MSP

MESTRADO EM
SAÚDE PÚBLICA

UNIVERSIDADE DO PORTO
FACULDADE DE MEDICINA
INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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Diabetes and the incidence of cancer in a Portuguese urban cohort

Porto, 2012

Diabetes and the incidence of cancer in a Portuguese urban cohort

Dissertação de candidatura ao grau de Mestre em Saúde Pública apresentada à Faculdade de Medicina e ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

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Porto, 2012

Este trabalho foi realizado no Departamento de Epidemiologia Clínica, Medicina Preditiva e Saúde Pública da Faculdade de Medicina da Universidade do Porto e no Instituto de Saúde Pública da Universidade do Porto (ISPUP), sob orientação da Doutora Bárbara Peleteiro e co-orientação do Professor Doutor Nuno Lunet.

Neste trabalho colaborei na operacionalização das hipóteses em estudo, na análise dos dados e na interpretação dos resultados.

Agradecimentos

À minha família, agradeço pelo apoio, paciência e carinho. Pelos valores incutidos, pelo exemplo e pela confiança em mim depositada. Um sincero e profundo Obrigada!

À Doutora Bárbara Peleteiro, orientadora, agradeço o apoio desde o primeiro dia, pela confiança, compreensão e tolerância nos momentos difíceis atravessados. Um suporte fundamental para a realização da dissertação.

Ao Professor Doutor Nuno Lunet, co-orientador, agradeço o conhecimento científico e a presença nas alturas certas.

Aos Professores, com quem tive o privilégio de aprender ao longo do meu percurso académico, agradeço o estímulo e a transmissão de conhecimentos.

Aos colegas do Mestrado em Saúde Publica com quem tive o privilégio de percorrer esta jornada, agradeço a convivência, a boa-disposição e a partilha.

A todos os meus amigos, que não precisam de ser enumerados, agradeço a compreensão pela minha ausência, a força por vós inspirada, o carinho e a amizade neste meu percurso.

Por último, mas não menos importante, um especial agradecimento à Helena Carreira, companheira e amiga de longa data, pela amizade, pelo alento e disponibilidade nos vários momentos. Mas também pela partilha de conhecimentos e pela ajuda técnica para a elaboração da dissertação.

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Resumo

Introdução:

A diabetes mellitus e o cancro são duas doenças comuns, cuja incidência tem aumentado globalmente. Alguns estudos têm explorado uma possível associação entre ambas, mostrando uma maior frequência de cancro em indivíduos diabéticos do que em indivíduos da população geral.

Vários mecanismos biológicos têm sido propostos para explicar esta associação: a diabetes mellitus e o cancro partilham vários factores de risco (modificáveis e não-modificáveis), que podem influenciar o processo neoplásico por vários mecanismos metabólicos, incluindo hiperinsulinemia, hiperglicemia e adiposidade, particularmente a obesidade abdominal, que está associada com um estado de inflamação crónico de baixo grau. Adicionalmente, estudos observacionais sugeriram que alguns fármacos usados para tratar a hiperglicemia estão associados quer com um maior quer com um menor risco de cancro.

O principal objectivo deste estudo foi quantificar a associação entre diabetes mellitus tipo 2 e a incidência de cancro, numa coorte de indivíduos adultos de uma zona urbana Portuguesa.

Métodos:

Este estudo foi realizado no âmbito da coorte EPIPorto, constituída por uma amostra de 2485 habitantes não institucionalizados da cidade do Porto, com idades entre os 18 e os 92 anos, recrutados por aleatorização de dígitos telefónicos. Entrevistadores treinados efectuaram entrevistas presenciais, utilizando um questionário estruturado, para recolher informações sociodemográficas, clínicas e de estilos de vida, além de avaliações físicas, incluindo medições antropométricas. Colheram-se amostras de sangue venoso depois de um jejum de 12 horas durante a noite. Na avaliação inicial, os indivíduos foram classificados em: não diabéticos sem alterações no metabolismo da glicose se o valor da glicemia em jejum fosse abaixo de 1,10 g/L; não diabéticos com alterações no metabolismo da glicose, se o valor da glicemia em jejum se situasse entre 1,10 g/L e 1,25 g/L; ou diabéticos se o valor da glicemia em jejum fosse acima de 1,25 g/L, ou se estivessem a ser tratados com insulina ou anti-diabéticos orais, ou se reportassem um diagnóstico prévio de diabetes tipo 2. Foram elegíveis para este estudo os indivíduos sem um diagnóstico prévio de cancro e excluíram-se os indivíduos sem a informação necessária para a sua caracterização relativamente à principal exposição em estudo, tendo a amostra final sido constituída por 2035 indivíduos. Identificaram-se os indivíduos que posteriormente desenvolveram cancro (com excepção de melanoma da pele) através do Registo Oncológico Regional do Norte (RORENO). O tempo mediano de seguimento da coorte foi de 8,1 anos (percentil 25-75: 6,2-11,6). Para quantificar a associação entre diabetes e cancro, calcularam-se razões de taxas de incidência (RR) ajustadas para o sexo, idade e escolaridade, e os correspondentes

intervalos de confiança a 95% (IC95%), utilizando a regressão de Poisson. Também se efectuaram análises estratificadas por exposição aos principais factores de risco para cancro, nomeadamente, excesso de peso, perímetro da cintura aumentado, tabagismo, etilismo, baixo consumo de vitamina C, fruta, e vegetais, além dos padrões alimentares.

Resultados:

Durante o período de seguimento da coorte, foram diagnosticados 115 tumores primários, correspondendo a uma taxa de incidência (TI) de 586/100000 pessoas-ano (IC95%: 488-703). As topografias tumorais mais frequentes foram mama (16,5%), próstata (13,9%), cólon e recto (13,9%), estômago (7,0%), pulmão (7,0%), fígado (5,2%) e bexiga (5,2%).

Comparativamente com os não diabéticos (TI=508/100000, IC95%: 413-626), os indivíduos não diabéticos mas com alterações do metabolismo da glicose medida em jejum apresentaram um risco aumentado de cancro (RR=1,99, IC95%: 1,04-3,83). Contudo, este resultado deixou de ser estatisticamente significativo após ajuste para o sexo, idade e escolaridade. O risco de cancro foi superior entre os indivíduos diabéticos, independentemente do sexo, idade e escolaridade (RR=2,04, IC95%: 1,18-3,51). Quando se considerou os participantes não diabéticos, com ou sem alterações do metabolismo da glicose em jejum, como categoria de referência, o risco de cancro diminuiu ligeiramente (RR=1,95, IC95%: 1,14-3,34). A diabetes associou-se significativamente com um risco aumentado de tumores do tracto gastrointestinal (RR=2,89, IC95%: 1,23-6,82), particularmente cancro colorectal (RR=3,34, IC95%: 1,05-10,66). Os cancros de mama (RR=2,49, IC95%: 0,69-8,99), estômago (RR=3,68, IC95%: 0,72-18,84), pulmão (RR=3,77, IC95%: 0,73-19,41), fígado (RR=2,17, IC95%: 0,24-19,37), tracto urinário (RR=2,57, IC95%: 0,54-12,14) e órgãos genitais femininos (RR=3,83, IC95%: 0,40-37,08) também apresentaram um risco aumentado entre os diabéticos e observou-se uma associação inversa para o cancro da próstata (RR=0,88, IC95%: 0,12-6,76). Não se verificaram diferenças significativas na associação entre diabetes e cancro entre os estratos de exposição aos principais factores de risco para as doenças oncológicas.

Conclusão:

A incidência de cancro foi superior nos indivíduos com diabetes do que nos não diabéticos, com associações de diferente magnitude de acordo com o tipo de cancro. Os indivíduos com alterações do metabolismo glicídico tiveram também um risco aumentado de cancro.

Este estudo sugere que o desenvolvimento de programas de gestão da diabetes poderão passar a ter de incluir também uma especial atenção à prevenção primária e secundária de cancro nestes indivíduos.

Abstract

Introduction:

Diabetes mellitus and cancer are two common diseases whose incidence is increasing globally. Some studies have explored a possible association between these two conditions, pointing that cancer is more frequent in diabetic patients than in the general population.

Several mechanisms have been proposed to explain this association: type 2 diabetes and cancer share many risk factors (modifiable and nonmodifiable), that may influence the neoplastic process by metabolic mechanisms, including hyperinsulinemia, hyperglycemia and adiposity, particularly central obesity, creating a low-grade chronic inflammation state. Furthermore, observational studies suggest that some medications used to treat hyperglycemia are associated with either an increased or reduced risk of cancer.

The main goal of this study was to quantify the association between diabetes and cancer incidence, in a Portuguese urban cohort.

Methods:

Participants of the EPIPorto cohort were included in this study. This cohort comprises a representative sample of 2485 Porto non-institutionalised dwellers, aged 18 to 92 years, recruited by random digit dialling. A personal interview, using a structured questionnaire comprising data on socio-demographic, clinical, and lifestyle characteristics, and a physical examination, including anthropometric measurements, was performed by trained interviewers. A venous blood sample was drawn after a 12-hour overnight fast. At baseline, subjects were classified as non-diabetic without impaired fasting blood glucose (FBG) if FBG was below 1.10 g/L, non-diabetic with impaired FBG if FBG was between 1.10 g/L and 1.25 g/L, or diabetic if FBG was above 1.25 g/L or if being treated with insulin or oral anti-diabetic drugs or if reported a previous diagnosis of type 2 diabetes.

Subjects without a previous cancer diagnosis were eligible for this study, and after excluding those without the necessary data regarding the main exposure under study, the sample comprised 2035 individuals. The identification of participants who were prospectively diagnosed with a cancer (except skin non-melanoma) was accomplished using the North Region Cancer Registry (RORENO). The median follow-up time was 8.1 years (percentiles 25-75: 6.2-11.6). Age-, sex- and education-adjusted incidence rate ratios (RR) and corresponding 95% confidence intervals (95%CI) were computed using Poisson regression to quantify the association between diabetes and cancer. We also conducted these analyses among the subgroups of subjects exposed to the main cancer risk factors, namely, overweight, central obesity, smoking, alcohol, vitamin C, fruits and vegetables intake, and dietary patterns.

Results:

During follow-up, 115 primary tumours were diagnosed, corresponding to an incidence rate (IR) of 586/100000 person-years (95%CI: 488-703). The most frequent tumour topographies were breast (16.5%), prostate (13.9%), colon and rectum (13.9%), stomach (7.0%), lung (7.0%), liver (5.2%) and bladder (5.2%).

Comparing with the non-diabetic (IR=508/100000, 95%CI: 413-626), non-diabetic subjects with impaired FBG were at an increased risk of developing cancer (RR=1.99, 95%CI: 1.04-3.83). However, after adjustment for sex, age and education, the association was not statistically significant. The risk of cancer was higher for diabetic subjects, independently from sex, age and education (RR=2.04, 95%CI: 1.18-3.51). When considering all the non-diabetic participants, without and with impaired FBG, as the reference category, the risk of cancer was slightly lower (RR=1.95, 95%CI: 1.14-3.34). Diabetes was significantly associated with an increased risk of digestive tumours (RR=2.89, 95%CI: 1.23-6.82), particularly colorectal neoplasms (RR=3.34, 95%CI: 1.05-10.66). Breast (RR=2.49, 95%CI: 0.69-8.99), stomach (RR=3.68, 95%CI: 0.72-18.84), lung (RR=3.77, 95%CI: 0.73-19.41), liver (RR=2.17, 95%CI: 0.24-19.37), urinary tract (RR=2.57, 95%CI: 0.54-12.14) and female genital organs (RR=3.83, 95%CI: 0.40-37.08) cancer risk were also higher among the diabetic, and an inverse association was found for prostate cancer (RR=0.88, 95%CI: 0.12-6.76). No significant differences in cancer risk associated with diabetes were observed between strata of exposure to the main lifestyle risk factors for oncological diseases.

Conclusion:

The incidence of cancer was higher among diabetic subjects compared with non-diabetic, with differences in the magnitude of the association according to cancer type. Subjects with impaired fasting glucose also had an increased risk of cancer.

This study suggests that the development of diabetes management programs may have to include a special attention to the primary and secondary prevention of cancer in these subjects.

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DM	Diabetes Mellitus
T2D	Type 2 diabetes
T1D	Type 1 diabetes
IGT	Impaired glucose tolerance
IFG	Impaired fasting glucose
FBG	Fasting blood glucose
IDF	International Diabetes Federation
IGF	Insulin-like growth factor
IGF-1	Insulin-like growth factor 1
IGF-2	Insulin-like growth factor 2
IR	Insulin receptor
IR-A	Insulin receptor isoform A
IR-B	Insulin receptor isoform B
IGF-IR	Insulin-like growth factor receptor
IGFBP	Insulin-like growth factor binding proteins
GLUT	Glucose transporter type
BMI	Body mass index
MMSE	Mini Mental State Examination
OR	Odds ratio
RR	Relative risk
MetS	Metabolic syndrome
IRR	Incidence rate ratio
CI	Confidence interval
HCC	Hepatocellular carcinoma
Il-6	Interleukin-6
TZD	Thiazolidinediones

Background

1. Diabetes and cancer - definition, incidence and mortality

Diabetes mellitus (DM) and cancer are common diseases that have a tremendous impact on health worldwide, and are associated with severe acute and chronic complications [1-3].

DM is recognized as a group of heterogeneous disorders with the common elements of hyperglycemia and glucose intolerance, due to insulin deficiency, impaired effectiveness of insulin action, or both. Diabetes is typically divided into 2 major subtypes, type 1 and type 2, along with less common types like gestational diabetes. Type 1 diabetes is sometimes called insulin-dependent, immune-mediated or juvenile-onset diabetes. It usually accounts for only a minority of the total burden of diabetes in a population, but its incidence is increasing in both developing and developed countries [4]. Type 2 diabetes (T2D) constitutes about 85 to 95% of all diabetes in high-income countries and may account for an even higher percentage in low-and middle-income countries. T2D is characterized by insulin resistance and relative insulin deficiency, either of which may be present at the time that diabetes becomes clinically manifested. The diagnosis of T2D usually occurs after the age of 40 years but can occur earlier, especially in populations with high diabetes prevalence, where the number of reports of children developing T2D is increasing. Impaired glucose tolerance (IGT), along with impaired fasting glucose (IFG), is recognized as being a stage before diabetes when blood glucose levels are higher than normal. Thus, people with IGT are at high risk of developing T2D [5].

Both diabetes and cancer are common diseases whose incidence is increasing globally. According to the International Diabetes Federation (IDF), about 366 million people worldwide, or 8.3% of adults between the ages of 20 and 79, were estimated to have diabetes in 2011. About 80% of these live in low- and middle-income countries [5]. If these trends continue, by 2030, about 552 million people, or one adult in 10, will have diabetes. This equates to approximately 9 million new cases diagnosed every year [1]. This prevalence is more than double the prevalence observed in the year 2000 [6]. The largest increases will take place in the regions dominated by developing economies [5].

In Portugal, the prevalence of self-reported diabetes and mean fasting glucose increased in the last two decades (1987-2009) [7]. In 2010, the PREVADIAB study found a diabetes prevalence of 11.7% with a significant difference between men (14.2%) and women (9.5%). This study evaluated a national sample of Portuguese subjects, and applied objective criteria to define diabetes. While 6.6% of subjects had previously been diagnosed with diabetes, 5.1% were undiagnosed. If ‘pre-diabetes’ is also considered, about one-third (34.9%) of the population aged 20–79 years is affected [8].

Worldwide, the prevalence of cancer has been difficult to establish because many areas are not covered by cancer registries, but in 2008 there were an estimated 12.7 million new cancer cases diagnosed, of these 6.6 million cases were in men and 6.0 million in woman. This number is expected to increase to 21 million by 2030 [9]. The most commonly diagnosed cancers are those of the lung/bronchus, breast, and colorectal, whereas the most common causes of cancer deaths are lung, stomach, and liver cancer [2]. In the North region of Portugal, the most commonly diagnosed cancers were those of the prostate, colon, lung and stomach in men and the breast, colon, stomach and thyroid in women in 2007 [10].

Worldwide, cancer and diabetes are the 3rd and 11th leading cause of death, respectively [11]. Diabetes is a major global cause of premature mortality that is widely underestimated, because only a minority of persons with diabetes dies from a cause uniquely related to the condition [12].

Epidemiologic evidence suggests that people with diabetes are at a significantly higher risk of many forms of cancer [13-16].

2. Epidemiological findings regarding the association between diabetes and cancer

The association between diabetes and cancer was described long before the 21st century, as far back as 1885 [17]. Most studies were initiated after introduction of insulin treatment, which prolonged the patient´s life and therefore resulted in over-diagnoses of cancer cases. Some stages can be distinguished in the research of this association. The first substantial reports, based on *post-mortem* examinations, suggested an increased incidence of different cancers in diabetic patients. The second stage of the research focused on analyzing the association using more objective and thorough epidemiological methods. However, it provided only partial confirmation of the previous reports [17]. The most recent epidemiological research consisted of well-prepared and well-documented studies based on analysis of cancer diagnosis and mortality among diabetic patients and included both case-control and cohort studies as well meta-analysis [13, 14, 18-20].

Because of the different physiology between the two subtypes of diabetes, these findings cannot be extended to type 1 diabetes (T1D). There are few studies of T1D and cancer. One cohort study from Sweden showed modest excess risk for stomach, cervix and endometrial cancers in T1D [21].

Overall, 8% to 18% of individuals suffering from cancer also have diabetes. While the literature indicates a strong and consistent increased risk of cancer in people with T2D, the strength of association depends on the specific cancer site [15].

The strongest relations have been demonstrated for cancers of liver, pancreas, endometrium and kidney and lesser for cancers of the colon/rectum, breast and bladder [14, 15, 19]. The evidence for others cancer is inconclusive or have a small number of studies (*e. g.* non-Hodgkin lymphoma, lung, ovarium) [14, 15]. However, diabetes is associated with a lower risk of prostate cancer [14, 22, 23].

Previous studies reported a difference in magnitude of cancer risk associated with diabetes between men and women, such as liver, colon and stomach cancer [18, 24]. These sex-specific differences in the association between diabetes and cancer require more careful analyses.

The impact of T2D on the prognosis of patients with cancer has been studied less well than its impact on the risk of cancer. Results of some, but not all, epidemiological studies suggest that diabetes may significantly increase mortality in patients with cancer [3, 25-27]. For example, in one study, which included up to 50% of Swedish patients with T2D, of whom 16,123 were diagnosed with subsequent cancer, and 999,982 cancer patients without T2D, clearly revealed an impact of T2D on cancer mortality. After adjusting for the confounding factors, patients who had cancer with T2D experienced a significant increase in cause-specific mortality ranging from 1.11 to 1.80 for different types of cancer [26].

3. Mechanisms for the link between diabetes and cancer

Several hypotheses have been suggested to explain the potentially causal relationship between glycemic level and cancer (Figure 1). T2D and cancer share many risk factors, modifiable and non-modifiable. The environmental or lifestyle factors could contribute at the same time to metabolic factors underlying both T2D and cancer, including visceral adiposity and a consequent state of inflammation, hyperglycemia and hyperinsulinemia [3, 14, 20, 28].

Furthermore, insulin, glycemia, adiposity and inflammation can be modified by antidiabetic pharmacotherapy and so, a better understanding of their pathophysiologic links to cancer allow us to more effectively target T2D treatment [29, 30].

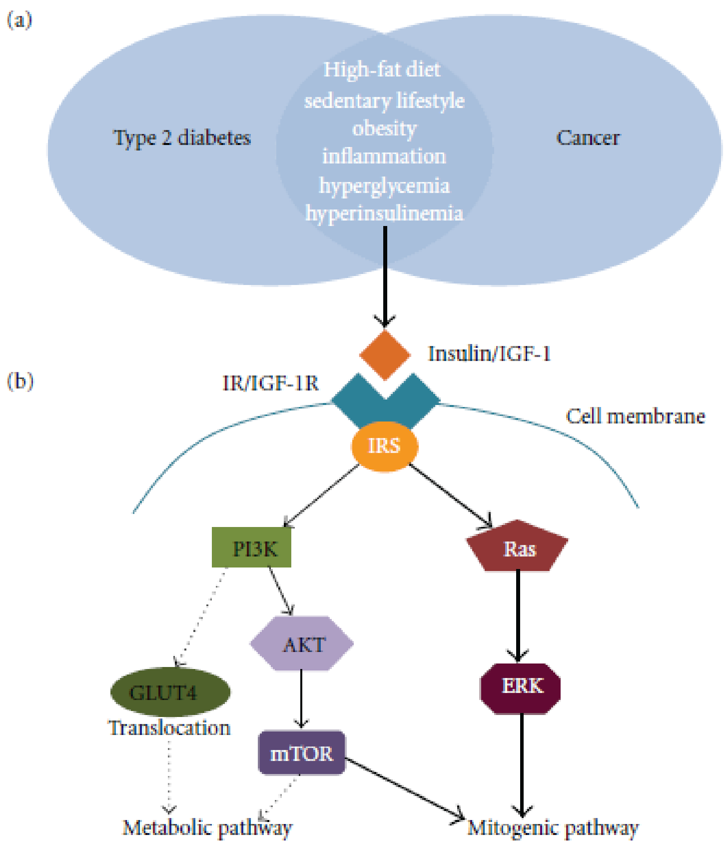

Figure 1. The link between T2D and cancer. (a) The shared metabolic factors underlying both T2D and cancer, including visceral adiposity, inflammation, hyperglycemia, and hyperinsulinemia. (b) The metabolic and mitogeneic pathways that are activated through different molecules [16]

3.1 Common risk factors to diabetes and cancer

It is known that both T2D and cancer increase with age; those aged ≥ 55 years comprise almost 80% of newly diagnosed cancers and 23.1% of patients aged ≥ 60 years develop T2M compared to 10.7% of younger adults [5]. Men have slightly more cancer and diabetes compared to women after adjusting for other risks [31]. African-Americans appear as a subgroup more prone to cancer-related deaths, T2D, and obesity compared to non-Hispanic white counterparts [31, 32].

T2D is strongly related to overweight and obesity, two conditions associated with an increased risk of several cancers [13]. The cancers most consistently associated with overweight and obesity are those of breast (in post menopausal women), colon/rectum, endometrium, pancreas, adenocarcinoma of the esophagus, kidney, gallbladder and liver [33]. Central obesity, a marker of insulin resistance and a key player in both T2D and the metabolic syndrome, has also been linked to breast, colorectal, liver, and endometrial malignancies [34].

A majority of studies, despite employing different study designs and populations, suggest that diets low in red and processed meat and high in vegetables, fruits, and whole grains are associated with a lower risk of many types of cancer [35]. Diets that are low in red and processed meat but high in monounsaturated fatty acids, fruits, vegetables, wholegrain cereals, and dietary fiber may protect against T2D, possibly through improving insulin sensitivity. Low-carbohydrate diets (which often include a greater consumption of red meats and fat) have also been associated with weight loss and improvements in insulin sensitivity and glycemic control [36].

Evidence from observational epidemiologic studies consistently shows that higher levels of physical activity are associated with a lower risk of the colon, postmenopausal breast, and endometrial cancer [35]. Prevention of diabetes with increased physical activity has also been proven by numerous trials [37, 38].

Moreover, tobacco and excess alcohol usage are linked to cancer and can also worsen diabetes complications [14, 35, 36, 39].

3.2 Biological hypotheses proposed to link diabetes to cancer

Diabetes may influence the neoplastic process by metabolic mechanisms, including hyperinsulinemia (either endogenous due to insulin resistance or exogenous due to administered insulin or insulin secretogogues), hyperglycemia and adiposity, particularly central obesity, creating a low-grade chronic inflammation state (Figure 1).

Hyperinsulinemia

The role of insulin in promoting cancer growth was first recognized by studies in experimental animals as early as 1972 [40].

In the genesis of T2D, reduced insulin sensitivity plays a key role, inducing compensatory hyperinsulinaemia with an increased level of circulating insulin-like growth factors (IGF), well-know to stimulate cell proliferation and inhibit apoptosis in many organs including the liver, pancreas, colon, ovary, breast, which are the most frequent sites with an increased risk of cancer in T2D patients [14, 20].

Insulin stimulates cell growth and differentiation acting within a complex system of interrelated hormones, receptors and binding proteins, known as the insulin/IGF axis. This system includes insulin, insulin-like growth factors (IGF-I, IGF-II), insulin receptor in two isoforms (IR-A, IR-B), growth factors receptors (IGF-IR, IGF-IIR), hybrid receptors and insulin-like growth factor binding proteins (IGFBP-1 to 6) [41].

Both insulin and IGF-1 have affinity to both the insulin receptor (IR) and IGF-1 receptor (IGF-1R) due of similar structural homology [42]. These receptors, expressed in majority of cancer cells tend to have stronger mitogenic and antiapoptotic effects, and the hyperinsulinemia that occurs in insulin-resistant individuals may enhance this effect [14, 28]. Insulin may also indirectly promote cancer development via IGF-1. Insulin reduces the hepatic production of IGF-binding protein-1, and possibility IGF-binding protein-2, with resultant increases in the levels of circulating free, bioactive form of the growth factor, IGF-1 [43].

In addition, malignant cells predominantly express the A isoform of the IR (IR-A) or hybrid IR-A/IGF-1R forms, which have more mitogenic effects that the B isoform IR. The A receptor isoform can stimulate insulin-mediated mitogenesis, even in cells deficient in IGF-1 [43, 44]. Thus, in the hyperinsulinemic individual, insulin´s mitogenic properties may further oncogenic proliferation.

Multiple signaling pathways are activated after insulin receptors or IGF-I receptors interact with their ligands. By phosphorylating adaptor proteins, most notably the insulin receptor substrate family, the initial kinase event is linked to downstream signaling pathways. Once activated, these signaling pathways may stimulate multiple cancer phenotypes including proliferation, protection from apoptotic stimuli, invasion, and metastasis, potentially enhancing the promotion and progression of many types of cancer cells. It is also clear that insulin/IGF may stimulate normal cells that are involved in cancer progression. All these effects might contribute to carcinogenesis [16, 45].

Hyperinsulinemia may ultimately upregulate the insulin mitogenic pathway compared to the insulin metabolic pathway.

Hyperglycemia

Hyperglycemia, one of the main characteristics of diabetes, is considered one possible reason for increased risk of cancer in diabetic patients [46]. Hyperglycemia may not only promote tumorigenesis indirectly by stimulating insulin production, but also may have a direct effect, since cancer cells depend on glycolysis for energy.

In order for cancer cells to maintain their intensive and uncontrolled proliferation, it requires a lot of energy and substrates. For these purposes, various substances can be used, *e.g*. glucose, whose input is ensured in tumor cells by an increase in the expression of glucose membrane transporters, especially forms of GLUT1, GLUT3 and GLUT12 [14] .

Heavy consumption of glucose is characteristic of neoplastic cells but glucose metabolism proceeds differently than in the unmodified cells. In healthy cells, glucose is converted to pyruvate, and later ATP is formed. This is done with the participation of oxygen in the Krebs cycle, and taking place in the mitochondria. Cancer cells convert the pyruvates into lactic acid and use glucose for the synthesis of DNA, RNA, proteins, lipids, namely for the cell construction. This different metabolism of cancer cells leads to an increased production of lactate, despite the full availability of oxygen [14, 41]. This phenomenon, which was described in 1927 is called the Warburg effect [47]. The excess of lactic acid production lowers the pH of the extra cellular matrix, thus contributing to the death of normal tissue cells and to the increased activity of collagenases, which facilitate tumor cells migration [41].

Nevertheless, increased intake of sugar and refined carbohydrates has been positively correlated with the risk of cancer. One mechanism by which glucose could induce cancer progression is by the induction of oxidative stress. Inducing hyperglycemia in the human breast cancer cell line MDA MB-231 leads to increased expression of the oxidative stress-responsive gene, thioredoxin interacting protein, and subsequent increased levels of reactive oxygen species. Thus, hyperglycemia in obesity and T2D could accelerate tumor growth and progression.

HbA1c is a test that measures the amount of glycated hemoglobin in blood, and gives a stable estimate of blood glucose control over the last 1 to 3 months. Inconsistent results were reported from previous observational studies on the relationship between HbA1c levels and cancer incidence or mortality. For example, Miao Jonasson *et al* performed a nationwide population-based observational study based on Swedish patient registers and they did not observed associations between HbA1c and incidence of all cancers or cancers of specific types in patients with type 2 diabetes [48]. Their results are not consistent with the Hong Kong study of T2D which was based on 973 new insulin users and 971 matched non-users of insulin. This study found that HbA1c per percentage was associated with a 1.24-fold increase in cancer risk [49].

Hyperglycemia, which is an independent risk factor for cancer, is usually associated with further metabolic changes - obesity, insulin resistance and hyperinsulinemia, which also contribute to the development of cancer.

Central obesity – inflammatory status

Obesity has been known for many years to increase the risk of T2D and is itself associated with an increase risk of cancer [50, 51]. Obesity potentially contributes to tumor progression through the effects of hyperinsulinemia, increased circulating estrogens, and chronic inflammation with altered regulation of cytokines and adipokines. Therefore, in many individuals with diabetes and obesity there are multiple physiological elements that may contribute to tumor development [52].

Central adiposity, particularly, is highly correlated with insulin resistance and is a key player in metabolic derangements associated with diabetes [34].

As recently reviewed, adipose tissue is an active endocrine organ, producing free fatty acids, interleukin-6 (IL-6), monocyte chemoattractant protein-1, (MCP-1) plasminogen activator inhibitor-1 (PAI-1), adiponectin, leptin, and tumor necrosis factor-α (TNF-α) [53]. Each of these factors has been shown to modify insulin resistance, lipid abnormalities, glucose intolerance, and grade inflammation that may collectively create an environment ripe for cancer development and progression. In some cases, the role of these molecules is well known.

Resistin, vastin, TNF-α, MCP-1, and IL-6 may increase cancer risk by perpetuating insulin resistance, hyperglycemia and inflammation [54, 55]. In contrast, adiponectin is of particular interest for cancer prevention because of its unique metabolic properties which include the ability to decrease hepatic gluconeogenesis, to increase insulin sensitivity, and to reduce adipogenic inflammation [56, 57].

3.3 Diabetes treatment and cancer

A potential role for pharmacological treatments of T2D in cancer had been suggested from pharmaco-epidemiological studies. Oral anti-diabetic drugs like metformin, sulfonylurea and thiazolidnediones (TZD), and human insulin and insulin analogues are the most recommended options for the treatment of T2D. The four major classes of antidiabetic drugs show different mechanisms of action, translating into different effects on circulating insulin levels. Metformin and TZD increase insulin sensitivity, thus decreasing hyperinsulinemia, while both sulfonylureas increase insulin levels. Since hyperinsulinemia is considered to play a role in increasing cancer risk, many studies have tried to investigate the link between the use of different drug classes and the risk of developing cancer.

The biguanide metformin is a first-line drug, recommended in the treatment of T2D, particularly when associated with overweight or obesity, given its safety profile and lower cost [58].

In the past few years, some epidemiological studies have shown that metformin exerts a protective effect against the development of different neoplasms and improves prognosis in patients with cancer [59-63]. The anti-cancer effect of metformin is probably related to a dual mechanism. In addition to the lowering of circulation levels of insulin and glycemia, metformin stimulates adenosine 5´-mono-phosphate-activated protein kinase (AMPK), leading to the rapid inhibition of cellular protein synthesis and growth [59, 63, 64].

Noto *et al* investigated, with greater precision, the preventive effect of metformin on the cancer incidence and mortality in patients with diabetes, by systematic review and meta-analyses of worldwide reports, and demonstrated that metformin is associated with a substantially lower risk of all-cancer mortality and incidence, compared with other treatments for diabetes [64].

For TZD, few studies are available due to their recent arrival on the market and their results are sparse and inconsistent [65, 66].

The TZDs are insulin-sensitizing peroxisome prolifeterol-activated receptor (PPARy) agonists that do not increased insulin secretion directly or cause hypoglycemia when used alone. Two drugs in this class, pioglitazone and rosiglitazone, are currently available. The mechanism of action of TZDs is to activate PPAR receptors, and several *in vitro* models suggest that these compounds can have a potential anticancer effect, such as inhibiting growth and inducing apoptosis and cell differentiation. Furthermore, PPARy is currently considered a potential target for both chemoprevention and cancer therapy based on preclinical studies, although conflicting results are reported from *in vivo* models [14, 15, 20].

Sulfonylureas (*e.g.* glyburide, glipizide, and glimepiride) are a class of oral antidiabetic drugs with long clinical usage that stimulate β cells to release insulin by binding to specific cell receptors, resulting in β cell depolarization and the release of insulin stores. Previous studies showed that associations with cancer remain uncertain [67-69].

Currie et al. studied the risk of specific cancers in a cohort of 62,809 individuals with >40 years of age with primary T2D who started treatment with oral antidiabetic drugs or insulin between 2001 and 2006. After an average of 2 years of follow-up, the relative risk of cancer (per 100 person-years) was 0.9 for metformin monotherapy, 1.6 for sulfonylurea monotherapy, 1.1 for metformin plus sulfonylurea and 1.3 for insulin initiators [70]. Although it is possible that the association between sulfonylureas and cancer risk is genuine, it is difficult to determine whether the findings reflect excess cancer among users of the secretagogues or a reduced risk in those using comparator drugs, which often include metformin therapy.

However, the majority of these studies had very few cancer cases among users of sulfonylureas, and therefore power was limited to examine associations with specific cancer sites. Studies regarding dose, duration, recency, and persistence of use are limited

Insulin is required for all patients with T1D. It is also necessary for many patients with T2D to treat hyperglycemia, in part due to the progressive loss of β cell function over time. Between 40% to 80% of individuals with T2D will ultimately be considered for insulin therapy in an effort to achieve glycemic targets [14]. Several formulations of insulin exist: short-acting human regular insulin, intermediate-acting human neutral protamine Hagedorn (NPH) insulin, and both rapid-acting and long-acting analogs of human insulin. Subcutaneous injection of insulin results in significantly higher levels of circulating insulin in the systemic circulation than endogenous insulin secretion, thereby possibly amplifying links between hyperinsulinemia and cancer risk.

During the past decades, long-acting insulin preparations have become widely used as a basal insulin supplement for diabetic patients due to their stable action and lower risk of nocturnal hypoglycemia. However, modification of amino acids on the insulin chain for these new insulin analogues may not only change metabolic properties but also alter their mitogenic effects, probably through prolonged binding to insulin receptor or by increased cross-reactivity with IGF-1 receptor [30]. Indirect mechanisms have been less well studied but would involve interactions of signaling molecules whose levels (*e. g.* glucagon, adiponectin) or activity are influenced by administration of insulin into these target cells [14].

The debate about the potential risk of developing cancer associated with insulin use has been fueled by the recent publication of several studies. Several studies showed that as compared with human insulin, insulin glargine – a long-acting insulin analogue – might substantially increase cellular proliferative potential, while the mitogenic potency of the other insulin analogues, including insulin detemir, were similar to or lower than human insulin [71]. In addition, it has been shown that insulin glargine, but not human insulin, increases resistance to apoptosis in several tumor cell lines including colorectal, breast, and prostate cancers [72].

However, available epidemiological studies results are not consistent. In a Swedish study consisting of 114,481 patient records, no differences were found between patients treated with glargine and patients treated with other insulin in terms of overall cancer risk (RR=1.09 95% CI 0.91-1.27). Nevertheless, women treated with glargine alone had a twofold increased risk for breast cancer (RR=1.99 95%CI 1.33-3.03), while those using insulin glargine in combination with another insulin did not show an increased risk (RR=1.10 95%CI 0.77-1.56) [73]. Similarly, in a Chinese study no increased risk of developing cancer in people using glargine compared to those with intermediate/long-acting human insulin [30].

Although the results of in vitro studies with insuline glargine show that it may act via the insulin/ IGF axis, potentially escalating the development of cancer, glargine oncogenic effect has not been sufficiently confirmed in epidemiological studies. Because of all the controversy, the Food and Drug Administration has concluded that glargine is safe, but it is awaiting longer-term prospective studies regarding malignancy potential [41].

The relations between different types of therapies used in diabetes and the incidence of cancer still under investigation. However, one may conclude that the use of metformin alone or in combination with another diabetic drug has a protective effect and is associated with lower risk of cancer, whereas exogenous insulin and oral medications that increase insulin secretion increase the risk of cancer development. Nevertheless, these findings should be viewed in light of the emerging role of metformin as a protective agent. In other words, it is not clear whether secretagogues increase cancer risk or simply metformin lowers the average risk in those who are taking this drug.

Furthermore, large cohort studies will provide the best evidence on the impact of anti-diabetic treatment on cancer occurrence including time- and dose-dependent relationships [19].

Main objective

Using data from a Portuguese urban cohort, the main objective of this dissertation was to quantify the association between diabetes and cancer incidence:

- in subjects presented with T2D and in those in a pre-diabetic stage;
- according to tumour topography;
- and taking into account the possible effect modification by the exposure to the main cancer risk factors.

Methods

Study participants

The present investigation was based on the EPIPorto cohort. It comprises a representative sample of 2485 Portuguese adults (61.8% women), aged 18 to 92 years, residing in Porto, an urban center in northwest Portugal, recruited between 1999 and 2003. As previously described [74], the participants were recruited by random digit dialing using households as the sampling unit. Once a household was selected, all residents were identified by age and sex, and one resident (aged more than 17 years) was randomly selected as the respondent, without allowing a replacement if there was a refusal. A participation rate of 70 % was achieved [74].

A visit to the Department of Clinical Epidemiology, Predictive Medicine and Public Health was scheduled by telephone according to the participant's convenience. A personal interview, using a structured questionnaire, and a physical examination was performed by trained interviewers. A venous blood sample was drawn after a 12-hour overnight fast. All the samples were analyzed at the central laboratory of the university hospital.

In individuals aged above 64 years, a rapid evaluation of cognitive function was done using the Mini-Mental State Examination test (MMSE) [75], and subjects were excluded when scoring below than 24 points [76].

At baseline, subjects were classified as non-diabetic without impaired fasting blood glucose (FBG) if FBG was below 1.10 g/L, non-diabetic with impaired FBG if FBG was between 1.10 g/L and 1.25 g/L, or diabetic if FBG was above 1.25 g/L or if being treated with insulin or oral anti-diabetic drugs or if reported a previous diagnosis of T2D.

Data collection

A structured standard questionnaire was applied by trained interviewers to obtain data on socio-demographic characteristics, personal and family medical history, and behavioral features.

Subjects were classified as daily smokers (current consumers of at least one cigarette per day, on average), occasional smokers (current consumers of less than one cigarette per day, on average), ex-smokers (not smoking for more than six months) and never-smokers [77]. Daily and occasional smokers were grouped into current smokers for data analysis. Regarding the consumption of alcoholic beverages, participants were classified as daily drinkers (current consumers of at least one alcoholic drink per day, on average), occasional drinkers (current consumers of less than an alcoholic drink per day, on average), ex-drinkers (not drinking for more than six months) and never-drinkers. For analysis, daily and occasional drinkers were grouped into current drinkers. Daily ethanol intake during the previous year was estimated, and cut-offs for men and women were defined according to the proposed by the American Heart Association [78].

Dietary intake during the previous year was estimated using a validated semi-quantitative food-frequency questionnaire [79, 80], comprising 82 food item/group and beverage categories. For each item, participants were asked to indicate the average frequency of consumption (nine possible responses, ranging from never to six or more times per day), as well as the amount consumed (using a photograph manual with three portion sizes, small, medium and large), and the number of months during which the foods had been consumed in the previous year. Any foods that were not specified in the food-frequency questionnaire but eaten regularly (once a week or more frequently) were listed in an open section.

The consumption of fruit and vegetables intake was estimated by adding up the frequencies of consumption of medium servings, corrected for seasonality, of the items referring to different fruits (apple or pear, orange or mandarin, banana, kiwi, strawberry, cherry, peach or plum, melon or watermelon, persimmon, fig or loquat or apricot, and grapes) and vegetables (lettuce, watercress, tomato, cucumber, green and white cabbages, broccoli, cauliflower or Brussels sprout, spinach or spring greens or turnip greens, spinach, bean pod, carrot, turnip, green pepper, and onion), as previously described in detail [81, 82]. For analysis, the cut-off of five servings per day recommended by the World Cancer Research Fund on their 2007 report on “Food, Nutrition, Physical activity and the Prevention of Cancer” was used to classify participants [83].

Energy and nutrient intake were obtained from consumption frequency and portion size data using the software Food Processor Plus R (ESHA Research), based on values from the US Department of Agriculture, further adapted for typical Portuguese foods using the Portuguese tables of food composition [84] and data from other studies that analysed the composition of Portuguese foods [79].

Dietary patterns were defined by principal components and cluster analyses, as previously described in detail [85]. The four different dietary patterns were identified in each sex. In women, the pattern with the highest consumption of vegetables, including vegetable soup, fruits, and dairy products, and with the lowest consumption of red meat, fast foods and soft drinks was named “healthy”; the pattern characterized by the lowest intake of all food group in particular fruit and vegetables, was called “low fruit and vegetables”; the pattern with the highest intake of red meat and alcohol, but also with the lowest consumption of dairy products and vegetable soup, was designated as “red meat and alcohol”; lastly, the pattern with the highest intake of white meat, sweets, and fast foods and the second highest intakes of red meat, vegetables (including vegetable soup), and dairy products was named “in transition to fast food.” In men, the pattern with the highest consumption of vegetable soup, fruits, dairy products, and cereals, and the lowest of red meat, fast foods, and alcoholic beverages, was designated as “healthy”; the highest intake of fish and vegetables characterized the “fish” pattern; the pattern with the highest consumption of red meat, alcohol, and fast foods, and the lowest of fruits, vegetable soup, dairy products, and cereals was named “red meat and alcohol”; lastly, a pattern with an intermediate consumption of most food groups but of white meat was designated as “intermediate intake”. For analysis, the “healthy” dietary pattern in both men and women was compared with the others.

The concentrations of total cholesterol were also assessed in the overnight fasting serum samples. If total cholesterol concentration was above 200 mg/dl or if subjects were on specific treatment for this lipid abnormality, ‘dyslipidemia’ was considered.

Trained interviewers measured blood pressure on a single occasion using the recommendations of the American Heart Association [86]. A standard mercury sphygmomanometer with the cuff on the right upper arm was used. Blood pressure levels were calculated as the mean of three readings. If systolic blood pressure was 140 mg/Hg and/or diastolic blood pressure was 90 mg/Hg or if subjects were on anti-hypertensive therapy, ‘hypertension’ was considered.

Anthropometric measurements were obtained after a 12-hour overnight fast, with the participant wearing light clothing and no footwear. Body weight was measured to the nearest 0.1kg using a digital scale, and height was measured to the nearest centimetre in the standing position using a wall stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m), and further divided into three categories: normal and underweight (< 24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) or obese (≥ 30.0 kg/m^2) [87, 88].

Central obesity was defined by waist circumference, which was measured midway between the lower limit of the rib cage and the iliac crest, to the nearest centimeter, with the subject standing, using a flexible and non-distensible tape and avoiding exertion of pressure on the tissues. Subjects were classified as having central obesity, according to the World Health Organization criteria [88].

Identification of cancer cases

The identification of participants with a cancer diagnosis (except skin non-melanoma) was accomplished using the North Region Cancer Registry (RORENO) and death certificates information. This population-based cancer registry was set up in 1988 and covers the whole northern region of Portugal (approximately 3.3 million inhabitants). The main procedure for gathering data is passive notification of cases, both by public or private hospitals/clinics and private pathology laboratories.The identification of cohort participants in the cancer registry database was performed using the name and date of birth of each participant, up to December 2009.

Data analysis

Subjects without a previous cancer diagnosis were eligible for this study (n=2,432), comprising a median follow-up time of 8.1 years (percentiles 25-75: 6.2-11.5). After excluding participants with a MMSE score lower than 24 points (n=74), those without information on diabetes (n=303) and those previously diagnosed with T1D (n=20), our sample comprised 2,035 participants, of whom 115 were diagnosed with cancer after the initial cohort evaluation. During follow-up, 35 participants with a tumour and 45 without a tumour had died.

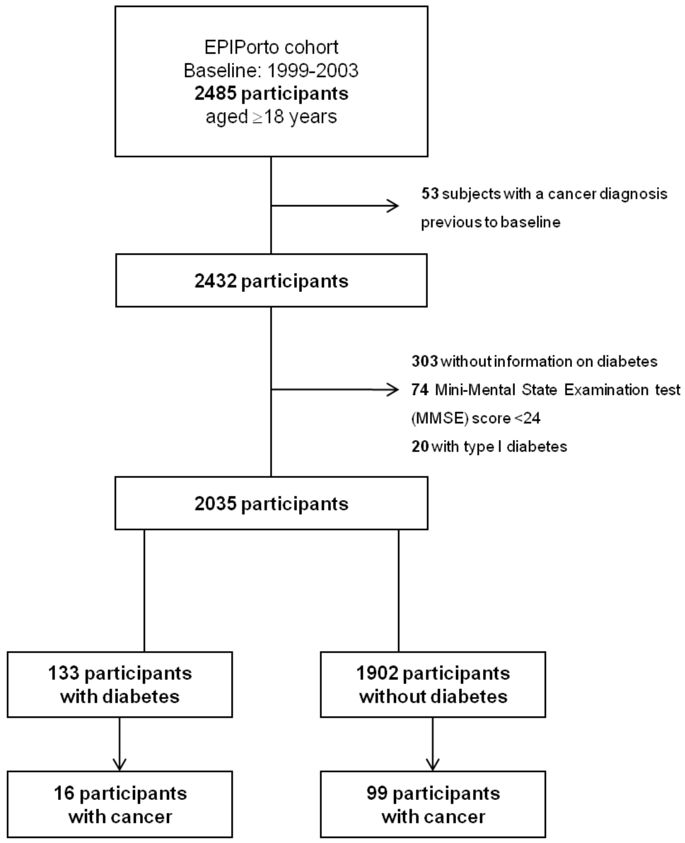

Figure 2. Flow-chart of the participants included in this study.

Age-, sex- and education-adjusted incidence rate ratios (IRR) and corresponding 95% confidence intervals (95%CI) were computed using Poisson regression to quantify the association between diabetes and cancer. We also conducted these analyses among subgroups of subjects exposed to the main cancer risk factors like BMI, waist circumference, smoking, alcohol, fruits and vegetables consumption, total fiber and vitamin C intake, and dietary patterns.

Ethics

The local ethics committee (Centro Hospitalar de São João) approved the study protocol. All participants gave written informed consent to participate; the study was carried out in accordance with the Helsinki Declaration II.

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Results

Table 1 presents the characteristics of the cohort participants. In our sample, approximately 60% were females, with a median age of 52 years and a median 9 schooling years. More than half of the subjects were overweight (40.3%) or obese (22.4%), and about 30% had central obesity.

At baseline, approximately one-quarter were current smokers and three-quarters were current drinkers, with more than half having moderate alcohol consumption. More than half of the subjects had a fruits and vegetables intake below the five a day recommendation (59.8%), but vitamin C intake was according the recommendations for most participants (79.9%). Total fiber intake was also below the sex- and age-specific recommendations for most subjects (77.4%). About 20% of the men and women had a healthy dietary pattern, but most women had a fast food dietary pattern (48.2%) while most men had a fish and vegetables dietary pattern (44.0%).

Table 1. Baseline characteristics of the cohort participants (N=2035).

	N (%) [a]	Median (percentile 25-percentile 75)
Sex		
Women	1247 (61.3)	
Men	788 (38.7)	
Age (years)		52 (42-63)
Education (years)		9 (4-13)
BMI (Kg/m^2)		
< 25.0	759 (37.3)	
25.0-29.9	821 (40.3)	
≥ 30.0	455 (22.4)	
Waist circumference (cm)		
≤ 88.0 in women or ≤ 102.0 in men	1405 (69.8)	
> 88.0 in women or > 102.0 in men	609 (30.2)	
Smoking status		
Never smokers	1103 (54.3)	
Former smokers	426 (21.0)	
Current smokers	503 (24.7)	
Alcohol consumption status		
Never drinkers	373 (18.4)	
Former drinkers	147 (7.2)	
Current drinkers	1512 (74.4)	
Alcohol consumption (g ethanol/day)		
0 [b]	520 (25.7)	
≤ 15 in women or ≤ 30 in men	1007 (49.7)	
> 15 in women or > 30 in men	500 (24.7)	
Fruits and vegetables intake (servings/day) [c]		4.42 (3.09-6.02)
Vitamin C intake (mg/day) [c]		119.41 (88.40-162.32)
Total fiber intake (g/day) [c]		18.57 (13.78-24.44)
Dietary patterns		
Women		
'Lower fruit and vegetables'	102 (8.3)	
'Red meat and alcohol'	239 (19.5)	
'Healthy'	293 (23.9)	
'In transition to fast food'	590 (48.2)	
Men		
'Healthy'	157 (20.6)	
'Fish and vegetables'	335 (44.0)	
'Red meat and alcoho'l	168 (22.0)	
'Intermediate intake'	102 (13.4)	

BMI – body mass index.
[a] The sum of the number of participants may be lower than the total due to missing data, and percentages may add up more than 100% due to rounding;
[b] includes never and former drinkers;
[c] data available only for 2024 subjects who fulfilled the food frequency questionnaire.

During follow-up, 115 primary tumours were diagnosed, corresponding to an incidence rate of 586/100000 person-years (95%CI: 488-703). The most frequent tumour topographies were breast (n=19), prostate (n=16), colon (n=11), lung (n=8), stomach (n=8), bladder (n=6), liver (n=6), kidney (n=5), rectum (n=5) and cervix (n=4). For 4 tumours the topography was unknown.

Non-diabetic subjects with impaired FBG were at an increased risk of developing cancer (RR=1.99, 95%CI: 1.04-3.83) (Table 2). However, after adjustment for sex, age and education, the association was not statistically significant. The risk of cancer was higher for diabetic subjects, and this association remained statistically significant after adjustment (RR=2.04, 95%CI: 1.18-3.51). When considering all non-diabetic participants, without and with impaired FBG, as the reference category, the risk was slightly decreased (RR=1.95, 95%CI: 1.14-3.34).

Diabetes was significantly associated with an increased risk of digestive tumours (RR=2.89, 95%CI: 1.23-6.82), particularly colorectal neoplasms (RR=3.34, 95%CI: 1.05-10.66) (Table 2). Breast, stomach, lung, liver, urinary tract and female genital organs cancer risk were also higher among the diabetic. Although not statistically significant, an inverse association was found for prostate cancer (RR=0.88, 95%CI: 0.12-6.76).

Table 2. Cancer risk associated with type 2 diabetes mellitus according to different criteria to define type 2 diabetes mellitus and according to cancer type.

	N (%)	Cases during follow-up N (%)	Crude RR (95%CI)	Adjusted [a] RR (95%CI)
According to different criteria to define type 2 diabetes mellitus				
According to FBG level and self-reported information [b]				
Non-diabetic				
without impaired FBG	1698 (88.4)	89 (5.0)	1	1
with impaired FBG	105 (5.5)	10 (8.7)	1.99 (1.04-3.83)	1.63 (0.85-3.15)
Diabetic	117 (6.1)	16 (12.0)	2.77 (1.63-4.72)	2.04 (1.18-3.51)
According to cancer type [c]				
All cancers				
Non-diabetic	1902 (93.5)	99 (5.2)	1	1
Diabetic	133 (6.5)	16 (12.0)	2.63 (1.55-4.46)	1.95 (1.14-3.34)
Breast cancer (C50*)				
Non-diabetic	1171 (93.9)	16 (1.4)	1	1
Diabetic	76 (6.1)	3 (4.0)	2.96 (0.86-10.15)	2.49 (0.69-8.99) [d]
Prostate cancer (C61*)				
Non-diabetic	731 (92.8)	15 (2.0)	1	1
Diabetic	57 (7.2)	1 (1.8)	1.14 (0.15-8.67)	0.88 (0.12-6.76) [d]
Colorectal cancer (C18-20*)				
Non-diabetic	1902 (93.5)	12 (0.6)	1	1
Diabetic	133 (6.5)	4 (3.0)	5.44 (1.75-16.86)	3.34 (1.05-10.66)
Stomach cancer (C16*)				
Non-diabetic	1902 (93.5)	6 (0.3)	1	1
Diabetic	133 (6.5)	2 (1.5)	5.40 (1.09-26.77)	3.68 (0.72-18.84)
Digestive cancer (C15-C16,C18-C20*)				
Non-diabetic	1902 (93.5)	25(1.3)	1	1
Diabetic	133 (6.5)	7 (5.3)	4.51 (1.95-10.42)	2.89 (1.23-6.82)
Lung cancer (C34*)				
Non-diabetic	1902 (93.5)	6 (0.3)	1	1
Diabetic	133 (6.5)	2 (1.5)	5.45 (1.10-26.99)	3.77 (0.73-19.41)
Liver cancer (C22*)				
Non-diabetic	1902 (93.5)	5 (0.3)	1	1
Diabetic	133 (6.5)	1 (0.8)	3.34 (0.38-27.7)	2.17 (0.24-19.37)
Urinary tract (C64*-C67*)				
Non-diabetic	1902 (93.46)	9 (0.47)	1	1
Diabetic	133 (6.54)	2 (1.50)	3.64 (0.79-16.85)	2.57 (0.54-12.14)
Female genital organs (C51*, C53-55*)				
Non-diabetic	1171 (93.91)	6 (0.51)	1	1
Diabetic	76 (6.09)	1 (1.32)	2.63 (0.32-21.83)	3.83 (0.40-37.08) [d]

FBG – fasting blood glucose;

* cancer was classified according the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10), 2010 [89];

[a] Adjusted for sex, age and education, except if otherwise specified;

[b] subjects were classified as non-diabetic without impaired FBG if FBG ≤ 1.10 g/L, non-diabetic with impaired FBG if 1.10 g/L < FBG ≤ 1.25 g/L, or diabetic if FBG > 1.25 g/L or if being treated with insulin or oral anti-diabetic drugs or if reported a previous diagnosis of type 2 diabetes mellitus;

[c] subjects were considered diabetic if FBG > 1.25 g/L or if being treated with insulin or oral anti-diabetic drugs or if reported a previous diagnosis of type 2 diabetes mellitus;

[d] adjusted for age and education.

No significant differences in cancer risk associated with diabetes were observed between strata of exposure to the main lifestyle risk factors for oncological diseases. However, the risk was lower in those having healthier lifestyles, such as never smokers (RR=1.71, 95%CI: 0.82-3.54), in those having moderate alcohol consumption (RR=1.75, 95%CI: 0.76-3.84), an intake of five or more servings of fruits and vegetables *per* day (RR=1.57, 95%CI: 0.59-4.16) and a total fiber intake above the recommendations (RR=1.78, 95%CI: 0.51-6.14), except for vitamin C intake and dietary pattern. Furthermore, lower risk estimates were also found among subjects presenting other cardiometabolic risk factors, namely overall (RR=1.83, 95%CI: 1.00-3.35) and central obesity (RR=1.71, 95%CI: 0.82-3.54), and hypertension (RR=1.86, 95%CI: 1.04-3.35).

Table 3. Cancer risk associated with T2DM according to the exposure to main risk factors for oncological diseases.

	Adjusted [a] RR (95%CI)	P for interaction
All participants [b]	1.95 (1.14-3.34)	
BMI (Kg/m^2)		
< 25.0	2.47 (0.72-8.42)	
≥ 25.0	1.83 (1.00-3.35)	0.659
Waist circumference (cm)		
≤ 88.0 in women or ≤ 102.0 in men	2.28 (0.97-5.36)	
> 88.0 in women or > 102.0 in men	1.71 (0.82-3.54)	0.592
Hypertension [c]		
No	2.63 (0.63-11.03)	
Yes	1.86 (1.04-3.35)	0.623
Dyslipidemia [d]		
No	0.76 (0.24-2.48)	
Yes	2.52 (1.32-4.80)	0.096
Smoking status		
Never smokers	1.71 (0.76-3.84)	
Ever smokers	2.13 (1.02-4.43)	0.428
Alcohol consumption (g ethanol/day)		
≤ 15 in women or ≤ 30 in men [e]	1.75 (0.89-3.44)	
> 15 in women or > 30 in men	2.50 (1.02-6.14)	0.606
Fruits and vegetables intake (servings/day)		
< 5	2.28 (1.18-4.38)	
≥ 5	1.57 (0.59-4.16)	0.721
Vitamin C intake (mg/day)		
< 75 in women or < 90 in men	1.74 (0.61-4.99)	
≥ 75 in women or ≥ 90 in men	2.11 (1.12-3.98)	0.608
Total fiber intake (g/day)		
< 25 in women aged ≤ 50 years or < 21 in women aged > 50 years or < 38 in men aged ≤ 50 years or < 30 in men aged > 50 years	2.18 (1.19-3.99)	
≥ 25 in women aged ≤ 50 years or ≥ 21 in women aged > 50 years or ≥ 38 in men aged ≤ 50 years or ≥ 30 in men aged > 50 years	1.78 (0.51-6.14)	0.725
Dietary patterns		
Healthy	2.61 (0.95-7.21)	
Others	1.91 (1.00-3.64)	0.603

[a] Adjusted for sex, age and education;
[b] subjects were considered diabetic if FBG > 1.25 g/L or if being treated with insulin or oral anti-diabetic drugs or if reported a previous diagnosis of type 2 diabetes mellitus;
[c] subjects were considered hypertensive if systolic blood pressure ≥ 140 mm Hg or if diastolic blood pressure ≥ 90 mm Hg or if being treated with antihypertensive drugs;
[d] dyslipidemia was considered present if total cholesterol > 200mg/dl or if subjects were on specific treatment for this lipid abnormality;
[e] includes never and former drinkers.

When investigating the impact of diabetic treatment on cancer occurrence within the cohort, we noted that metformin, either as a monotherapy or in combination was associated with a decreased (RR=0.48, 95%CI: 0.05-4.56) and sulforynureas with an increase risk of cancer (RR=3.54, 95%CI: 0.41-30.5).

Discussion

This study addressed the association between T2D and the incidence of cancer, using a representative population-based sample of the urban area of Porto. Our results suggest a two-fold increased cancer risk among diabetic subjects. An increased risk of cancer was also noticed among subjects with impaired fasting glucose, though diabetes was not established at that time. Particularly, diabetes was significantly associated with an increased risk of digestive tumours.

These results are in accordance with previous investigations [14, 18, 90]. In China, an increased risk of overall cancer in T2D patients was found in men and women, with standardized incidence ratios of 1.33 (95%CI: 1.14-1.52) and 1.74 (95%CI: 1.48-2.00), respectively [24].

Diabetes may be a risk factor for some cancers; however the impact of pre-existing DM was also visible among individuals with intermediate, non diabetic glycaemic levels, suggesting that factors associated with abnormal glucose metabolism may play an important role in the etiology of cancer [28, 91-93]. In studies that evaluated the impact of pre-diabetes on overall cancer prognosis, including cancer recurrence, cancer mortality, and all-cause mortality, the results were unclear [25, 27, 94].

Common risk factors to both conditions – cancer and diabetes – may explain the association described in the present study. Metabolic syndrome (MetS) is a combination of metabolic disorders, which include central obesity, hyperglycemia, hypertension and dyslipidaemia (high density lipoprotein cholesterol and triglycerides) [95]. Initially defined as a risk for cardiovascular diseases, MetS has recently been associated to the development of various cancers [96-101]. We also explored the association between MetS and cancer risk in our study (data not shown). Similarly to what has been described in other studies, our study indicates that the overall condition of MetS is an important risk factor for cancer. However, when assessing the independent contribution of each component, only diabetes was independently associated with an increased risk of cancer, as other studies have reported [98, 101].

No significant differences in cancer risk associated with diabetes were observed between strata of exposure to the main lifestyle risk factors for oncological diseases. However, lower risk estimates were found among subjects presenting other cardiometabolic risk factors, namely overall and central obesity, and hypertension. Although not statistically significant, these results argue in favor of a more pronounced role of diabetes in the absence of other cardiometabolic changes [98, 101].

Our results suggest an increased risk of cancer associated with diabetes in subjects who were grouped in the “healthy” dietary pattern. In women, this pattern is characterized by the highest consumption of vegetables, including vegetable soup, fruits, and dairy products, and with the lowest consumption of red meat, fast foods and soft drinks; in men, it is the pattern with the highest consumption of vegetable soup, fruits, dairy products, and cereals, and the lowest of red meat, fast foods, and alcoholic beverages [85]. In spite of this being the healthiest dietary pattern identified a posteriori, some of the remaining patterns were also characterized by healthy dietary habits, which could have contributed to attenuate the differences, when compared with the healthiest. For instance, in men, the pattern of "Fish" was characterized by the highest intake of fish and vegetables. When we considered the adequacy of vitamin C consumption, those who had higher intakes were suggested to be at higher risk of cancer associated with diabetes. Nevertheless, the imprecision of the estimate did not allow sound conclusions regarding the higher risk of cancer among diabetic subjects in the healthiest pattern of food or with adequate intakes of vitamin C.

More research is needed to understand the role of specific components of a healthy lifestyle independent of others (*e.g.*, diet quality independent of body weight). In addition, further study of those who are of normal body weight but have hyperinsulinemia or are sedentary and those who are obese but have normal metabolic parameters is necessary to better understand the independent relation between diabetes and cancer risk.

The cancer risk in diabetic patients is different according to the topography [90, 102]. Our findings indicate that diabetes was significantly associated with an increased risk of colorectal neoplasms. Similar results have been reported in a set of retrospective and prospective studies [103-105]. A recent meta-analysis, including 24 eligible cohort studies, showed an increase in the risk of developing colorectal cancer in persons with T2D compared with those without T2D (RR=1.28; 95%CI: 1.19-1.39). In studies reporting standardized incidence ratios (SIRs), there was an increased incidence of colorectal cancer with T2D (SIR=1.27; 95%CI: 1.14-1.42) and the association was stronger among men (SIR=1.47; 95%CI: 1.15-1.86) than women (SIR=1.08; 95%CI: 1.00-1.17) [106].

In the present study, although not statistically significant, the relative risk of breast, stomach, lung, liver, urinary tract and female genital organs cancers was also higher among the diabetics. In other hand, diabetes was also associated with lower risk of prostate cancer. However, this study has a limited power to detect significant associations. Our cohort included 2035 subjects that were followed up for a median time of 8.1 years, which may not be enough to detect a large number of cancer cases of a specific topography. Due to the small number of cancers diagnosed in the cohort it was not possible to stratify our analyses by sex.

Besides the biological mechanism previously described, other particular mechanisms may justify the association between T2D and the risk of cancer in some topographies found in this study, such as breast, liver, stomach and prostate.

Several case-control and cohort studies have evaluated the relationship between diabetes and breast cancer and showed an increased risk of breast malignancy in individuals with diabetes [107, 108]. This relation is more evident in postmenopausal women with T2D than their younger, premenopausal counterparts. Elevations in plasma estrogen levels have been associated with postmenopausal breast cancer risk in non diabetes population. In other hand, in insulin-resistant postmenopausal women, elevated serum insulin levels reduce sex hormone-binding globulin, resulting in increased estrogen bioavailability possibly explaining this increased risk. Furthermore, insulin has been show to be a mitogenic in human mammary epithelial cells.

Because the liver is one main target organ for insulin metabolism, it is not surprising that T2D and hepatocellular carcinoma (HCC) share a link. The association between diabetes and HCC has been investigated in several studies, and summarized in a systematic review, showing a higher risk of liver cancer for individuals with diabetes when compared to controls [109-111]. Metabolic abnormalities (hyperinsulinemia, hyperglycemia, and dyslipidemia) may contribute to the increasing risk of nonalcoholic fatty liver disease, including its most severe form, nonalcoholic steatohepatitis, and that HCC may be a late subsequent consequence of cirrhosis caused by nonalcoholic fatty liver disease [92]. The alternative mechanism to explain this association may be related to hepatitis C infection or cirrhosis. In fact, one study developed in Japan observed a statistically significant association between diabetes and liver cancer and the association was modified by hepatitis and cirrhosis [111]. Additionally, reverse causality is a major concern for causal inference because in some cases diabetes might itself be a result of cirrhosis [92].

The presence of diabetes has also been associated with an increased risk of gastric cancer. In a cohort study conducted in Japan, this excess risk was documented only in patients with *Helicobacter pylori* infection, suggesting that hyperglycemia is a possible cofactor increasing the risk posed by *H. pylori* [112]. Increase in gastrin level, decrease in somatostatin levels, and increase in neutrophilic and monocytic infiltration of the gastric mucosa in patients with *H. pylori* infection may lead to insulin resistance [113]. However, the prevalence findings of *H. pylori* in T2D patients has been variable, and the implications in this population are not yet clearly understood [114].

The association between diabetes and endometrial cancer has been frequently reported and it is the one showing the strongest association, though obesity can represent an important confounder, being strongly related to both conditions [115]. Other biological mechanisms have been proposed to potentially underlie the development of endometrial cancer in diabetic women. Insulin has been shown to stimulate the growth of endometrial stromal cells by binding to insulin receptors on endometrial cells. Epidemiological studies have observed an elevated risk of endometrial cancer in relation to high prediagnostic C-peptide concentrations indicating hyperinsulinaemia [48]. As with breast cancer hyperinsulinaemia may also increase levels of bioactive estrogens by decreasing concentrations of circulating sex hormone-binding globulin. Estrogens have been shown to increase endometrial cancer risk by stimulating proliferation of endometrial cells [51], when unopposed by progesterone

People with T2D are at increased risk of several types of cancer, including a 40% increased risk of bladder cancer, compared with those without diabetes [65]. Analysis of 16 studies showed that diabetes was associated with a modest increase in the risk of bladder cancer, compared with no diabetes. The possible mechanisms underlying the association of diabetes with bladder cancer risk are uncertain. Beyond the effect of insulin, IGF-1, diabetes is also associated with an increased risk of urinary tract infection, which has been related to bladder cancer risk [116]. Furthermore the limited evidence available supports the hypothesis that TZD, particularly pioglitazone, are associated with an increased risk of bladder cancer among adults T2D [65].

In our study, lung cancer was one of the most frequent cancers diagnosed during follow-up. This may be explained by the higher incidence of lung cancer in Portugal, regardless of the diabetes condition. Smoking is the main risk factor for lung cancer. Portugal is thought to be at earlier stages of the smoking epidemic compared with most European countries [117, 118], what may explain the higher incidence of lung cancer in our study. Specifically, women are in the stage II and men are at the later stages of the tobacco epidemic [119]. In Portugal, between 1987 and 2008, the prevalence of smoking increased significantly among women aged ≤70 years. The steepest increase was observed in those aged 31-50 and 51-70 years (from 4.6% and 0.1% in 1988, respectively, to 16.4% and 4.5% in 2008, respectively). In the same period, among men, smoking decreased in all age-groups, with steepest declines observed in those aged ≤30 years (from 41.8% in 1988 to 28.8% in 2008) and those aged ≥71 years (from 15.1% in 1988 to 4.6% in 2008) [119].

In contrast, we observed a non significant inverse association between T2D and prostate cancer, which is consistent with most previous epidemiologic studies [23, 120]. A meta-analysis of 19 studies found an inverse association between diabetes mellitus and prostate cancer (RR=0.84; 95%CI: 0.76-0.90) [120].

The reduced risk for prostate cancer in diabetic men could be due to the lower level of plasma androgens, as higher androgens levels are known to be associated with an increased risk of prostate cancer [20]. Furthermore elevated testosterone levels have been associated with an increased risk of prostate cancer [120]. Men with T2D and obesity often have low testosterone levels and this may help explain the decreased rates of prostate cancer in these men. In fact, studies have suggested that as many as 43% of men with T2D have reduced total testosterone levels and as many as 57% have decreased free testosterone levels [121]. Thus, as prostate cancer is positively associated with testosterone levels and men with T2D and obesity have low testosterone levels, it is reasonable to conclude that men with T2D and obesity have a lower risk of prostate cancer than men without these conditions. Stocks *et al* reported on a prospective study and found that men with increased insulin resistance and worsening glycemic control had a decreased risk of prostate cancer [122].

The association observed in our study could alternatively be explained by an increased use of health care services by diabetic subjects. Diabetes is a chronic condition, with increased medical attention, and may contribute to earlier detection of any present but previously undiagnosed cancer. In this study, diabetic subjects had a doubled number of medical appointments in the year before the interview, compared with non-diabetic (median: 4 appointments vs. 2 appointments, p<0.001). Notwithstanding, the median number of appointments was similar for those who had developed a tumour and those who did not regardless of diabetes status (median of two appointments in the last year, p=0.611). Furthermore, it has been described that individuals more recently diagnosed with diabetes have an increased risk of cancer due to the same indication bias [102]. However, in our study we found no difference in the risk of cancer according to the duration of diabetes (RR=1.19, 95%CI: 0.39-3.68 for patients diagnosed with diabetes for more than 5 years compared with those diagnosed in the last 5 years). These results strengthen the hypothesis of a causal relation between diabetes and cancer.

When investigating the impact of diabetic treatment on cancer occurrence within the cohort, we noted that metformin, either as a monotherapy or in combination was associated with a decreased (RR=0.48, 95%CI: 0.05-4.56) and sulforynureas with an increase risk of cancer (RR=3.54, 95%CI: 0.41-30.5). This finding is in the line with a study conducted by Hsieh *et al*. They showed that diabetic patients treated with insulin or sulfonylureas had significantly higher risk of all cancers, compared to those treated with metformin [odds ratio (OR)=1.58; 95%CI: 1.39-1.80, and OR=1.78; 95%CI: 1.41-2.26, respectively] [123].

Associations between other oral anti-diabetic drugs or insulin analogues, either alone or combination have also been reported [30, 63, 67]. Unfortunately, the number of T2D patients taking these medications in our cohort was low and no risk estimates could be computed. Furthermore, the distinction between long and fast acting analogues was not possible, nor did we have data on drug dosages.

Although we had data on mortality for our cohort participants, we opted for not conducting a survival analysis for those diagnosed with cancer during follow-up, aiming to disclosure the impact of type 2 diabetes on cancer survival. This methodological option was due to the non-exhaustiveness of the mortality data and to the probably low quality of these data in our cohort, since the available information for death was collected from different sources, such as the death certificates and response by relatives.

To best of our knowledge, this is the first study conducted in Portugal addressing this association, using a prospective approach. Our study confirms previous reports on the association of T2D with cancer, however, the impact varied across different cancer types suggesting the need to evaluate specific cancers individually. Our results highlight an additional attention that health care professionals need to take into account when dealing with diabetic patients. They point out how important is to monitor these subjects properly, as cancer prevention and early detection should be important components of diabetes management in light of the exponentially increasing prevalence of this chronic condition.

Our prospective study confirms previous reports on the association of T2D with cancer, which is independent of other risk factors for oncological diseases. However, the impact of diabetes varied across different cancer types suggesting the need to evaluate specific cancers individually.

We also found that subjects with impaired fasting have an increased risk of cancer, though diabetes was not established at that time. Furthermore specific antihyperglycemic medications are associated with increase or decrease cancer risk.

This study may support the development of primary and secondary prevention programs aimed to increased awareness of diabetes patients and healthcare personnel to the importance of cancer prevention efforts targeted to this specific population.

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