. Furthermore, our results suggest that supplementation with T3 is a potential target for improving prognosis in HFpEF. In future studies, low-dose T3 should be used and detailed monitoring of adverse events including arrhythmic events is recommended. Ideally, double-blind randomized clinical trials of patients with HFpEF assessing the effects of low-dose T3 (vs. placebo) on hard outcomes (e.g., cardiovascular death or heart failure hospitalization) should be performed to evaluate the effects of T3 supplementation.

In the field of prediabetes, our findings of an increased risk of cardiovascular events, which are concordant with a vast bibliography showing similar results, highlight the importance of further research to address this unmet need. In the past decade, several randomized clinical trials have shown that GLP-1 receptor agonists and SGLT2 inhibitors decrease the risk of cardiovascular events in patients with type 2 diabetes.^{89,152} In all clinical trials of GLP-1 receptor agonists and SGLT2 inhibitors assessing their cardiovascular effects, the benefits were independent of the duration of type 2 diabetes and baseline HbA1c.^{89,152} As hyperglycemia and associated complications are probably a continuum of risk, we can expect to observe similar benefits among people with prediabetes. From a societal perspective, this is very relevant, as 20-30% of adults in developed countries have prediabetes. In this field, double-blind randomized clinical trials evaluating the effects of

GLP-1 receptor agonists and/or SGLT2 inhibitors on cardiovascular events are needed to evaluate if these drugs can also be used in prediabetes to reduce their cardiovascular risk.

The modulation of the incretin system is a very active field of research. At this moment, there are ongoing clinical trials evaluating the effect of GLP-1 receptor agonists on non-alcoholic fatty liver disease,¹⁵³ chronic kidney disease,¹⁵⁴ peripheral artery disease¹⁵⁵ and even to prevent dementia.¹⁵⁶ This thesis shows that more studies are needed to evaluate the effects of GLP-1 receptor agonists in patients with heart failure. Currently, two ongoing randomized clinical trials (STEP-HFpEF and STEP-HFpEF-DM)¹⁵⁷ are evaluating semaglutide in patients with HFpEF and obesity, and the randomized clinical trial SUMMIT is evaluating tirzepatide (dual GIP/GLP-1 receptor co-agonist) in HFpEF.¹⁵⁸ These are relevant trials to evaluate the effects of incretins in HFpEF but those will not address the concerns on adverse events with GLP-1 receptor agonists in HFrEF. Whether it is ethical to perform a randomized clinical trial in HFrEF with GLP-1 receptor agonists is controversial. From a practical perspective, at this moment, the awareness of an increased risk of adverse events with GLP-1 receptor agonists in those with HFrEF is very low, and clinicians do not appear to prescribe less GLP-1 receptor agonists in the presence of HFrEF.¹⁵⁷ As such, more studies evaluating the safety of GLP-1 receptor agonists in patients with other indications for treatment (e.g. high atherosclerotic risk) and with concomitant HFrEF may help to inform clinical practice. Whether the benefits of GLP-1 receptor agonists in atherosclerotic complications can counterbalance the risk in HFrEF is uncertain, and a randomized clinical trial is needed to evaluate this intervention. Simultaneously, basic research and precision-medicine approaches to the mechanisms of increased risk of heart failure adverse events with GLP-1 receptor agonists are needed. There is a considerable interindividual variation in the expression of GLP-1 receptor in the myocardium.¹⁵⁹ Whether this interindividual variation or other mechanisms can explain interindividual differences in the risk/benefit ratio of modulation of the incretin system is unknown and further research is needed to clarify it.

Chapter 5

CONCLUSIONS



From the results of this thesis, the following conclusions can be made (the main conclusions are shown schematically in Figure 4):

Variations of thyroid function within the euthyroid range are associated with cardiovascular risk factors and cardiac function. A decreased conversion of T4 to T3 is associated with a higher prevalence of diabetes, a more adverse lipid profile, a lower eGFR and an increased 10-year risk of cardiovascular events. Increasing thyroid function is associated with a higher heart rate, a lower diastolic blood pressure, and larger LV volumes. LV wall thickness and ejection fraction have a U-shaped association with thyroid hormones.

Thyroid function and body weight regulation are significantly interconnected. Higher FT3 levels are associated with greater weight loss after bariatric surgery. On the other hand, TSH decreases after bariatric surgery and this decrease correlates with weight loss.

Lower FT3 levels within the euthyroid range are associated with higher cardiovascular mortality. Lower FT3 is associated with cardiovascular mortality even after adjustment for demographic variables, comorbidities, FT4 and TSH levels. These results support that a lower FT3 level is an independent predictor of cardiovascular mortality.

Modulation of thyroid hormones may be a potential therapeutic target in HFpEF; however, such approaches must consider a narrow therapeutic window of T3 in HFpEF. In an animal model of HFpEF treatment with T3 improved metabolic function, myocardial calcium handling, and diastolic and systolic function. While the low dose was well tolerated and safe, the replacement dose was associated with a higher risk of sudden death.

Prediabetes is associated with an increased risk of adverse cardiovascular events and progression of proteinuria. While these results were observed for those with prediabetes defined by HbA1c, prediabetes defined by fasting plasma glucose did not increase the risk of adverse cardiorenal events.

In patients with type 2 diabetes and cardiovascular disease, treatment with GLP-1 receptor agonists reduced the risk of cardiovascular events independently of SGLT2 inhibitors use. These findings support the use of GLP-1 receptor agonists in combination with SGLT2 inhibitors to reduce cardiovascular risk in type 2 diabetes with established cardiovascular disease.

Treatment with GLP-1 receptor agonists reduces heart failure events in patients with type 2 diabetes without heart failure but does not reduce this outcome in patients with heart failure. The reduction of atherosclerotic events with GLP-1 receptor agonists is not influenced by heart failure status. These results are clinically relevant highlighting that in patients with type 2 diabetes without heart failure, treatment with GLP-1 receptor agonists may prevent not only atherosclerotic events but also new-onset heart failure.

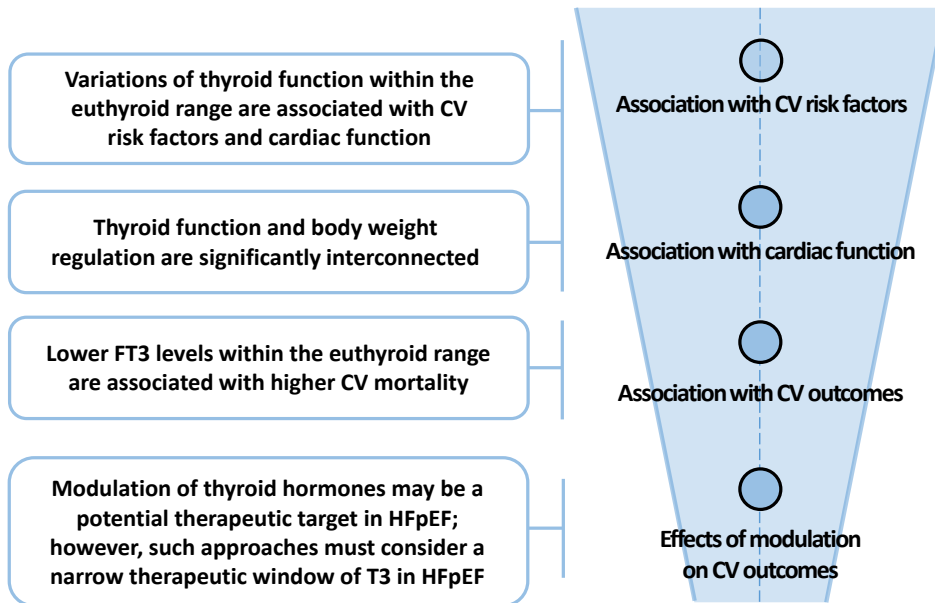
GLP-1 receptor agonists may increase the risk of heart failure hospitalization in patients with a reduced left ventricle ejection fraction. In advanced HFrEF, the increased risk of adverse cardiovascular effects with GLP-1 receptor agonists is possibly driven by the excess risk of arrhythmias and worsening heart failure. An increased risk of heart failure hospitalization with GLP-1 receptor agonists may also occur in stable patients with type 2 diabetes and reduced left ventricle ejection fraction.

From a clinical perspective, subclinical endocrine dysfunction must be considered an important factor that influences cardiac function and cardiovascular risk. Modulation of thyroid function and glucose homeostasis are relevant approaches to improve cardiovascular health. These modulations must consider thyroid function, glycemic status, previous atherosclerotic cardiovascular events and heart failure status. In patients with cardiovascular disease or at risk for cardiovascular dysfunction, a significant improvement in cardiovascular prognosis can be achieved by assessing and modulating thyroid function and glucose homeostasis.

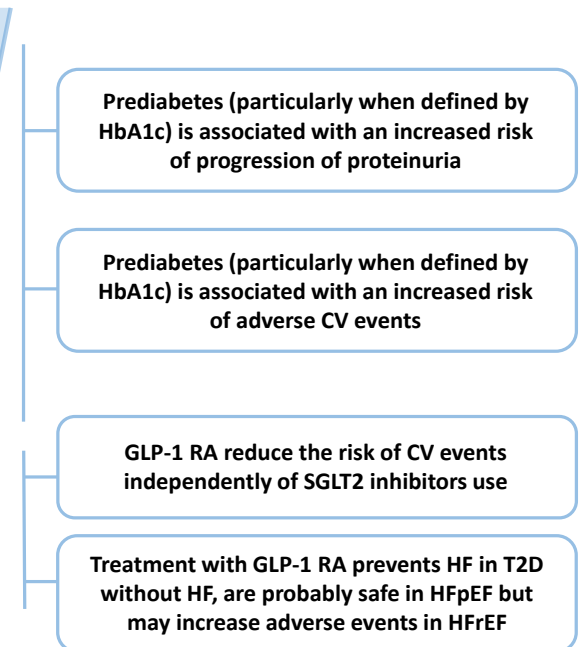
MAIN CONCLUSIONS

ROLE OF SUBCLINICAL ENDOCRINE DYSFUNCTION IN CARDIOVASCULAR RISK AND CARDIAC FUNCTION

Thyroid function



Glucose homeostasis



Overall conclusion:

Subclinical endocrine dysfunction must be considered an important factor that influences cardiac function and CV risk

Figure 4. Main conclusions. CV, cardiovascular; FT3, free triiodothyronine; T3, triiodothyronine; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; SGLT2i, sodium-glucose cotransporter 2 inhibitors; GLP-1 RA, glucagon-like peptide 1 receptor agonists.

Chapter 6

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This section includes bibliography for introduction and discussion (bibliography of the results section is included in the references section at the end of each individual articles).

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