U.PORTO

2.º CICLO
NUTRIÇÃO CLÍNICA

Impact of intestinal biomarkers in cardiac reverse remodelling induced by pregnancy

Maria Adelina Azevedo Almeida

M

2025

Impacto de biomarcadores intestinais na remodelagem cardíaca reversa induzida pela gravidez

Impact of intestinal biomarkers in cardiac reverse remodelling induced by pregnancy

Maria Adelina Azevedo Almeida

Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto

Faculdade de Medicina da Universidade do Porto

Orientador: Ana Filipa Ferreira, Faculdade de Medicina da Universidade do Porto

Coorientador: Juliana Morais, Faculdade de Medicina da Universidade do Porto

Coorientador: Isabel Miranda, Faculdade de Medicina da Universidade do Porto

Dissertação de candidatura ao grau de Mestre em Nutrição Clínica apresentada à Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto

2025

Financiamento

"Impact of intestinal biomarkers in cardiac reverse remodelling induced by pregnancy"

Esta investigação foi financiada por:

-Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto

-Bolsa de Investigação Sociedade Portuguesa de Obstetrícia e Medicina Materno-Fetal 2025

Acknowledgements

Antes de mais, quero agradecer à minha mãe pelo apoio constante na minha vida e prestar uma homenagem ao meu pai, que, apesar da sua partida recente, apoiou-me durante todo o meu percurso académico e profissional até agora. Agradeço-lhes por todo o amor, valores e força que me transmitiram, e por acreditarem sempre em mim. Esta conquista é, em grande parte, deles.

À minha orientadora, Doutora Ana Filipa Ferreira, o meu agradecimento pela orientação dedicada, pela disponibilidade constante e pelo rigor científico que me ajudou a manter ao longo deste trabalho.

À minha coorientadora, Mestre Juliana Morais, o meu agradecimento pelo suporte constante no desenvolvimento deste trabalho. Foi incansável no apoio ao trabalho laboratorial, sempre pronta a ajudar e a esclarecer dúvidas com entusiasmo e dedicação.

À minha coorientadora Professora Isabel Miranda, deixo ainda um carinho adicional por ter aceite coorientar este trabalho e o gesto generoso de ceder materiais essenciais para o desenvolvimento do projeto, bem como pelo incentivo e apoio contínuo.

O meu agradecimento estende-se ainda à comissão científica do mestrado em Nutrição Clínica e a toda a comunidade científica da FCNAUP, pelo acolhimento, pelos ensinamentos que contribuíram de forma significativa para o meu crescimento académico e pessoal.

Por último, agradeço aos colegas de mestrado e amigos próximos (eles sabem quem são), pelas gargalhadas, companheirismo, boa disposição e suporte nestes últimos 2 anos.

Resumo

A gravidez acarreta alterações fisiológicas profundas, particularmente a nível cardiovascular, que tendem a regredir durante o período pós-parto. Alterações na microbiota intestinal, na permeabilidade intestinal e na inflamação sistémica, surgem como um possível fator modulador deste fenómeno.

Esta dissertação investigou a associação entre biomarcadores intestinais e a remodelagem cardíaca reversa induzida pela gravidez, com especial atenção na calprotectina fecal, na zonulina sérica e na bactéria *Akkermansia muciniphila*.

Foi conduzido um estudo de coorte observacional retrospetivo em que as participantes foram avaliadas no terceiro trimestre de gravidez (entre as 30 e 35 semanas de gestação) e aos seis meses após o parto. Foram recolhidos dados clínicos, alimentares, ecocardiográficos, bioquímicos e antropométricos.

Um sub-grupo de 34 mulheres grávidas da coorte PERIMYR & OralBioBorn com mediana de idade de 33 [31;36] anos foram incluídas neste estudo O IMC pré-gestacional das participantes 28,9 [25.0; 34.5] kg/m^2. A adesão à dieta mediterrânica, avaliada pelo MEDAS score, foi classificada como moderada a elevada na maioria das participantes no terceiro trimestre. Através de ecocardiografia transtorácica, observou-se que entre a gravidez e os seis meses após o parto a massa cardíaca reduziu significativamente (-10,84%) associando-se a uma melhoria da função diastólica e a um aumento significativo da rigidez arterial. No que diz respeito aos biomarcadores intestinais explorados, observou-se uma redução significativa dos níveis de calprotectina fecal (2,90 [1,81; 7,92] µg/g vs 2,12 [1,34; 2,98] µg/g; $p < 0,001$), de zonulina sérica (456 [409; 480] ng/mL vs 378 [327; 443] ng/mL; $p < 0,001$), e de *Akkermansia muciniphila* (0,74 [0,00; 1,22] vs 0,29 [0,00; 1,65] Log10 de cópias do gene16S rRNA/10 ng de DNA; $p = 0,034$) entre o terceiro trimestre e os seis meses após o parto. A calprotectina fecal apresentou uma correlação positiva com a massa ventricular esquerda no terceiro trimestre ($\rho = 0,405$; $p = 0,024$) e com o índice E/e' no pós-parto ($\rho = 0,424$; $p = 0,017$). A zonulina sérica correlacionou-se positivamente com a pressão arterial diastólica ($\rho = 0,468$; $p = 0,021$), com a massa cardíaca ($\rho = 0,517$; $p = 0,010$) e com o volume ventricular esquerdo ($\rho = 0,484$; $p = 0,019$). Não foram observadas correlações significativas entre os níveis de *Akkermansia muciniphila* e os parâmetros cardiovasculares.

Uma maior adesão à dieta mediterrânica no pós-parto associou-se a melhor função diastólica (ρ = –0,510; *p* = 0,015) e a menor espessura relativa da parede ventricular (ρ = –0,560; *p* = 0,007). Embora não se tenham verificado associações significativas entre a adesão à dieta e os níveis de calprotectina ou zonulina de forma global, o consumo mais frequente de carne vermelha associou-se a níveis mais elevados de zonulina (*p* = 0,004), assim como com o peso no pós-parto (ρ = 0.637, *p* = 0.020).

Foram observadas diversas associações entre maior adesão à dieta mediterrânica e parâmetros ecocardiográficos maternos. O uso do azeite como principal fonte de gordura durante a gravidez correlacionou-se negativamente com várias variáveis cardíacas, sugerindo um efeito benéfico sobre a função cardiovascular. O menor consumo de carnes vermelhas e processadas associou-se a perfis ecocardiográficos mais saudáveis, incluindo menores valores de pressão diastólica. Além disso, o consumo de produtos de pastelaria industrial mostrou uma correlação negativa com o volume auricular esquerdo, sugerindo uma possível ligação com menor sobrecarga cardíaca. Seis meses após o parto, observou-se uma correlação negativa significativa entre o consumo de gordura saturada (como manteiga, margarina e natas) e variáveis cardíacas adversas, bem como entre o consumo de vinho e parâmetros ecocardiográficos. O uso de azeite como principal fonte de gordura associou-se a menor massa cardíaca, reforçando a persistência dos efeitos protetores da dieta mediterrânica no período pós-parto.

Em conclusão, os resultados indicam que biomarcadores de inflamação e permeabilidade intestinal materna se associam a parâmetros de remodelagem cardíaca reversa e poderão constituir indicadores precoces de risco cardiovascular. A dieta mediterrânica revela-se uma estratégia promissora na promoção da saúde intestinal e cardiovascular durante o período perinatal, com possíveis implicações relevantes na prevenção de doenças cardiovasculares em mulheres a longo prazo. Este estudo reforça a importância de uma abordagem integrada e multidimensional na vigilância da saúde materna.

Palavras-Chave

Gravidez, Remodelagem cardíaca reversa, Biomarcadores intestinais, Dieta Mediterrânica

Abstract

Pregnancy entails profound physiological changes, particularly at the cardiovascular level, which tend to regress during the postpartum period. Alterations in gut microbiota, intestinal permeability, and systemic inflammation have emerged as potential modulators of this phenomenon.

This dissertation investigated the association between intestinal biomarkers and pregnancy-induced reverse cardiac remodelling, with special attention to faecal calprotectin, serum zonulin, and the bacterium *Akkermansia muciniphila*.

A retrospective observational cohort study was conducted, in which participants were assessed during the third trimester of pregnancy (between 30 and 35 weeks of gestation) and at six months postpartum. Clinical, dietary, echocardiographic, biochemical, and anthropometric data were collected.

A subgroup of 34 pregnant women from the PERIMYR & OralBioBorn cohort with a median age of 33 [31;36] years were included in this study. The participants' pre-gestational BMI was 28.9 [25.0; 34.5] kg/m^2. Adherence to the Mediterranean diet, assessed by the MEDAS score, was classified as moderate to high in the majority of participants during the third trimester. Transthoracic echocardiography showed that between pregnancy and six months postpartum, cardiac mass significantly decreased (–10.84%), which was associated with an improvement in diastolic function and a significant increase in arterial stiffness.

Regarding the intestinal biomarkers explored, a significant reduction was observed in faecal calprotectin levels (2.90 [1.81; 7.92] µg/g vs 2.12 [1.34; 2.98] µg/g; $p < 0.001$), serum zonulin (456 [409; 480] ng/mL vs 378 [327; 443] ng/mL; $p < 0.001$), and *Akkermansia muciniphila* (0.74 [0.00; 1.22] vs 0.29 [0.00; 1.65] Log10 16S rRNA gene copies/10 ng of DNA; $p = 0.034$) between the third trimester and six months postpartum. Faecal calprotectin showed a positive correlation with left ventricular mass in the third trimester ($\rho = 0.405$; $p = 0.024$) and with the E/e′ index postpartum ($\rho = 0.424$; $p = 0.017$). Serum zonulin was positively correlated with diastolic blood pressure ($\rho = 0.468$; $p = 0.021$), cardiac mass ($\rho = 0.517$; $p = 0.010$), and left ventricular volume ($\rho = 0.484$; $p = 0.019$). No significant correlations were observed between *Akkermansia muciniphila* levels and cardiovascular parameters.

Greater adherence to the Mediterranean diet postpartum was associated with better diastolic function (ρ = –0.510; p = 0.015) and lower relative thickness of the ventricular wall (ρ = –0.560; p = 0.007). Although no significant associations were found between overall dietary adherence and calprotectin or zonulin levels, more frequent consumption of red meat was associated with higher zonulin levels (p = 0.004), as well as higher postpartum weight (ρ = 0.637, p = 0.020).

Several associations were observed between greater adherence to the Mediterranean diet and maternal echocardiographic parameters. The use of olive oil as the main source of fat during pregnancy was negatively correlated with various cardiac variables, suggesting a beneficial effect on cardiovascular function. Lower consumption of red and processed meats was associated with healthier echocardiographic profiles, including lower diastolic pressure values. Additionally, the consumption of industrial pastries showed a negative correlation with left atrial volume, suggesting a possible link to lower cardiac overload. Six months after childbirth, a significant negative correlation was observed between saturated fat consumption (such as butter, margarine, and cream) and adverse cardiac variables, as well as between wine consumption and echocardiographic parameters. Continued use of olive oil as the main fat source was associated with lower cardiac mass, reinforcing the persistent protective effects of the Mediterranean diet in the postpartum period.

In conclusion, the results indicate that maternal inflammation and intestinal permeability biomarkers are associated with parameters of reverse cardiac remodelling and may constitute early indicators of cardiovascular risk. The Mediterranean diet appears to be a promising strategy in promoting intestinal and cardiovascular health during the perinatal period, with potentially relevant implications for the long-term prevention of cardiovascular disease in women. This study reinforces the importance of an integrated and multidimensional approach to maternal health surveillance.

Keywords

Pregnancy, cardiac reverse remodelling, intestinal biomarkers, Mediterranean diet

Table of Contents

Abbreviations

3T - 3rd trimester of pregnancy

AHT - Arterial hypertension

BMI - Body mass *index*

EFSA - European Food Safety Authority

FC - Faecal calprotectin

GDM - Gestational diabetes *mellitus*

LV - Left ventricle

LVM – Left ventricular mass

LPS - Lipopolysaccharide

MED - Mediterranean Diet

MEDAS - Mediterranean Diet Adherence Screener

PIH - Pregnancy-induced hypertension

PP2 - 6 months postpartum

RR - Reverse remodelling

SCFAs - Short-chain fatty acids

ULS São João - Unidade Local de Saúde de São João

Figures list

Tables list

1. Introduction

1.1 Physiological Cardiovascular Reverse Remodelling induced by Pregnancy

Pregnancy is a complex physiological process during which the maternal organism experiences profound systemic adaptations to support foetal growth and development[1]. The cardiovascular system undergoes a series of adaptations primarily driven by hemodynamic overload, which accommodate the foetus's growing demands and support the normal course of pregnancy[2]. Among the most prominent are cardiovascular changes, which begin as early as the first trimester and intensify throughout gestation[2].

The continuous increase in plasma volume drives a progressive rise in cardiac output, by approximately 30–50%, driven primarily by elevations in stroke volume and heart rate, which peaks by the end of the second trimester and tends to plateau or slightly decline thereafter[3]. Systemic vascular resistance declines due to the vasodilatory effects of hormonal changes, including increased levels of progesterone and estrogen during pregnancy that stimulate the production of renin substrate, leading to increased angiotensin concentrations[4, 5]. As a result, activation of the renin–angiotensin–aldosterone system leads to a progressive rise in plasma volume, beginning around the 6th to 8th week and continuing until approximately the 28th to 30th week of gestation[5]. This expansion in plasma volume plays a key role in maintaining blood pressure and promoting sodium and water retention throughout pregnancy[4, 5].

Structurally, the maternal heart undergoes physiological remodelling characterised by left ventricular hypertrophy associated with increased cardiac chamber volumes to accommodate the expanded plasma volume and metabolic demands of pregnancy[3]. Although cardiac systolic function remains preserved during pregnancy, a deterioration in left ventricular relaxation has been observed[6]. These changes are generally adaptive and reversible. In uncomplicated pregnancies, cardiac morphology and function typically return to pre-pregnancy states within a few months after delivery, a process called cardiac reverse remodelling (RR)[3, 4, 7]. The duration of RR is not consensual. However, the presence of comorbidities such as gestational hypertension, pre-eclampsia, obesity, or gestational diabetes *mellitus* (GDM) can compromise cardiac recovery[8].

In obese pregnant women, especially in the 3rd trimester (3T), studies have shown increased left ventricle mass (LVM) and reduced stroke volume index, suggesting a limited adaptive capacity to meet increased hemodynamic demands[9, 10]. These women also exhibit signs of subclinical diastolic and systolic dysfunction[9, 10]. Having pre-gestational obesity and excessive gestational weight gain are associated with a higher risk of pregnancy-induced hypertension (PIH) and reduced postpartum cardiac recovery, particularly in cases of peripartum cardiomyopathy[11].

GDM is associated with increased vascular resistance, left ventricular hypertrophy, and systolic and diastolic dysfunction during the 3T[12]. While some cardiac changes resolve postpartum, particularly functional impairments, may persist longer[12].

Hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia, are associated with increased LVM, relative wall thickness, and a predominance of concentric remodelling, often persisting postpartum despite pharmacological therapy[8, 13]. These alterations correlate with subclinical systolic and diastolic dysfunction and predict adverse outcomes in future pregnancies[8]. Long-term studies show a higher prevalence of maladaptive cardiac remodelling, including impaired RR, in women with a history of preeclampsia[13].

In this context, the presence of cardiovascular risk factor (such as obesity, diabetes *mellitus* type II, chronic arterial hypertension, or cardiovascular complications induced by pregnancy (such as gestational hypertension, preeclampsia or GDM) may compromise the cardiac remodelling and RR during pregnancy and postpartum, respectively, increasing maternal cardiovascular risk in long-term. These findings highlight the importance of early cardiac assessment and long-term follow-up in affected women[8, 11].

1.2 Gut microbiome and heart axis

Currently, scientific evidence supports the existence of the gut–heart axis, a dynamic and bi-directional communication system between the gastrointestinal microbiome and the cardiovascular system [14] . This interaction is mediated through a variety of signalling molecules, including microbial metabolites, such as short-chain fatty acids (SCFAs), endotoxins (e.g., lipopolysaccharide (LPS)), and host inflammatory responses[14]. The term microbiome is defined as an ecological collection of microorganisms (eukaryotes, archaea, bacteria, and viruses), their

associated genomes and metabolites, and their interaction with the host environment (mainly in the gut)[15, 16]. These microorganisms play a crucial role in nutrient absorption, immune regulation, gut barrier integrity, and systemic metabolic homeostasis[15]. The development of the human gut microbiome is affected by factors including maternal oral microbiome, mode of delivery, maternal and infant diet, and environmental exposures[17]. Gut microbiome may participate in physiologic activities and further regulate cardiovascular metabolism, affecting the health of both mother and offspring[18].

During pregnancy, the maternal gut microbiome undergoes profound compositional and functional shifts[19, 20]. A marked reduction in alpha diversity occurs into the 3T, accompanied by an expansion of Proteobacteria and Actinobacteria, as well as increased levels of LPS - a component of the outer membrane of gram-negative bacteria that plays a central role in the pathogenesis of cardiovascular diseases, particularly in arterial hypertension (AHT), through its ability to induce systemic inflammation and endothelial dysfunction[19, 21]. These changes are thought to be adaptive, supporting increased energy storage, insulin resistance, and immune tolerance required for healthy foetal development[20]. Some factors, such as diet, excessive gestational weight gain and obesity, are associated with the gut microbiome of mothers, and an increase in the ratio of Bacillota/Bacteroidota and inflammatory markers is observed[20]. Emerging evidence also indicates that maternal microbial components, such as LPS and extracellular vesicles, can cross the placental barrier and modulate foetal immune programming[19]. This prenatal microbial priming may influence the maturation of regulatory T-cells and antigen-presenting cells, shaping immune resilience and cardiometabolic risk in infancy and beyond[19].

1.3 *Akkermansia muciniphila* in metabolic and cardiovascular regulation during pregnancy

One of the most studied microbes is *Akkermansia muciniphila (A. muciniphila),* which has attracted considerable attention for its role in maintaining host well-being, being regarded as a promising “next-generation beneficial microbe” for metabolic disease prevention or therapy[22]. This species exhibits various beneficial properties, by producing SCFAs, improving intestinal integrity, and reducing

endotoxemia through inhibiting the translocation of LPS from the intestine to circulation[22-24]. The relative abundance of *A. muciniphila* is positively associated with healthy status in comparison to patients with obesity, diabetes, and high blood pressure[25, 26]. In fact, *A. muciniphila* was approved by the European Food Safety Authority (EFSA) to be used in food supplements and foods for special medical purposes[27]. In both animal and human studies, supplementation with *A. muciniphila*, live or pasteurised, has led to reductions in fat mass, insulin resistance, plasma cholesterol, and metabolic endotoxemia, with some evidence suggesting greater efficacy from pasteurised forms[28]. Its cardiovascular benefits are underscored in hypertensive models, where it has improved endothelial function via the nitric oxide pathway and modulated the renin–angiotensin system[28]. Dietary strategies, such as increased fibre intake, polyphenols, or even the Mediterranean Diet (MED), have increased the abundance of *A. muciniphila*, correlating with improved metabolic and cardiovascular outcomes[28] . At the mechanistic level, *A. muciniphila* releases extracellular vesicles that strengthen the intestinal barrier and may cross the placenta, influencing foetal stem cell programming. Additionally, membrane proteins (e.g., Amuc_1100) activate TLR2-mediated anti-inflammatory pathways, helping modulate chronic inflammation linked to obesity and cardiovascular risk[29].

1.4 Faecal calprotectin as a marker of Intestinal Inflammation

Alongside microbial modifications, pregnancy represents a period of systemic low-grade inflammation[1], with the gut mucosa experiencing elevated inflammatory responses with high levels of pro-inflammatory cytokines as the pregnancy advances[18]. Calprotectin is a cytosolic protein in neutrophils, and it is secreted in stool when the intestinal mucosa is infiltrated by these cells[30]. Faecal calprotectin (FC) is considered a surrogate marker of intestinal inflammation, and FC was found to be increased in subjects with insulin resistance, obesity, and cardiovascular risk[31-33]. In healthy pregnancy, FC levels remain stable and within normal ranges <50 µg/g and do not significantly vary across trimesters[34, 35]. This stability, even as other systemic markers fluctuate, highlights FC specificity as a biomarker of intestinal inflammation during gestation[35]. However, in pregnant women with complications such as gestational diabetes and hypertensive disorders, systemic

inflammation is elevated, and this is reflected in higher serum calprotectin levels[36]. While data on FC in these groups are limited, the inflammatory milieu suggests that FC likely increases in these conditions[36]. Notably, healthy pregnancies show only slight FC elevations throughout gestation, but more pronounced inflammation as seen in GDM or hypertensive pregnancies[36].

1.5 Zonulin as a Key Marker of Gut Barrier Function

Zonulin is a protein that modulates intestinal tight junctions and has emerged as a non-invasive marker of intestinal permeability[37]. Elevated serum levels of zonulin reflect a compromised gut barrier, commonly referred to as "leaky gut", which allows the translocation of microbial components into the systemic circulation and can promote low-grade systemic inflammation[38]. In this context, "leaky gut" has gained attention as a potential upstream contributor to maternal and foetal pathology[39]. Circulating level of zonulin has been associated with autoimmune diseases, lung diseases, intestinal diseases, metabolic disorders and cardiovascular diseases[38]. Recent evidence points to the increased permeability of the gut epithelial barrier and an inflammatory state being associated with blood pressure elevation[38, 40]. During the second trimester of pregnancy, abnormal placentation occurs due to remodelling of the spiral arteries, release of syncytiotrophoblast pro-inflammatory factors, which are believed to cause systemic inflammatory response and clinical manifestations of pregnancy-induced hypertension (PIH)[40]. Zonulin may contribute to this process by increasing intestinal permeability, allowing the translocation of inflammatory microbial products into the circulation, thereby amplifying systemic inflammation and potentially worsening endothelial dysfunction associated with PIH.

Elevated zonulin levels during pregnancy have been linked to gestational complications, possibly by promoting systemic inflammation and impairing placental vascular function[38, 40]. It is characterised by impaired gut barrier function, allowing translocation of microbial components into systemic circulation[39]. This can shift the maternal immune profile from a physiologically adaptive inflammatory state to a pathologically excessive one, potentially leading to conditions such as preeclampsia or foetal growth restriction[17].

1.6. The Mediterranean Diet as a Nutritional Strategy for Gut Integrity and Cardiac Adaptation

Diet has multiple effects on the gut microbiota, being able to modify its composition and function.

The Mediterranean Diet (MED), characterised by high consumption of fruits, vegetables, whole grains, legumes, nuts, extra virgin olive oil, and moderate intake of fish and dairy, is widely recognised for its anti-inflammatory and cardioprotective properties[41, 42]. During pregnancy, adherence to the MED has been associated with a reduced risk of major obstetric complications such as GDM, hypertensive disorders of pregnancy and excessive gestational weight gain[41-43].

Strong evidence suggests that MED appears to counteract the effects of cardiometabolic disorders by modulating the gut microbiome, increasing beneficial taxa while reducing levels of intestinal inflammatory markers[44, 45]. Improved intestinal barrier integrity may play a central role in maintaining vascular homeostasis and reducing myocardial stress throughout gestation[45].

Evidence also suggests that MED adherence is linked to favourable lipid and glucose profiles, attenuated blood pressure rises, and lower systemic inflammatory markers, all relevant for mitigating the progression of gestational hypertensive or diabetic phenotypes and their cardiac consequences[42, 43]. In GDM, for instance, poor glycaemic control and LV hypertrophy can persist postpartum and elevate long-term cardiovascular risk. However, MED interventions have been shown to improve glycaemic markers and insulin sensitivity in pregnant women, potentially limiting structural cardiac changes[43, 46].

Furthermore, dietary patterns rich in polyphenols, omega-3 fatty acids, and fibre may influence cardiac remodelling via modulation of the gut–heart axis, including suppression of pro-inflammatory pathways and enhancement of nitric oxide bioavailability[41]. Olive oil consumption can reduce adverse maternal and foetal effects, such as GDM, preeclampsia, cardiovascular risk, and gestational weight gain[47].

MED diet is optimal to ensure an adequate supply of nutrients during pregnancy. It provides energy and nutrients required to allow adequate foetal growth and development, protecting from the development of obstetric pathologies, including childbirth complications and infections[46, 48].

1.7. Other strategies to modulate the gut microbiome

Beyond dietary patterns such as the MED, other interventions have emerged as promising strategies to modulate the maternal gut microbiota and improve metabolic and cardiovascular outcomes during pregnancy.

Include probiotic and prebiotic supplementation with evidence to improve glucose, lipid metabolism, and anti-inflammatory responses in gestational diabetes patients [49, 50]. More recently, postbiotics, including bacterial-derived metabolites and extracellular vesicles, have also been explored for their immunomodulatory potential and gut barrier stabilization[51]. In particular, *A. muciniphila* has demonstrated postbiotic effects through its membrane protein Amuc_1100 and extracellular vesicles, improving intestinal integrity, modulating host immunity, beneficial metabolic effects and attenuating systemic inflammation [52].

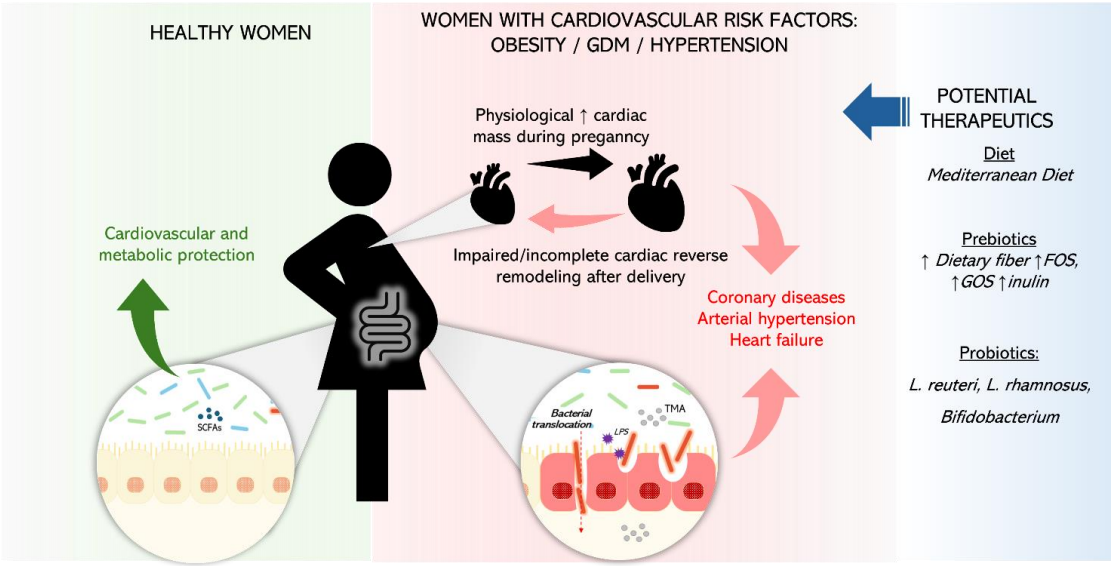
Moderate physical activity, when adapted to gestational needs, has been shown to promote gut microbial diversity, increase beneficial taxa (e.g., SCFA producers), and reduce systemic inflammation, ultimately supporting cardiovascular adaptation and metabolic homeostasis [53, 54]. Additionally, limiting unnecessary antibiotic exposure is critical, particularly during early pregnancy, as antibiotics can disrupt microbial homeostasis, reduce bacterial diversity, and promote increased gut permeability, thereby enhancing inflammatory responses associated with adverse obstetric outcomes [55, 56].

In contrast, supplementation with polyphenols, found in berries, green tea, cocoa, and extra virgin olive oil, has demonstrated prebiotic effects by selectively enriching beneficial bacteria, improving gut barrier integrity and exerting anti-inflammatory and antioxidant actions [57].

Together, these complementary strategies represent feasible, non-pharmacological tools that may enhance gut and cardiovascular health during pregnancy, with potential long-term benefits for both maternal and offspring outcomes.

Despite growing scientific interest, the mechanistic pathways linking intestinal health, microbial composition, gut-derived metabolites, and cardiac adaptation in pregnancy remain poorly characterised. Understanding the interactions within the gut–heart axis during this critical period may offer novel insights into maternal cardiovascular health and future prevention strategies. A conceptual figure (Fig. 1)

is presented below to illustrate these interconnected domains and their potential implications in pregnancy-related cardiac remodelling.



GDM – Gestational diabetes *mellitus*; FOS- Fructooligosaccharides; GOS- galactooligosaccharides; *L. Lactobacillus*; LPS – Lipopolysaccharide; SCFAs – Short chain fatty acids; TMA – Trimethylamine

Figure 1: Schematic overview of the proposed gut–heart axis during pregnancy. Maternal gut dysbiosis and increased gut permeability may promote systemic inflammation through translocation of bacterial components such as LPS, while reduced production of SCFAs impairs anti-inflammatory signalling. These processes, influenced by dietary patterns (e.g., MED) and probiotic intake, may modulate cardiovascular adaptation and cardiac remodelling in pregnancy, especially in the presence of comorbidities. Image originally submitted as part of a review article to the journal *Acta Portuguesa de Nutrição* (submitted, decision pending).

2. Aims

The primary goal of this master's thesis is to assess the interplay between intestinal biomarkers, cardiovascular reverse remodelling, weight dynamics, and dietary patterns in women during the 3T of pregnancy and the postpartum period.
The specific objectives are:

1. To assess the relation between cardiac reverse remodelling and intestinal inflammation (FC), permeability (serum zonulin) and the levels of *A. muciniphila* at 3rd trimester (3T) and at 6 months postpartum (PP2);
2. To explore the association between gestational weight gain and postpartum weight recovery and FC, zonulin and *A. muciniphila*;
3. To explore the association between MED adherence and FC, zonulin and the levels of *A. muciniphila*;
4. To investigate the association between MED adherence and echocardiographic parameters, gestational weight gain and postpartum weight recovery.

3. Material and Methodology

This research was a retrospective observational cohort study that was inserted in the ongoing observational cohort study PERIMYR & OralBioBorn conducted at the Department of Surgery and Physiology of the Faculty of Medicine of the University of Porto.

Participant recruitment and follow-up:[58]

34 participants with completed follow-up were included in this master´s thesis. Adult pregnant women (>18 years old) have been recruited during their medical appointment (in the 3T of pregnancy) at the Obstetrics Departments of *Unidade Local de Saúde de São João (ULS São João)*.

Inclusion criteria were adult pregnant women with or without cardiovascular risk factors, namely, chronic and/or gestational hypertension, GDM, and/or obesity. Chronic arterial hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg measured in the office or in-hospital before 20 weeks of gestation[59]. Gestational hypertension was defined as arterial hypertension diagnosed after 20 weeks of gestation that resolved within 42 days postpartum[59]. GDM was considered if fasting glucose was ≥92 and ≤ 126 mg/dL in the first trimester or ≥180 mg/dL or ≥153 mg/dL 1 or 2 h after an oral glucose tolerance test (75-g oral glucose load) performed at 24–28 weeks of pregnancy. Obesity was defined as a body mass index (BMI) ≥ 30 kg/m^2 before pregnancy. Exclusion criteria included twin pregnancies, pre-existing cardiomyopathy, renal disease, chronic obstructive pulmonary disease, active systemic infections, genetic syndromes, or type 1 diabetes. Participants who experienced pregnancy loss during the follow-up period were also excluded.

Included participants underwent the following evaluations: (1) clinical and sociodemographic characterisation; (2) assessment of dietary habits; (3) collection of faecal and blood samples; (4) transthoracic echocardiography, and (5) vascular stiffness evaluation. All of the mentioned evaluations were conducted at two time points: the 3T (30–35 weeks of gestation), representing the peak of cardiac remodelling, and PP2.

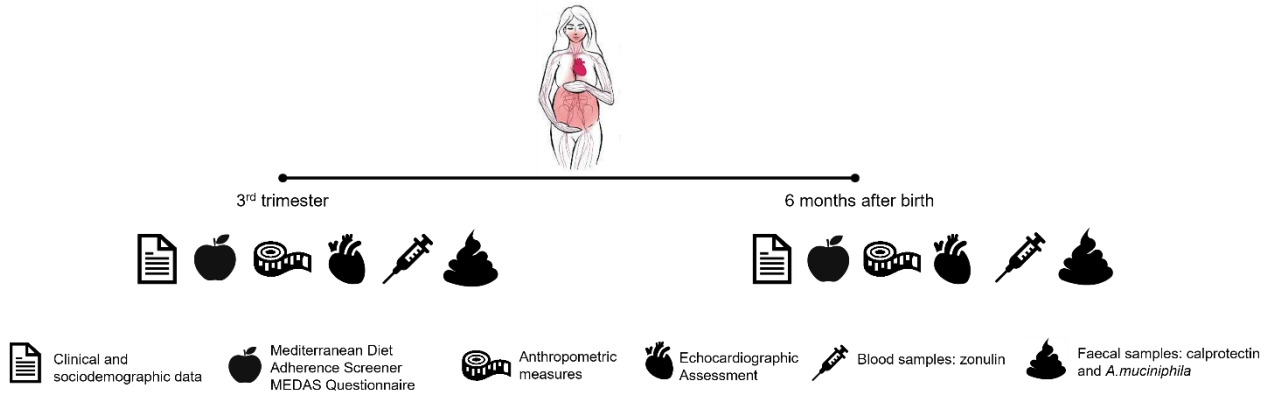


Figure 2: Schematic representation of the study design.

All eligible participants were able and willing to provide written informed consent to be included in this study. The Ethics Committees of ULS São João approved the study (PERIMYR: ID 201/18 and OralBioBorn: ID 294/18). All data management complied with professional confidentiality and used pseudonymization. Only PERIMYR & OralBioBorn team members have access to the data. An exclusively dedicated server stores study information, and access to it is restricted to the study team. Data collection and management comply with the General Data Protection Regulation (GDPR) 2016/679, 27 April 2016, and Law 58/2019 (Portugal). Biological samples are kept throughout the project and destroyed five years after study completion.

Data and sample collection: Detailed clinical data were collected during the first evaluation time (30–35 gestational weeks) through extensive questionnaires that included information about obstetric outcomes (gestational hypertension, gestational diabetes, weight gain), maternal smoking habits and medical history. Dietary habits were assessed by the Mediterranean Diet Adherence Screener Questionnaire (MEDAS)[60]. In the following moments, 6 months after delivery, new questionnaires were applied to obtain more up-to-date clinical and personal information. Blood and faeces were collected from the mother at every time point (Figure 2).

Echocardiographic Assessment:

The transthoracic echocardiographic assessment was performed by a single operator, using a 3MHz phased array probe (ACUSON S2000 PRIME ™). The resulting measurements are in conformity with the recommendations of the European Society

of Cardiology for cardiac chamber quantification and diastolic function evaluation[61, 62]. Images of at least three cardiac cycles were obtained for data analysis. Two certified cardiologists independently interpreted, analysed and harmonized the results. LV mass was calculated using the following equation: 0.8 x {1.04 x [(LV end-diastolic diameter + LV posterior wall diastolic thickness + interventricular septum diastolic thickness) 3 - (LV end diastolic diameter) 3] + 0.6} g [54]. Relative wall thickness was determined using the following equation: 2 x LV posterior wall diastolic thickness / LV end diastolic diameter. The diastolic volume, ejection fraction and the LV end systolic and end diastolic volumes were calculated using the Simpson biplane method. Allometric normalisation was applied to cardiac mass and volumes by indexing these echocardiographic parameters to a height raised to the power of 2.7. Systemic vascular resistance was determined using: 80 x mean blood pressure / LV cardiac output (dyn.s.cm -5), with mean arterial pressure calculated as: systolic blood pressure + (2 x diastolic blood pressure) /3. Additionally, E/e' was calculated using the ratio of E mitral inflow velocity to the average early diastolic septal velocity with lateral mitral annular velocity.

Vascular stiffness evaluation:

Brachial blood pressure was measured in the non-dominant arm after 10 minutes of resting. Vascular stiffness was evaluated using the Complior device (Alam Medical, France) by calculating the carotid-femoral pulse wave velocity (PWV). This parameter was estimated using the carotid-femoral distance/transit time. Each participant underwent at least two pulse wave velocity measurements (with a difference ≤0.5 m/s) per each cardiovascular evaluation. The average of these measurements was then recorded in the database.

Faecal Sample Analysis for Calprotectin:

Intestinal inflammation was assessed by measuring FC. Participants collected stool samples at home (the day before or on the day of the visit) and kept them refrigerated. On the day of the visit, aliquots were prepared and stored at -80ºC until analysis. Faecal calprotectin was quantified using the S100A8/S100A9 heterodimer DuoSet ELISA (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions, except for the initial sample preparation. Specifically,

20 mg of stool was weighed, diluted 1:50 by adding 1 mL of extraction buffer (0.1M Tris; 0.015 M NaCl, 1.0M Urea, 1.0mM $CaCl_2$), and the resulting faecal extract was filtered through a 0.2 µm filter. All subsequent steps were performed according to the manufacturer's protocol. Absorbance of standards and samples was measured at 450 nm. The results were expressed in µg/g.

Blood Sample Collection and Zonulin Quantification:

Blood samples were centrifuged at 15,000 × g for 15 minutes at 4 °C to obtain serum and stored at -80ºC until used. Serum zonulin levels were quantified using the Enzyme-linked Immunosorbent Assay EEL072 (Invitrogen/Thermo Fischer, Ulm, Germany). The manufacturer's instructions were followed, and a dilution factor 1:10 was used. Absorbance of standards and samples was measured at 450 nm. The results were expressed in ng/mL.

DNA extraction and *A. muciniphila* quantification:

For DNA extraction, 200 mg of stool was weighed, and TE buffer (10mM Tris/KCl, pH 8.0) was added. The mixture was vortexed vigorously until homogeneous and then centrifuged at 4000 x g for 15 minutes. Subsequently, 180 µL of Buffer NT1 were added to the pellet, followed by vortexing and the addition of 25 µL of Proteinase K. The samples were incubated at 56 °C for 1 hour. After this step, DNA extraction was carried out using the NZY Tissue gDNA Isolation Kit (NZYTech), according to the manufacturer's protocol. DNA concentration was measured using a NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific).

Real-time quantitative PCR (RT-qPCR) was used to assess the abundance of *A. muciniphila*in faecal samples. The PCR amplification was performed using specific primers[63] F: 5'-CAGCACGTGAAGGTGGGGAC-3′, R: 5′- CCTTGCGGTTGGCTTCAGAT-3′ targeting *Akkermansia muciniphila*, with SYBR™ Green Universal Master Mix (Applied Biosystems™). The bacterial concentration in each sample was determined by comparing the Ct values to a standard curve. This standard curve was made using DNA extracted from a pure culture of *Escherichia coli* with specific primers F: 5'-CATTGACGTTACCC GCAGAAGAAGC-3′, R: 5'-CTCTACGAGACTCA AGCTTGC-3′ as a reference strain[63].

Statistical analysis:

Statistical analyses were conducted using IBM SPSS® Statistics, (version 30.0.0.0) for Windows.

In the descriptive analysis, categorical variables were presented as absolute (n) and relative frequencies (%), while continuous variables were expressed as medians and interquartile ranges [P25; P75] due to their non-normal distribution. The normality of continuous variables was assessed using the Shapiro–Wilk test, which is appropriate for small sample sizes (n < 50). Since most variables did not follow a normal distribution, non-parametric tests were applied in the inferential analysis.

The association between continuous variables was evaluated using Spearman's rank correlation coefficient (ρ). The strength of the correlation was interpreted according to the following criteria:

- [0; 0.25[– very weak
- [0.25; 0.5[– weak
- [0.5; 0.75[– moderate
- [0.75; 0.9[– strong
- [0.9; 1] – very strong

To compare paired samples the Wilcoxon signed-rank test was used. For comparisons between independent groups, the Mann–Whitney U test was applied. To explore the associations between binary dietary variables and continuous cardiometabolic parameters, we computed point-biserial correlations. All binary variables (e.g., dietary habits, coded as "Yes"/"No") were numerically recoded as 1 and 0, respectively. Then, Pearson's correlation test was applied between each binary predictor and each continuous outcome. All statistical analyses were performed using R (version 4.3.1). Correlation coefficients and p-values were computed using the cor.test() function. Heatmaps were generated with the ggplot2 package.

Statistical significance was set at $p < 0.05$, with a 95% confidence interval (CI).

4. Results

4.1 Sample characterization

A total of 34 pregnant women were included in the present study. The median age was 33 [31; 36] years old. Regarding educational level, 5.9% of the participants had basic education, 38.2% had completed secondary education, 38.2% had a first-cycle degree, and 17.6% held a second-cycle degree. Most participants were employed (88.2%), while 8.8% were unemployed and 2.9% reported being domestic workers. The median pre-pregnancy weight was 76.0 [69.0; 93.0] kg, with a median height of 1.63 [1.60; 1.69] m, resulting in a median pre-pregnancy BMI of 28.9 [25.0; 34.5] kg/m^2. In relation to BMI, 23,5% had normal weight, 32.3 % were overweight, and 44.1 % were obese. The gestational weight gain was a median of 6.2 [0.0; 9.0] kg, and the median of weight recovery of the pregestational weight gain at PP2 was 3.0 [-3.1; 6.3 kg. Table 1 presents the sociodemographic and clinical characteristics of the study sample.

Regarding cardiovascular risk factors, 47.1% of women had pre-pregnancy obesity, 23.5% had chronic AHT, and 58.8% developed GDM. A small number had combinations of these conditions. One woman (2.9%) had both chronic AHT and GDM, while 17.6% had obesity and GDM. No cases of preeclampsia were recorded, although 14.7% were identified as at risk according to the result of first-trimester combined screening (Table 1).

Most participants were non-smokers (73.5%), and 35.3% reported drinking alcohol during pregnancy. The median gestational age at delivery was 38 weeks (38; 39). Vaginal delivery was more frequent (38.2%) than vaginal delivery (26.5%). Primiparous women represented 29.4% of the sample (Table 1).

Table 1: Sociodemographic and clinical characterisation of participants. Values are expressed by median [interquartile range].

Participants characterization (n = 34)	
Age, years	33 [31; 36]
Education level, n (%)	
Basic education	2 (5.9)
Secondary education	13 (38.2)
1st cycle of studies	13 (38.2)
2nd cycle of studies	6 (17.6)
Work situation, n (%)	
Employed	30 (88.2)
Unemployed	3 (8.8)
Domestic	1 (2.9)
Prepregnancy weight, kg	76.0 [69.0; 93.0]
Height, m	1.63 [1.60; 1.69]
Pre pregnancy BMI, kg/m²	28.9 [25.0; 34.5]
[18.6; 25.0[n (%)	8 (23.5)
[25.0; 30.0[n (%)	11 (32.3)
≥30.0 n (%)	15 (44.1)
Gestational weight gain, kg	6.2 (0.0; 9.0)
Weight recovery at 6 months, kg	3.0 (-3.1; 6.3)
Cardiovascular risk factors	
Obesity	16 (47.1)
Diabetes *mellitus* type 2	1 (2.9)
Chronic Arterial hypertension	8 (23.5)
Gestational diabetes *mellitus*	20 (58.8)
Preeclampsia risk positive	5 (14.7)
Smoking habits, n (%)	
Non-smoker	25 (73.5)
Smoker n	9 (26.4)
Alcohol habits	
Alcohol habits during pregnancy	12 (35.3)
Alcohol habits postpartum	8 (23.5)
Previous pregnancies	1 [0; 2]
Primiparous women, n (%)	10 (29.4)
Gestational week at delivery	38 (38; 39)
Mode of delivery, n (%)	
Vaginal delivery	13(38.2)
Caesarean delivery	9 (26.5)
Exclusively breastfeeding, n (%)	11 (32.4)

During pregnancy, several women required pharmacological interventions. Statins, insulin, and metformin were each used by 11.8% of participants, indicating the presence of lipid and glycaemic disturbances (Table 2). Beta-blockers were prescribed to 17.6%, followed by diuretics (14.7%) and calcium channel blockers (8.8%), likely reflecting management of hypertensive disorders. Additionally, 5.9%

of participants received antidepressants, anxiolytics, or thyroid hormone therapy. Antibiotic use during pregnancy was reported in 8.8%, and probiotic therapy in one participant (2.9%).

Postpartum, pharmacologic therapy patterns changed. Metformin was continued in 11.8% of cases, while the use of insulin, statins, and beta-blockers ceased. A small proportion of women continued treatment with calcium antagonists (5.9%), diuretics (2.9%), antidepressants, anxiolytics, and thyroid hormones (each 5.9%). At PP2, antibiotic therapy was reported by 8.8% of participants, and probiotic use remained low (2.9%).

Table 2: Pharmacological therapy during pregnancy and during the first 6 months of postpartum

Participants' pharmacologic therapy (n = 34)		
	During pregnancy	During 6 months postpartum
Statins, **n (%)**	4 (11.8)	0 (0.0)
Insulin, **n (%)**	4 (11.8)	0 (0.0)
Metformin, **n (%)**	4 (11.8)	4 (11.8)
Betablockers, **n (%)**	6 (17.6)	0 (0.0)
Diuretics, **n (%)**	5 (14.7)	1 (2.9)
Calcium antagonists, **n (%)**	3 (8.8)	2 (5.9)
Antidepressants, **n (%)**	2 (5.9)	2 (5.9)
Anxiolytics, **n (%)**	2 (5.9)	2 (5.9)
Thyroid hormones, **n (%)**	2 (5.9)	2 (5.9)
Antibiotic therapy, **n (%)**	3 (8.8)	3 (8.8)
Probiotic therapy, **n (%)**	1 (2.9)	1 (2.9)

4.2. Maternal Mediterranean Diet adherence

The median score of MED adherence at late pregnancy was 9.0 (7.0; 10.0), and showed a tendency to decline at PP2 7.5 (6.0; 9.25) (p = 0.149) (Table 3). At 3T, 23.5% of participants showed high adherence (>9 points), decreasing to 14.7% at PP2. Meanwhile, the proportion of women with low adherence (≤5 points) increased from 2.9% in 3T to 14.7% at PP2 (Table 3).

Although most women reported using olive oil as the primary fat for cooking, usage decreased from 76.5% in the 3T to 55.9% postpartum, but not reaching significant difference (ρ = 0.260). Daily consumption of ≥4 tablespoons of olive oil remained low across both time points (5.9% at 3T vs. 11.8% at PP2; p = 1.000). Eating two

or more servings of vegetables per day and fruit (≥3 pieces/day) also decreased from 44.1% at 3T to 26.5% at PP2 (p = 1.000).

Consumption of less than one serving of red or processed meats per day dropped from 50.0% to 20.6% (p = 0.172), and intake of butter, margarine, or cream <1 serving/day decreased from 58.8% to 32.4% (p = 1.000). The percentage of participants consuming ≥1 sweetened and/or carbonated beverage daily increased from 8.8% to 20.6% (p = 0.318), and although wine consumption remained absent across both time points, no participant reported drinking ≥7 glasses/week.

Weekly intake of legumes (≥3 servings/week) remained stable (26.5% at 3T vs. 29.4% at PP2; p = 1.000), fish or seafood intake (≥3 servings/week) changed not significantly from 32.4% to 17.6% (p = 0.283). Interestingly, the number of women consuming fewer than three commercial pastries per week showed an tendency to increase from 14.7% at 3T to 47.1% at PP2 (p = 1.000), and nut consumption (≥3 servings/week) rose slightly from 14.7% to 17.6% (p = 0.532), though neither change was statistically significant.

A statistically significant reduction was observed in the preference for white meat over red meat, declining from 64.7% in the 3T to 55.9% postpartum (p = 0.013). Finally, consumption of "sofrito" (≥2 servings/week) decreased from 70.6% to 52.9%, with no statistical significance (p = 1.000).

Table 3: Results of the Mediterranean Diet Adherence Screener (MEDAS) Questionnaire at late pregnancy and postpartum.

	3T (n =29)	**PP2 (n=24)**	***p*-value**
MEDAS total score, median [interquartile range]	9 [7;10]	7.5 [6; 9.25]	**0.149**
Levels of MED adherence, n (%)			
Low Adherent (≤ 5 points)	1 (2.9)	5 (14.7)	**0.074**
Moderate Adherent (≤ 9 points to >5 points)	20 (58.8)	12 (35.3)	
High Adherent (> 9 points)	8 (23.5)	5 (14.7)	
MEDAS Questions, n (%)			
			***p*-value**
Do you use olive oil as the principal source of fat for cooking?			
Yes	26 (76.5)	19 (55.9)	0.260
No	3 (8.8)	3 (8.8)	
How much olive oil do you consume per day (including that used in frying, salads, meals eaten away from home, etc.)? (≥4 tablespoons per day?)			

	Yes	2 (5.9)	8(23.5)	1.000
	No	27 (79.4)	14 (41.2)	
How many servings of vegetables do you consume per day? (≥2 servings per day?)				
	Yes	17 (50.0)	8(23.5)	0.204
	No	12 (35.3)	14 (41.2)	
How many pieces of fruit (including fresh-squeezed juice) do you consume per day? (≥3 pieces per day?)				
	Yes	15 (44.1)	9 (26.5)	1.000
	No	14 (41.2)	13 (38.2)	
How many servings of red meat, hamburger, or sausage do you consume per day? (<1 serving per day?)				
	Yes	17 (50.0)	7 (20.6)	0.172
	No	12 (35.3)	15 (44.1)	
How many servings of butter, margarine, or cream do you consume per day? (<1 serving (12g) per day?)				
	Yes	20 (58.8)	11 (32.4)	1.000
	No	9 (26.5)	11 (32.4)	
How many carbonated and/or sugar-sweetened beverages do you consume per day ?(≥1 sweetened per day?)				
	Yes	3 (8.8)	7 (20.6)	
	No	26 (76.5)	15 (44.1)	0.318
Do you drink wine? How much do you consume per week? (≥7 glasses per week?)				
	Yes	0 (0.0)	0 (0.0)	-
	No	29 (85.3)	21 (61.8)	
How many servings of pulses do you consume per week? (≥3 servings?)				
	Yes	9 (26.5)	10 (29.4)	1.000
	No	20 (58.8)	12 (35.3)	
How many servings of fish/seafood do you consume per week? (≥3 servings?)				
	Yes	11 (32.4)	6 (17.6)	0.283
	No	18 (52.9)	16 (47.1)	
How many times do you consume commercial (not homemade) pastries such as cookies or cake per week? (<3 commercial pastries per week?)				
	Yes	5 (14.7)	16 (47.1)	1.000
	No	24 (70.6)	6 (17.6)	
How many times do you consume nuts per week? (≥3 servings per week?)				
	Yes	5 (14.7)	6 (17.6)	0.532
	No	24 (70.6)	16 (47.1)	
Do you prefer to eat chicken, turkey, or rabbit instead of beef, pork, hamburgers, or sausages? (white meat over red meat?)				
	Yes	22 (64.7)	19 (55.9)	**0.013**
	No	7 (20.6)	3 (8.8)	
How many times per week do you consume boiled vegetables, pasta, rice, or other dishes with a sauce of tomato, garlic, onion, or leeks sautéed in olive oil? (≥2 servings per week?)				
	Yes	24 (70.6)	18 (52.9)	1.000
	No	5 (14.7)	4 (11.8)	

Fisher test CI 95%

4.3 Cardiovascular reverse remodelling (RR)

Cardiovascular function was evaluated in the 3T and at PP2. There was a statistically significant decrease in heart rate (80 [72–86] bpm to 71 [65–76] bpm, $p < 0.001$), suggesting a return to baseline autonomic tone. Cardiac mass index decreased significantly from PT3 to PP2 (134.83 [120.00; 156.78] g/m$^{2.7}$ to (115.23 [103.64;143.53] g/m$^{2.7}$, $p = 0.003$), while relative wall thickness remained stable across time points ($p = 0.768$). The absolute LVM regression was 13.88 [–1.7; 28.14] g/m$^{2.7}$, corresponding to a median percentage reduction of 10.84% [–1.5; 20.18]. A significant decrease in left ventricle (LV) volume index was observed, (28.67 [24.84; 30.13] mL/m$^{2.7}$ to 23.16 [20.88; 26.36] mL/m$^{2.7}$, ρ < 0.001) and left atrial volume index (12.77 [11.50; 14.04] to 11.02 [8.81; 12.9] mL/m$^{2.7}$, $p = 0.017$), aligning with the reduction in cardiac dimensions. Additionally, E/e', a marker of diastolic filling pressure, improved significantly from 3T to PP2 (6.60 [5.71; 8.08] to 5.90 [5.00; 6.48], $p = 0.001$), supporting recovery of diastolic function.

Table 4: Cardiovascular assessment at 3T and PP2. Values are expressed by median [interquartile range].

	3T (N=34)	PP2 (N=33)	*p*-value
Systolic blood pressure, mm Hg	127 [121; 137]	130 [120; 139]	0.187
Diastolic blood pressure, mm Hg	79 [75; 86]	83 [76; 90]	0.034
Heart rate, beats/min	80 [72; 86]	71 [65; 76]	<0.001
Pulse wave velocity, m/s	7.1 [6.3; 7.5]	7.4 [7.1; 8.1]	<0.001
Systemic vascular resistance, dyn-s/cm^5	1866.29 [1696.60; 2701.90]	2360.26 [2136.26; 2612.72]	0.160
Cardiac mass index, g/m$^{2.7}$	134,83 [120.00; 156.78]	115.23 [103.64; 143.53]	0.003
E/e'	6.60 [5.71; 8.08]	5.90 [5.00; 6.48]	0.001
Relative wall thickness	0.38 [0.35; 0.45]	0.39 [0.35; 0.43]	0.768
Left atrium volume index, mL/m$^{2.7}$	12.77 [11.50; 14.04]	11.02 [8.81; 12.99]	0.017
LV end-diastolic volume index, mL/m$^{2.7}$	28.67 [24.84; 30.13]	23.16 [20.88; 26.36]	<0.001
LVM regression, g/ m$^{2.7}$	13.88 [-1.7; 28.14]		-
LVM regression, %	10.84 [-1.5; 20.18]		-

Wilcoxon test, CI 95%
3T- 3rd trimester; PP2- 6 months postpartum; LV-Left ventricle; LVM-Left ventricular mass.

Pulse wave velocity increased significantly during the follow-up period (7.1 [6.3; 7.5] m/s to 7.4 [7.1; 8.1] m/s, $p < 0.001$), reflecting increased arterial stiffness postpartum. Diastolic blood pressure also rose significantly (79 [75;86] mmHg to

83 [76; 90] mmHg, p = 0.034), while systolic pressure and systemic vascular resistance did not change significantly.

4.4 Faecal calprotectin, serum zonulin, and *A. muciniphila* at late pregnancy and postpartum

Intestinal inflammation, measured by FC concentrations (Fig.3), was found to be at non-pathological levels at both timepoints. Median FC concentration significantly decreased (from 2.90 [1.81; 7.92] µg/g faeces at 3T to 2.12 [1.34; 2.98] µg/g faeces at PP2, p < 0.001). Similarly, median zonulin levels (Fig. 3) significantly decreased between the same period (456 [409; 480] ng/mL to 378 [327; 443] ng/mL, p < 0.001).

Regarding *A. muciniphila* (Fig.3), also significantly decreased (from (0.74 [0.00; 1.22] Log10 16S rRNA genes copies/10ng DNA at 3T to 0.289 [0.00; 1.65] Log10 16S rRNA genes copies/10ng DNA at PP2, p = 0.034).

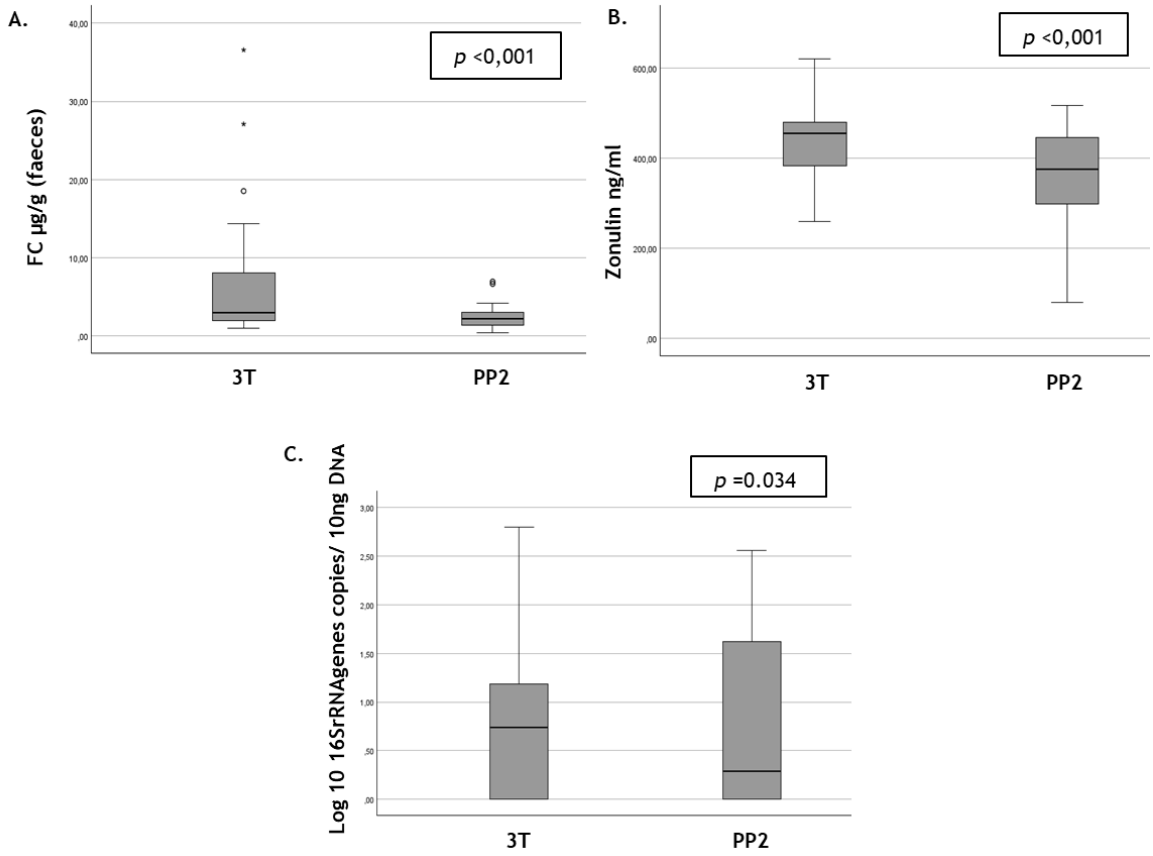


Figure 3: Concentrations of faecal calprotectin (A), serum zonulin (B), and *A. muciniphila (C)* at timepoints 3rd trimester (3T) and 6 months postpartum (PP2). Boxplots display the median and interquartile range.

4.4.1 Cardiac RR and Faecal Calprotectin

Correlations between FC levels and various cardiovascular parameters were assessed at both time points (3T and PP2) using Spearman correlation. At 3T (Table 5), a significant positive correlation was observed between calprotectin levels and cardiac mass index (ρ = 0.405, p = 0.024).

Table 5: Correlation between faecal calprotectin levels (µg/g) and echocardiographic parameters.

	Faecal calprotectin (ug/g)			
	3T		PP2	
	ρ	*p*-value	ρ	*p*-value
Systolic blood pressure, mm Hg	0.021	0.912	0.023	0.904
Diastolic blood pressure, mm Hg	0.021	0.912	-0.158	0.421
Heart rate, beats/min	-0.123	0.502	-0.032	0.864
Pulse wave velocity, m/s	0.062	0.750	-0.044	0.817
Systemic vascular resistance, dyn-s/cm^5	-0.350	0.062	-0.178	0.347
Cardiac mass index g/m$^{2.7}$	**0.405**	**0.024**	0.061	0.741
E/e´	-0.231	0.203	**0.424**	**0.017**
Relative wall thickness	-0.187	0.304	-0.333	0.860
Left atrium volume index, mL/m$^{2.7}$	0.030	0.673	0.165	0.383
LV end-diastolic volume index, mL/m$^{2.7}$	0.208	0.279	0.218	0.246
LVM regression	0.114	0.512	0.286	0.119

Spearman correlation CI 95%; ρ (rho) as representative of Spearmen coefficient
LV - Left ventricle; LVM - Left ventricular mass

At PP2 (Table 5), FC was positively correlated with the E/e′ ratio (ρ = 0.424, p = 0.017), an indicator of diastolic dysfunction, reinforcing the potential impact of intestinal inflammation on cardiac function. No other cardiovascular parameters showed statistically significant correlations with calprotectin at either time point (p > 0.05).

4.4.2 Cardiac RR and Zonulin

Spearman correlation between serum zonulin levels and cardiovascular parameters was also assessed at both time points (Table 6). At PP2, several statistically significant correlations were found. Serum zonulin was positively correlated with diastolic blood pressure (ρ = 0.468, p = 0.021), suggesting a

potential link between intestinal permeability and increased vascular tone or diastolic load. A strong positive correlation was observed between zonulin and cardiac mass index (ρ = 0.517, p = 0.010), reinforcing a possible association between gut barrier dysfunction and cardiac remodelling (Table 6).

Table 6: Correlation between serum zonulin levels (ng/mL) and echocardiographic parameters.

	Serum zonulin (ng/mL)			
	3T		PP2	
	ρ	*p*-value	ρ	*p*-value
Systolic blood pressure, mm Hg	0.228	0.263	0.315	0.134
Diastolic blood pressure, mm Hg	0.150	0.464	**0.468**	**0.021**
Heart rate, beats/min	0.247	0.197	0.155	0.459
Pulse wave velocity, m/s	0.119	0.563	0.064	0.768
Systemic vascular resistance, dyn-s/cm^5	0.055	0.788	-0.384	0.064
Cardiac mass index g/m$^{2.7}$	-0.168	0.392	**0.517**	**0.010**
E/e´	-0.099	0.609	0.172	0.412
Relative wall thickness	0.335	0.075	0.306	0.137
Left atrium volume index, mL/m$^{2.7}$	-0.02	0.992	0.331	0.114
LV end-diastolic volume index, mL/m$^{2.7}$	-0.015	0.941	**0.484**	**0.019**
LVM regression	-0.117	0.552	-0.202	0.332

Spearman correlation CI 95%; ρ (rho) as representative of Spearmen coefficient
LV - Left ventricle; LVM - Left ventricular mass.

Additionally, left ventricular end-diastolic volume index was significantly correlated with zonulin (ρ = 0.484, p = 0.019), further supporting the connection between zonulin and structural cardiac changes. At 3T, no statistically significant correlations were observed between zonulin and the cardiovascular variables (p > 0.05) (Table 6).

4.4.3 Cardiac RR and *A. muciniphila*

No significant correlations were found between the abundance of *A. muciniphila* (expressed as Log10 16S rRNA gene copies/10ng DNA) and cardiovascular parameters at the 3T and PP2 (Table 7). Spearman correlation coefficients ranged from -0.268 to 0.251 with all p values well above the threshold for significance (p

≥ 0.05), indicating no meaningful associations with systolic or diastolic blood pressure, heart rate, pulse wave velocity, systemic vascular resistance, cardiac mass indexed to height$^{2.7}$, E/e' ratio, relative wall thickness, left atrium volume index, left ventricular end-diastolic volume index, or LVM regression.

Table 7: *A. muciniphila* and echocardiographic parameters.

	A. muciniphila **(Log 10 16S rRNA gene copies/10ng DNA)**			
	3T		**PP2**	
	ρ	***p*-value**	**ρ**	***p*-value**
Systolic blood pressure, mm Hg	-0.025	0.892	-0.101	0.583
Diastolic blood pressure, mm Hg	-0.155	0.404	-0.107	0.562
Heart rate, beats/min	-0.127	0.475	-0.268	0.132
Pulse wave velocity, m/s	-0.029	0.877	0.029	0.875
Systemic vascular resistance, dyn-s/cm^5	0.015	0.937	-0.049	0.789
Cardiac mass index g/m$^{2.7}$	0.077	0.937	-0.248	0.171
E/e´	0.153	0.387	-0.122	0.500
Relative wall thickness	-0.170	0.337	-0.100	0.581
Left atrium volume index, mL/m$^{2.7}$	-0.123	0.511	-0.149	0.415
LV end-diastolic volume index, mL/m$^{2.7}$	0.014	0.941	-0.208	0.260
LVM regression	0.019	0.918	0.251	0.159

Spearman correlation CI 95%; ρ (rho) as representative of Spearmen coefficient
LV-Left ventricle; LVM-Left ventricular mass.

4.5 Gestational weight gain and postpartum weight recovery, Faecal Calprotectin levels, zonulin levels and *A. muciniphila*

No statistically significant correlations were observed between FC and weight parameters (Table 8). At 3T, the correlation between FC and gestational weight gain was weak and not significant (ρ = -0.093, *p* = 0.623), as was the association with weight at 3T (ρ = 0.112, *p* = 0.512). Similarly, at PP2, FC was not significantly correlated with weight recovery (ρ = -0.126, p = 0.558) or postpartum weight (ρ = -0.032, *p* = 0.875).

Regarding zonulin at 3T, no statistically significant correlations were found between zonulin and gestational weight gain (ρ = -0.273, *p* = 0.168) or maternal weight at 3T (ρ =0.294, *p* =0.137) (Table 8). However, at PP2 zonulin showed a

significant positive moderate correlation with postpartum weight (ρ = 0.637, *p* = 0.020).

No significant correlations were found between the levels of *A. muciniphila* (Table 8) and maternal weight parameters, namely with weight at the 3T (ρ = 0.089, *p* = 0.628), postpartum weight (ρ = -0.067, *p* = 0.736), gestational weight gain (ρ = -0.117, *p* = 0.523), and weight recovery (ρ = -0.070, *p* = 0.975).

Table 8: Results of gestational weight gain and postpartum weight recovery and FC, serum zonulin and *A. muciniphila*.

		ρ	*p*-value
Faecal calprotectin (ug/g)	**Weight at 3T (kg)**	0.112	0.512
	Weight at PP2 (kg)	-0.032	0.875
	Gestational weight gain (kg)	-0.093	0.623
	Weight recovery (kg)	-0.126	0.558
Seruym Zonulin (ng/mL)	**Weight at 3T (kg)**	0.294	0.137
	Weight at PP2 (kg)	**0.637**	**0.020**
	Gestational weight gain (kg)	-0.273	0.168
	Weight recovery (kg)	-0.365	0.124
A.muciniphila (Log 10 16S rRNA gene copies/10ng DNA)	**Weight at 3T (kg)**	0.089	0.628
	Weight at PP2 (kg)	-0.067	0.736
	Gestational weight gain (kg)	-0.117	0.523
	Weight recovery (kg)	-0.07	0.975

Spearman correlation CI 95%; ρ (rho) as representative of Spearmen coefficient

4.6 Maternal diet and faecal calprotectin, zonulin and the levels of *A. muciniphila*;

No significant correlations were found between adherence score of the MED diet and FC levels (Table 9). At 3T, the correlation was weak and not statistically significant (ρ = -0.040, *p* = 0.841), and the same was observed at PP2 (ρ = 0.047, p = 0.839). Similarly, zonulin levels were not significantly correlated with dietary adherence at either time point (3T: ρ = 0.018, *p* = 0.929; PP2: ρ = -0.063, *p* = 1.000) (Table 9)

FC and zonulin levels were compared between groups based on responses to individual MED diet adherence questions at both time points, 3T and PP2 (Table 10).

Regarding FC, no statistically significant associations were found with any individual components of the MED diet at either 3T or PP2 ($p > 0.05$).

At 3T, no statistically significant differences in zonulin concentrations were found for any of the dietary components evaluated ($p > 0.05$). At PP2, a significant difference in zonulin levels was observed based on red and processed meat consumption. Participants who reported consuming one or more servings per day had significantly higher zonulin levels, 445 [432; 457] ng/mL, compared to those who consumed less than one serving per day, 374 (255;437) ng/mL (p =0.004).

No statistically significant associations were found between the levels of *A. muciniphila* and MEDAS scores during pregnancy or postpartum (Table 9). A negative trend was observed between *A. muciniphila* levels and the MEDAS score at the 3rd trimester (ρ = -0.342, p = 0.069), suggesting a possible inverse association that did not reach statistical significance. No association was observed postpartum (ρ = 0.174, p = 0.439).

Table 9: Results of adherence to the MED (MEDAS score) and intestinal biomarkers at 3T and PP2.

	MEDAS score at 3T		**MEDAS score at PP2**	
	ρ	***p*-value**	**ρ**	***p*-value**
FC (µg/g)	-0.040	0.841	0.047	0.839
Zonulin (ng/mL)	0.018	0.929	-0.063	1.000
A. muciniphila (Log 10 16S rRNA gene copies/10ng DNA)	-0.342	0.069	0.174	0.439

Spearman correlation CI 95%; ρ (rho) as representative of Spearmen coefficient
FC- Faecal calprotectin. 3T- 3rd trimester; PP2- 6 months postpartum; MEDAS- Mediterranean Diet score.

Table 10: Maternal diet and FC, zonulin and *A. muciniphila* levels. Levels of FC of (ug/g), zonulin (ng/ml) and A. *muciniphila* (Log 10 16SrDNA gene copies/10ng DNA) are expressed as median and interquartile range between brackets.

	Feacal calprotectin						**Serum zonulin**						***A.muciniphila***					
	3T			**PP2**			**3T**			**PP2**			**3T**			**PP2**		
	Yes	No	*p*	Yes	No	*p*	Yes	No	*p*	Yes	No	*p*	Yes	No	*p*	Yes	No	*p*
1	2.96 [1.88 ;8.34]	2.86 [2.04; 4.92]	0.700	1.90 [1.35; 2.94]	1.81 [1.32; 1.96]	0.632	461 [416; 480]	453 [418; 455]	0.393	378 [325; 440]	455 [298; 485]	0.363	1.76 [0.981;2.00]	0.638 [0.00;1.13]	0.144	0.846 [0.000; 1.770]	0.456 [0.000; 0.507]	0.071
2	10.7 [3.05; 18.5]	2.86 [1.80; 6.98]	0.308	1.86 1.61; 32.70]	1.72 [1.28; 2.91]	0.420	456 [416; 469]	451 [376; 526]	0.858	375 [336; 411]	428 [212; 445]	0.798	0.728 [0.00; 1.26]	0.486 [0.00; .972]	0.510	0.737 [0.00; 0.812]	0.637 [0.00; 1.81]	0.228
3	3.01 [2.12; 8.34]	2.74 [1.51; 5.42]	0.474	1.96 [1.57, 3.44]	1.37 [1.28; 2.61]	0.218	455 [447; 475]	456 [383; 469]	0.638	399 [371; 438]	409 [212; 455]	0.733	0.811 [0.00; 1.18]	0.465 [0.00; 1.38]	0.603	1.376 [0.075; 2.02]	0.00 [0.00; 1.44]	0.115
4	2.89 [1.43; 8.66]	2.95 [2.10; 3.87]	0.752	1.97 1.50; 3.13]	1.41 [1.30; 2.61]	0.664	460 [391; 481]	456 [444; 461]	0.818	421 [149; 440]	378 [353; 455]	0.367	0.128 [0.00; 1.12]	0.891 [0.20; 1.76]	0.189	0.150 [0.00; 2.01]	0.428 [0.00; 1.44]	0.420
5	3.01 [2.03; 6.95]	2.38 [1.61; 8.34]	0.748	1.81 [1.50; 2.79]	1.66 [2.91; 1.38]	0.861	450 [399; 475]	459 [421; 481]	0.456	**445 [432; 457]**	**374 [255; 437]**	**0.004**	0.972 [0.00;1.34]	0.260 [0.00; 1.12]	0.456	0.846 [0.00; 1.77]	0.407 [0.00; 0.796]	0.318
6	2.95 [2.03; 5.95]	4.83 [1.58; 7.82]	0.958	1.55 [1.30; 2.33]	1.90 [1.24; 3.30]	0.439	456 [379; 481]	455 [453; 461]	0.883	378 [176; 439]	440 [371; 450]	0.224	0.638 [0.00; 1.12]	1.13 [0.20; 1.64]	0.312	0.846 [0.00; 1.77]	0.000 [0.00; 1.37]	0.512
7	4.92 [2.86; 6.98]	2.95 [1.80; 8.03]	0.643	1.56 [1.30; 3.34]	2.33 [1.49; 2.76]	0.823	453 [418; 457]	456 [403; 475]	0.577	378 [361; 444]	440 [223; 443]	0.916	0.471 [0.00; 1.12]	0.801 [0.08; 1.74]	0.064	0.00 [0.00; 1.47]	0.428 [0.00; 1.68]	0.630
8	2.95 [1.88; 7.50]	2.96 [1.80; 8.03]	0.523	1.85 [1.32; 2.95]	1.85 [1.31; 2.96]	0.555	465 [455; 482]	45 [403; 464]	0.856	378 [298; 445]	378 [255; 446]	0.694	0.728 [0.00; 1.18]	0.980 [0.00; 1.12]	0.588	0.428 [0.00; 1.74]	0.718 [0.00; 0.813]	0.321
9	4.14 [1.11; 13.6]	2.95 [2.19; 5.43]	0.595	1.76 [1.08; 2.12]	2.33 [1.34; 2.95]	0.137	458 [425; 465]	454 [391; 480]	0.476	428 [369; 446]	375 [181; 444]	0.510	0.128 [0.00; 0.39]	1.08 [0.00; 1.64]	0.075	0.639 [0.00: 1.99]	0.214 [0.00; 1.68]	0.533

10	3.02 [1.43; 8.66]	2.95 [1.96; 6.98]	0.920	1.56 [1.34; 1.81]	2.12 [1.18: 3.16]	0.484	453 [387; 475]	461 [451; 467]	0.792	363 [80; 378]	437 [333; 450]	0.122	0.811 [0.20; 1.15]	0.375 [0.00; 1.64]	0.801	0.637 [0.00; 1.80]	0.075 [0.00; 1.74]	0.615
11	2.86 [1.88; 5.43]	17.01 [3.96; 53.6]	0.290	1.41 [1.29; 2.47]	2.41 [1.72; 2.98]	0.436	461 [444; 461]	459 [403; 475]	0.280	406 [361; 444]	359 [149; 469]	0.883	0.471 [0.00; 1.15]	1.132 [1.05; 1.34]	0.250	0.214 [0.00; 1.68]	0.796 [0.00; 1.99]	0.588
12	2.97 [2.10; 3.75]	2.91 [1.43; 8.03]	0.901	2.12 [1.28; 3.54]	1.76 [1.32; 2.76]	0.741	456 [416; 467]	455 [417; 478]	0.928	405 [369; 442]	400 [255; 446]	1.000	0.728 [0.55; 1.13]	0.602 [0.00; 1.49]	0.883	0.812 [0.00; 2.06]	0.289 [0.00; 1.59]	0.615
13	2.97 [1.96; 8.03]	2.84 [1.80; 8.03]	0.793	1.81 [1.31; 2.76]	4.13 [1.30; 6.95]	0.632	453 [387; 475]	461 [455; 465]	0.937	421 [333; 444]	353 [247; 410]	0.667	0.769 [0.00; 1.31]	0.128 [0.00; 1.49]	0.815	0.428 [0.214; 1.08]	0.150 [0.00; 1.71]	1.000
14	2.90 [1.80; 8.03]	2.97 [2.10; 6.93]	0.950	1.86 [1.30; 2.91]	1.37 [0.90; 2.35]	0.600	451 [398; 455]	433 [390; 445]	0.308	406 [353; 445]	317 [176; 438]	0.551	0.891 [0.00; 1.14]	0.549 [0.20; 0.73]	0.746	0.423 [0.00; 1.41]	0.289 [0.00; 1.74]	0.789

3T- 3rd trimester; PP2- 6 months postpartum; 1- Do you use olive oil as the principal source of fat for cooking? (Do you use olive oil as the principal source of fat for cooking?); 2- How much olive oil do you consume per day (including that used in frying, salads, meals eaten away from home, etc.)? (D ≥4 tablespoons per day?); 3- How many servings of vegetables do you consume per day? (≥2 servings per day?); 4- How many pieces of fruit (including fresh-squeezed juice) do you consume per day? (≥3 pieces per day?); 5- How many servings of red meat, hamburger, or sausages do you consume per day? (<1 serving per day?); 6- How many servings (12 g) of butter, margarine, or cream do you consume per day? (<1 serving per day?); 7- How many carbonated and/or sugar-sweetened beverages do you consume per day? (<1 per day?); 8- Do you drink wine? How much do you consume per week? (≥7 glasses per week?); 9- How many servings of pulses (legumes) do you consume per week? (≥3 servings per week?); 10- How many servings of fish/seafood do you consume per week? (≥3 servings per week?); 11-How many times do you consume commercial (not homemade) pastry such as cookies or cake per week? (≤3 per week?); 12- How many times do you consume nuts per week? (≥3 servings per week?); 13- Do you prefer to eat chicken, turkey, or rabbit instead of beef, pork, hamburgers, or sausages? (Do you prefer white meat over red meat?); 14- How many times per week do you consume boiled vegetables, pasta, rice, or other dishes with a sauce of tomato, garlic, onion, or leeks sautéed in olive oil? (≥2 servings of “sofrito” per week?)

4.7 Association between Mediterranean diet adherence and cardiac RR (echocardiographic parameters), gestational weight gain and postpartum weight recovery

At 3T, the only significative yet weak negative correlation was MED adherence with systemic vascular resistance (ρ = -0.409, p = 0.038) (Table 11).

At PP2, two statistically significant inverse correlations were identified: a moderate negative correlation between MED adherence and E/e' ratio (ρ = -0.510, p = 0.015), suggesting improved diastolic function in those with higher dietary adherence; and a stronger negative correlation between MED adherence and relative wall thickness (RWT) (ρ = -0.560, p = 0.007) (Table 11).

All other cardiovascular parameters showed no significant correlation with MED adherence score at either time point.

Table 11: Results of MEDAS score and echocardiographic parameters.

	MEDAS score at 3T		**MEDAS score at PP2**	
	ρ	***p*-value**	**ρ**	***p*-value**
Systolic blood pressure, mm Hg	-0.169	0.408	0.168	0.466
Diastolic blood pressure, mm Hg	-0.185	0.367	0.024	0.918
Heart rate, beats/min	0.039	0.842	-0.321	0.145
Pulse wave velocity, m/s	-0.048	0.815	-0.175	0.449
Systemic vascular resistance, dyn-s/cm^5	**-0.409**	**0.038**	-0.401	0.072
Cardiac mass index g/m$^{2.7}$	-0.206	0.285	-0.103	0.658
E/e´	0.276	0.147	**-0.510**	**0.015**
Relative wall thickness	0.022	0.911	**-0.560**	**0.007**
Left atrium volume index, mL/m$^{2.7}$	0.076	0.713	0.086	0.710
LV end-diastolic volume index, mL/m$^{2.7}$	-0.038	0.858	0.107	0.657
LVM regression	0.094	0.633	-0.099	0.663

Spearman correlation CI 95%; ρ (rho) as representative of Spearmen coefficient
LV - left ventricle; LVM - left ventricle mass; 3T - 3rd trimester; PP2 - 6 months postpartum; MEDAS - Mediterranean Diet score.

The relationship between the MED score to verify the adherence to the MED diet and weight-related variables was analysed using Spearman correlation (Table 12). At 3T, no statistically significant correlation was observed between adherence to the MED and gestational weight gain (ρ = 0.100, p = 0.618) or weight at 3T (ρ = -

0.171, p = 0.394). Similarly, at PP2, no significant associations were found between MED adherence and postpartum weight recovery (ρ = 0.097, p = 0.692) or weight at PP2 (ρ = 0.062, p = 0.812).

Table 12: Results of MEDAS score, gestational weight gain, and postpartum weight recovery.

	MEDAS score at 3T			**MEDAS score at PP2**	
	ρ	***p*-value**		**ρ**	***p*-value**
Gestational weight gain (kg)	0.100	0.618	**Weight recovery (kg) at PP2**	0.097	0.692
Weight (kg) at 3T	-0.171	0.394	**Weight (kg) at PP2**	0.062	0.812

Spearman correlation CI 95%; ρ (rho) as representative of Spearmen coefficient
3T- 3rd trimester; PP2- 6 months postpartum.

About MED parameters and cardiovascular parameters, at 3T, the consumption of commercial pastries (≤3 per week), showed a moderate negative correlation with left atrium volume index, mL/$m^{2.7}$ (r = -0.624, p < 0.001) (Fig 4A). The consumption of carbonated and sugar-sweetened beverages (<1 per day) was moderately negatively correlated with systolic blood pressure(r = -0.523, p = 0.006) and weakly with cardiac mass index (r = -0.409, p = 0.030). The use of olive oil as the principal source of fat for cooking showed a moderate negative correlation with systolic blood pressure (r = -0.506, p = 0.008) and a weak positive correlation with left ventricular mass regression (r = 0.437, p = 0.019). Additionally, consumption of fruit (≥3 per day), was weakly negatively correlated with diastolic blood pressure, mm Hg (r = -0.466, p = 0.016).

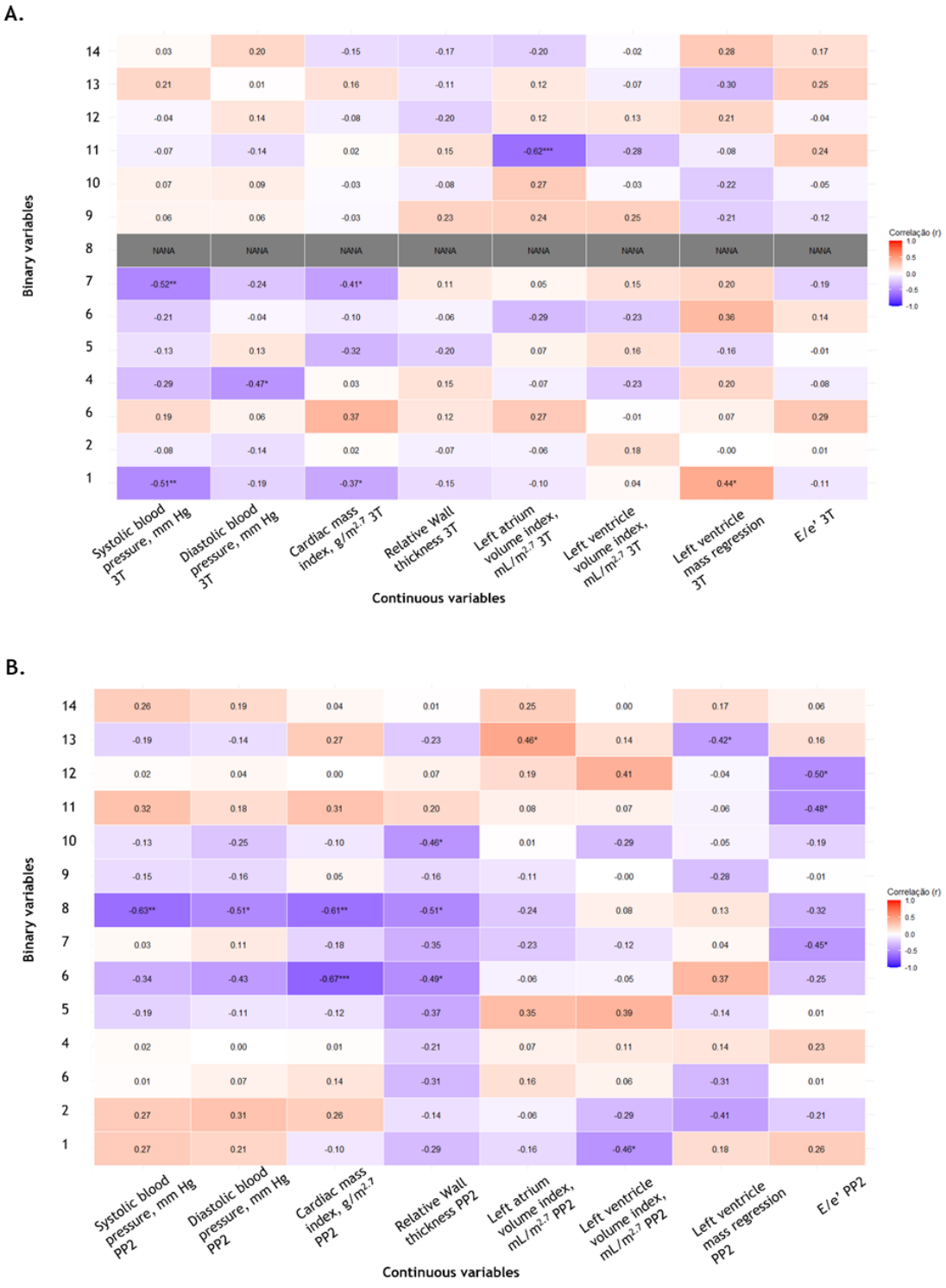


Figure 4: Correlations between MEDAS items and echocardiographic parameters at 3rd trimester (A) and at 6 months postpartum (B) .3T- 3rd trimester, PP2- 6 months postpartum; LVM-left ventricular mass. MEDAS Question: 1- Do you use olive oil as the principal source of fat for cooking?; 2- How much olive oil do you consume per day (including that used in frying, salads, meals eaten away from home, etc.)?(≥4 tablespoons per day?); 3- How many servings of vegetables do you consume per day?(≥2 servings per day?); 4- How many pieces of fruit (including fresh-squeezed juice) do you consume per day?(≥3 pieces per day?); 5- How many servings of red meat, hamburger, or sausages do you consume per day?(<1 serving per day?); 6-

How many servings (12 g) of butter, margarine, or cream do you consume per day? (<1 serving per day?); 7- How many carbonated and/or sugar-sweetened beverages do you consume per day?(<1 per day?); 8- Do you drink wine? How much do you consume per week?(≥7 glasses per week?); 9- How many servings of pulses (legumes) do you consume per week?(≥3 servings per week?), 10- How many servings of fish/seafood do you consume per week?(≥3 servings per week?); 11- How many times do you consume commercial (not homemade) pastry such as cookies or cake per week?(≤3 per week?); 12- How many times do you consume nuts per week?(≥3 servings per week?); 13- Do you prefer to eat chicken, turkey, or rabbit instead of beef, pork, hamburgers, or sausages?(Do you prefer white meat over red meat?); 14- How many times per week do you consume boiled vegetables, pasta, rice, or other dishes with a sauce of tomato, garlic, onion, or leeks sautéed in olive oil?(≥2 servings of "sofrito" per week?). Pearson correlation (r), CI 95%. Statistical significance was visually annotated on the heatmap ($p < 0.05$ as *, < 0.01 as **, and < 0.001 as ***).

At PP2, significant moderate negative correlation was observed between consuming (<1 serving per day) of butter, margarine, or cream and cardiac mass index, g/m$^{2.7}$ (r = -0.669, $p < 0.001$) (Fig. 4B). Drinking ≥7 glasses of wine per week was moderate negatively correlated with systolic blood pressure, mm Hg (r = -0.628, $p = 0.002$), cardiac mass index, g/m$^{2.7}$ (r = -0.614, $p = 0.003$), relative wall thickness (r = -0.508, $p = 0.015$), and diastolic blood pressure, mm Hg (r = -0.510, $p = 0.018$). Additionally, consuming ≥3 servings of nuts per week showed a weak negative correlation with the E/e' ratio (r = -0.496, $p = 0.018$), as consuming commercial pastries (≤3 servings per week) (r = -0.484, $p = 0.022$) and consuming <1 carbonated or sugar-sweetened beverage per day has a weak negatively correlation (r = -0.446, p = 0.037). Consumption more than 1 serving per day of butter, margarine, or cream was weak negatively correlated with relative wall thickness (r = -0.490, $p = 0.020$), as consuming ≥3 servings of fish or seafood per week (r = -0.461, p = 0.030). Preference for white meat over red meat was weak positively correlated with left atrium volume index, mL/m$^{2.7}$ (r = 0.456, p = 0.037) and otherwise using olive oil as the principal source of fat for cooking has a weak negatively correlation (r = -0.464, p = 0.039).

5. Discussion

This master's thesis explored the interplay between intestinal biomarkers (FC, zonulin, and *A. muciniphila*), cardiac RR induced by pregnancy, weight dynamics, and adherence to the MED in women during the 3T and PP2. Understanding these interactions is essential, as profound physiological adaptations characterise pregnancy, including changes in cardiovascular function, metabolic status, and gut microbiota composition.

Recent scientific evidence has emphasised the role of the gut as a central regulator of systemic health in modulating systemic inflammation and metabolic responses, with potential implications for cardiovascular regulation [14, 21]. Novel biomarkers like FC, serum zonulin, and *A. muciniphila have been studied* as intermediaries between gut-cardiovascular interactions and maternal health during and after pregnancy [14, 21]. In parallel, adherence to MED have been linked to favourable gut microbiota composition and cardiometabolic health, further underlining the importance of nutritional factors during the perinatal window[64]. Autonomic cardiovascular control undergoes significant changes during pregnancy and postpartum, driven by hormonal shifts, weight fluctuations, and lifestyle factors like diet.

The most relevant findings include evidence of partial cardiac RR postpartum, with significant reductions in heart rate, LVM, and diastolic function indices. Zonulin levels, a marker of intestinal permeability, were markedly elevated during pregnancy and remained high postpartum, while *A. muciniphila* abundance declined between the two timepoints. Faecal calprotectin remained low, excluding overt intestinal inflammation. Notably, zonulin and FC showed significant associations with cardiac parameters, supporting a potential gut–heart axis. Adherence to the MED tended to decline postpartum and was associated with favourable cardiac parameters (e.g., lower E/e′ ratio), though not with weight recovery. These findings suggest that gut permeability, diet quality, and metabolic factors may influence postpartum cardiovascular recovery.

The following discussion contextualises the findings with current scientific literature.

5.1 Cardiovascular RR induced by pregnancy

Systolic blood pressure showed no significant difference between 3T and PP2, indicating relative stability of systolic blood pressure across these periods. In contrast, diastolic blood pressure increased significantly postpartum, consistent with previous reports of increased systemic vascular resistance as vasodilation reverses after delivery[3, 4, 8]. Heart rate decreased significantly from pregnancy to postpartum, reflecting normalisation of autonomic regulation and reduced cardiac workload following delivery[3, 8]. Pulse wave velocity increased significantly postpartum, reversing the pregnancy-induced reduction in arterial stiffness. This finding is consistent with the physiological vasodilation mediated by hormones such as relaxin and nitric oxide during pregnancy, which lowers vascular tone and increases arterial compliance, changes that typically regress postpartum [65]. Although systemic vascular resistance did not reach statistical significance, the trend toward an increase postpartum supports the physiological recovery of vascular tone after delivery. Structural cardiac RR as evident in the significant decrease in LVM. This regression reflects the reversal of pregnancy-induced physiological hypertrophy due to normalisation of hemodynamic load postpartum [3, 4, 8]. The significant reductions in E/e' ratio, left atrial volume index, and LV end-diastolic volume index further support cardiac structural and functional postpartum normalisation.

No significant change was observed in relative wall thickness, indicating that geometric remodelling patterns may lag behind volume and mass changes or are less influenced by the early postpartum period.

These results confirm that cardiovascular adaptations in pregnancy, characterised by decreased arterial stiffness, vasodilation, increased cardiac mass index, and elevated heart rate, partially revert within the early postpartum period. However, the data of this thesis suggest that the normalisation process is ongoing at PP2, highlighting the need for extended postpartum cardiovascular monitoring to assess recovery and potential long-term implications [3, 4, 8, 11, 13]. Actually, at 6 months postpartum, it was only observed a 11% reduction of LVM regression, while LVM regression is approximately 20–30% at one year postpartum [13, 66], This may be due

to the early timing of the assessment, individual variability, or the influence of subclinical metabolic or vascular factors.[2, 3, 13, 67]

5.2. Intestinal biomarkers and cardiac function and structure

Faecal calprotectin, serum zonulin and *A. muciniphila* levels were measured at 3T and PP2 to assess intestinal inflammation and permeability. FC levels significantly decreased between 3T and PP2. These values remain well below the commonly accepted clinical threshold for inflammation (>50 µg/g), suggesting intestinal inflammation was absent in this cohort during pregnancy or postpartum[68] . The observed low FC levels exclude the presence of inflammatory bowel disease or other gut pathologies. In contrast, serum zonulin levels were markedly elevated in both periods, despite a significant decrease. These levels far exceed normal reference ranges, typically between 30–60 ng/mL in healthy individuals [38, 69]. Zonulin is a regulator of intestinal tight junctions, and elevated concentrations are associated with increased intestinal permeability [70]. In this study, the cohort included women with obesity, hypertension, and diabetes, which may help explain the markedly high zonulin concentrations observed, as these conditions are known to be associated with increased intestinal permeability. Such elevated zonulin levels have been previously reported in specific populations. For instance, pregnant women with GDM showed similarly high zonulin levels, suggesting a possible link between metabolic dysregulation and intestinal barrier dysfunction [71]. Likewise, elevated zonulin concentrations have been observed in non-pregnant populations such as asthmatic patients, indicating systemic inflammatory responses may also impact gut permeability[72]. The findings are consistence with existing literature, supporting the hypothesis that pregnancy is associated with a physiologically increased intestinal permeability, possibly modulated by hormonal, metabolic, and microbiota-driven changes [1, 19]. Although levels decreased significantly postpartum, the persistence of elevated zonulin levels at PP2 may reflect incomplete recovery of the intestinal barrier or individual variability in gut-mucosal healing. These findings highlight the importance of monitoring intestinal health during and after pregnancy, especially

given the emerging links between gut permeability, systemic inflammation, and cardiovascular and metabolic health in women.

Significantly, levels of *A. muciniphila*, a commensal bacterium known for its mucin-degrading and gut-barrier-strengthening properties, also decreased from 3T to PP2. Several studies have highlighted the beneficial effects of *A. muciniphila* on metabolic health, insulin sensitivity, and gut barrier function, with lower abundance linked to obesity, type 2 diabetes, and inflammation [25, 28, 29, 52]. Its reduction in this cohort aligns with the persistently elevated zonulin levels, reinforcing the hypothesis that microbial alterations during and after pregnancy may influence intestinal permeability and systemic health. Altogether, these findings suggest that although overt intestinal inflammation was not present, as reflected by low FC levels, pregnancy may be associated with a transient but significant increase in gut permeability, as indicated by elevated zonulin concentrations. This condition may be further influenced by a reduction in beneficial microbial taxa such as *A. muciniphila*, which is known to support intestinal barrier integrity.

Intestinal homeostasis may play a role in RR during and after pregnancy. At 3T, FC was positively moderately correlated with LVM, supporting the hypothesis that low-grade intestinal inflammation may contribute to cardiac structural hypertrophy during late pregnancy. At PP2, FC correlated significantly with the E/e' ratio, a surrogate marker of diastolic dysfunction, suggesting that gut-derived inflammation may persistently influence myocardial function postpartum. These associations align with emerging evidence that intestinal inflammation, even at subclinical levels, may be linked to systemic inflammation and RR [73].

Zonulin also demonstrated significant cardiovascular correlations postpartum. At PP2, zonulin was positively correlated with diastolic blood pressure, cardiac mass index and LV end-diastolic volume index. These findings support a potential role of gut barrier dysfunction in modulating vascular tone and cardiac structure after pregnancy. Disrupted intestinal permeability has been proposed to contribute to systemic inflammation, endothelial dysfunction, and myocardial remodelling via mechanisms involving LPS translocation and immune activation [38, 74].

Taken together, the correlations between FC and zonulin with distinct cardiac parameters reinforce the concept of a gut–heart axis during the pregnancy-

postpartum transition, where alterations in the intestinal environment may impact cardiovascular adaptation and recovery.

In contrast, no significant associations were observed between the levels of *A. muciniphila* and cardiovascular parameters at either time point. The absence of correlations across a wide range of hemodynamic and structural indices (including blood pressure, arterial stiffness, and cardiac reverse remodelling markers) suggests that, in this cohort, *A. muciniphila* levels were not a key modulator of maternal cardiovascular adaptation during late pregnancy or postpartum recovery. This may reflect the complexity of host-microbiota interactions, where microbial influence on cardiovascular health likely depends on broader compositional, functional, and metabolic profiles rather than isolated taxa. Additionally, it is possible that other members of the gut microbiota, or microbial metabolites such as SCFAs or endotoxins, play a more prominent role in the gut–heart axis during this period.

Previous human trials in overweight/obese individuals demonstrated that pasteurised *A. muciniphila* supplementation improved insulin sensitivity, lowered cholesterol, and showed trends toward reduced body weight, with no adverse blood pressure effects observed [75]. However, to date, no studies have directly examined its association with echocardiographic parameters in pregnancy, which aligns with our null findings and indicates a knowledge gap for maternal cardiovascular microbiota research.

5.3 Weight dynamics and cardiometabolic implications

One of the key physiological variables in this context is body weight, both before and after pregnancy. In this study, the median pre-pregnancy BMI was 28.9 kg/m^2, classifying most participants as overweight or obese. The prevalence of pre-pregnancy obesity in the present study was 44%, which is considerably higher than that reported in both national and regional studies. Viveiros et al. (2020), in a study conducted on Azores, found an obesity rate of 23.5% among 34 pregnant women [76]. Similarly, Silva et al. (2019), in a large multicentre national study, reported a prevalence of 13.6% [77]. This discrepancy may be attributed to sociodemographic factors, different inclusion criteria, or sample size effects. The

findings highlight the need for early nutritional screening and preconception care. Maternal obesity is strongly linked to adverse obstetric and metabolic outcomes. Pre-pregnancy excess weight is known to affect cardiovascular regulation and has been associated with altered autonomic function, increased systemic inflammation, and impaired endothelial response[78].

The observed median gestational weight gain was relatively modest at 6.2 kg, which may reflect improved antenatal monitoring or dietary caution among women with higher BMI. Despite a relatively low median gestational weight gain, participants retained a median of 3.0 kg at six months postpartum, indicating incomplete weight recovery. These weight dynamics may have contributed to the variability observed in reverse remodelling patterns and autonomic responses, as excess adiposity and poor postpartum weight recovery are linked to sustained cardiometabolic stress[79].

No statistically significant associations were observed between FC and weight-related parameters at either 3T or PP2. Specifically, FC did not correlate with gestational weight gain, maternal weight at 3T, postpartum weight, or weight recovery. These findings suggest that intestinal inflammation, as indicated by FC levels despite being low, may not directly influence weight dynamics during late pregnancy or the postpartum period in this cohort.

In contrast, zonulin demonstrated a notable relationship with postpartum weight. This suggests increased intestinal permeability may be associated with greater weight retention in the postpartum period, as referenced in the literature[80, 81]. This finding aligns with growing evidence linking gut barrier dysfunction to metabolic disturbances and weight regulation[80]. As a modulator of tight junctions, zonulin has been implicated in the pathophysiology of obesity, insulin resistance, and systemic inflammation, all of which can impact postpartum weight outcomes [80]. For example, studies have demonstrated elevated zonulin levels in individuals with obesity, AHT and GDM, supporting the role of increased gut permeability in metabolic dysregulation [40, 82, 83].

The initial expectation was that *A. muciniphila* levels would correlate with maternal weight parameters, given the growing body of evidence supporting its role in metabolic regulation and obesity prevention in non-pregnant populations[25]. It is essential to highlight emerging evidence from experimental

and clinical studies supporting the potential of *A. muciniphila* as a therapeutic probiotic for metabolic health and weight management. Preclinical studies in mice have demonstrated that oral supplementation with *A. muciniphila*, particularly in its pasteurised form, improves metabolic parameters, reduces body weight gain, and enhances insulin sensitivity in models of diet-induced obesity [84]. In humans, a landmark randomized controlled trial[75] provided the first clinical evidence of safety and efficacy: overweight and obese individuals who received daily oral supplementation of pasteurized *A. muciniphila* (10^{10} CFU) for three months showed improved insulin sensitivity, reduced plasma total cholesterol, and a trend toward reduced body weight and fat mass, compared to placebo *A. muciniphila* could theoretically influence gestational weight gain and postpartum weight recovery. However, in this study, no significant correlations were found between *A. muciniphila* levels and weight at 3rd trimester and postpartum weight, gestational weight gain, or weight recovery. These null results suggest that the influence of *A. muciniphila* on maternal weight regulation may be limited or more complex during pregnancy and postpartum periods.

Several factors could explain the lack of association observed. Pregnancy induces profound hormonal, immunological, and metabolic changes that affect both the gut microbiota composition and maternal physiology, potentially overriding the modulatory effects of single bacterial species such as *A. muciniphila*. Moreover, the interactions within the microbiota, pre and gestational dietary influences, and host genetics may modulate the abundance and activity of *A. muciniphila*, diluting simple correlations with anthropometric measures.

Additionally, studies on *A. muciniphila* during pregnancy are still scarce, and findings are inconsistent. It was suggested that *A. muciniphila* abundance may decrease during late pregnancy[85], while others indicate no clear pattern or a transient increase postpartum [86]. The variability in methodologies, sample sizes, and populations studied may contribute to these discrepancies.

5.4 Influence of maternal diet on intestinal biomarkers and cardiometabolic health

The MED is widely recognised for its beneficial effects on cardiovascular and gut health and weight management[64, 87].

The analysis of MED adherence revealed a tendency to decline in adherence to the MED from the 3T to PP2, but not statistically significant. This trend is consistent with previous research showing that postpartum is a vulnerable period for dietary deterioration due to multiple factors such as reduced time for food preparation, breastfeeding-related hunger, psychological stress, and shifting household priorities[88].

Several specific dietary components displayed a descending trend in postpartum adherence. Reducing olive oil as the principal cooking fat and daily vegetable and fruit intake suggests a shift from core MED practices. This change is concerning, given that these components are central to the anti-inflammatory and cardioprotective effects associated with the MED [89, 90]. Furthermore, the decreased tendency of fish consumption and the limited increase in pulse intake postpartum further reflect a drift from optimal dietary patterns.

Despite these shifts, the preference for white over red meat remained relatively stable and statistically significant, indicating some persistence in making cardiometabolic-conscious choices postpartum. Interestingly, an increase in the reported consumption of commercial pastries was noted, potentially reflecting greater reliance on convenience foods in the postpartum period.

The trend to decreased the overall MED adherence may affect postpartum recovery and long-term cardiovascular and metabolic health. MED has been associated with improved endothelial function, modulation of gut microbiota, and favourable lipid and glucose profiles, all of which are relevant in the context of postpartum regression of pregnancy-induced adaptations[64, 91]. In this light, strategies to support and maintain healthy dietary behaviours during the postpartum period, through counselling, social support, and accessibility are warranted.

Taken together, the findings underscore the need for continued nutritional guidance beyond pregnancy, particularly during postpartum recovery when nutritional neglect may negatively interact with residual cardiovascular remodelling, weight retention, and inflammation.

No significant correlations were observed between adherence to MED as assessed by the MEDAS score, and levels of FC, serum zonulin and *A. muciniphila* levels at either 3T or PP2. Analysis of individual MED dietary components yielded similarly

limited findings concerning FC, as no significant differences were found in FC levels across responses at either time point. This is consistent with prior research indicating that although diet can influence gut inflammation, FC may be more reflective of acute or clinically active gastrointestinal conditions rather than dietary patterns in otherwise healthy individuals [92].

In contrast, zonulin showed a more specific dietary association. At PP2, participants who reported consuming ≥1 serving per day of red and processed meat had significantly higher zonulin levels than those who consumed less than one serving per day. This finding supports the growing body of evidence linking high intake of red and processed meats with increased intestinal permeability. Diets high in red and processed meats contain excess cholesterol and sodium, which impair gut barrier function [93]. Cholesterol alters gut microbiota and promotes inflammatory metabolites. High salt intake disrupts tight junction proteins, increasing gut permeability and systemic inflammation. Scientific evidence has shown that diets rich in red meat and processed meats can impair intestinal barrier function by modulating gut microbiota composition and promoting systemic inflammation [94, 95].

Previous studies have suggested that adherence to MED may positively influence gut health by reducing intestinal inflammation and improving barrier function. Therefore, it would be expected that higher adherence to the MED could correlate with lower FC and zonulin levels. However, findings across studies are mixed, possibly due to differences in population characteristics, dietary assessment methods, and the complexity of gut barrier regulation. In this study, the lack of significant associations may reflect these complexities or the relatively small sample size.

No statistically significant correlations were found between *A. muciniphila* levels and adherence to the Mediterranean diet, during pregnancy or postpartum. The lack of correlation in this cohort may reflect pregnancy-specific microbiota dynamics, in which hormonal, immunological, and metabolic factors modulate gut microbial composition to attenuate dietary influence. Additionally, the negative trend, but not statistically significative, observed at the 3rd trimester may suggest that *A. muciniphila* levels are influenced by metabolic adaptations associated with

late pregnancy, such as increased insulin resistance and mild inflammation, which could counteract the expected positive effects of a healthy diet on this species.

The association between adherence to the MED and cardiovascular parameters was evaluated at both timepoints. At 3T, no statistically significant correlations were found, although a trend toward a negative correlation with systemic vascular resistance was observed. This suggests that while early effects may be subtle, the MED's influence on vascular tone could emerge more clearly postpartum. Indeed, at PP2, significant inverse correlations were identified between MED adherence and the E/e′ ratio and relative wall thickness. These findings suggest that greater adherence to the MED may promote improved diastolic function and more favourable RR postpartum. Such cardioprotective effects are consistent with previous literature showing that the MED dietary pattern, rich in antioxidants, unsaturated fats, and anti-inflammatory nutrients, can improve vascular function and reduce cardiac remodelling[41].

Interestingly, no significant correlations were found between MED adherence or MED parameters and weight parameters at either time point. This suggests that, within this cohort, the diet's impact on cardiovascular function may occur independently of weight changes during pregnancy and postpartum. While weight management is important, these results align with studies indicating that dietary quality can influence cardiovascular health markers beyond its effects on body weight. Overall, the data reinforce the potential role of the MED in supporting cardiovascular recovery postpartum, particularly by improving diastolic function and cardiac structure, even if no immediate effects on weight dynamics are apparent.

Adherence to the MED parameters showed significant associations with echocardiographic parameters during the 3T and PP2. The consistent negative correlations between the consumption of pastries and sugar-sweetened beverages with cardiac mass, blood pressure, and diastolic function suggest that limiting these items may benefit cardiovascular structure and performance. The regular use of olive oil and higher intake of fruits, fish, and nuts were also associated with favourable cardiac parameters, supporting their inclusion as dietary priorities. Interestingly, moderate wine consumption was inversely correlated with multiple cardiovascular markers, aligning with prior evidence of its protective effects when

consumed in moderation [96]. Moreover, reduced intake of saturated fats (butter, margarine, cream) showed strong associations with lower cardiac mass and relative wall thickness. These associations highlight the potential of specific dietary behaviours, rather than overall patterns alone, in influencing cardiovascular health. This underlines the importance of targeted nutritional counselling in cardiovascular risk prevention. These findings align with existing literature attributing cardioprotective properties to the Mediterranean diet, mainly due to its high monounsaturated fats, polyphenols, and dietary fibre, nutrients known to reduce oxidative stress, modulate systemic inflammation, and improve endothelial function [64].

Overall, these results support the importance of promoting healthy dietary patterns during and after pregnancy to enhance maternal cardiovascular recovery, even if these changes do not immediately translate into weight differences.

5.5 Strengths and limitations

This study presents several strengths that enhance the validity and relevance of its findings. Firstly, its multidimensional design, which simultaneously assessed intestinal biomarkers (FC, serum zonulin, *A. muciniphila*), cardiovascular function (through echocardiographic parameters), anthropometric data, and MED adherence, offers a comprehensive understanding of maternal physiological changes during the 3T and the PP2. Such an integrative approach is rare in the literature and provides novel insights into gut–heart–diet interactions during this critical transition.

Another key strength lies in the clinical relevance of the population studied. The cohort included pregnant women with metabolic risk factors, such as overweight, obesity, GDM and AHT, that are often underrepresented in microbiome and cardiovascular remodelling studies but who are at increased risk for adverse outcomes. Additionally, including the MED diet adherence assessment using the validated MEDAS score allows the identification of modifiable lifestyle factors, which could inform postpartum dietary interventions.

Using validated and widely studied biomarkers, such as FC and zonulin, adds biological reliability to the findings. Moreover, selecting two clinically meaningful

time points: late pregnancy and six months postpartum, enabled observing dynamic and potentially reversible physiological processes, particularly those related to cardiovascular remodelling and intestinal permeability.

However, the study is not without limitations. The small sample size (n = 34) restricts statistical power. Additionally, the retrospective cohort design limits control over confounding variables and introduces the possibility of incomplete or biased data collection, particularly regarding self-reported dietary information.

The lack of a control group of healthy, normal-weight pregnant women limits the generalizability of the findings and hinders the ability to distinguish physiological from pathological processes. Furthermore, being a single-centre study, the demographic and clinical characteristics of the cohort may not be representative of the wider pregnant population, reducing the external validity of the results.

Another limitation is the short-term follow-up: while six months postpartum is a relevant period for assessing early recovery, it does not capture long-term maternal cardiovascular or metabolic outcomes. However, this period was chosen due to a lack of samples available one year after delivery.

Finally, the reliance on self-reported dietary questionnaires introduces the risk of recall bias and social desirability bias, particularly in postpartum women who may experience time constraints, fatigue, or stress.

Despite these limitations, the study provides a valuable foundation for future research and highlights key areas for further investigation, including longer follow-up periods, broader microbiota profiling, and prospective, controlled designs.

6. Conclusion

This study contributes valuable insights into the interplay between intestinal biomarkers, pregnancy-induced cardiac RR, weight dynamics, and adherence to the MED in women during the 3T and PP2. The findings underscore the profound physiological adaptations that occur during pregnancy and the early postpartum period, highlighting the interconnected nature of metabolic, cardiovascular, and microbial systems in shaping maternal health.

The results confirm a partial reversal of cardiovascular adaptations by PP2, marked by significant reductions in cardiac mass and improvements in diastolic function. However, the persistence of elevated diastolic blood pressure and pulse wave velocity suggests that vascular recovery is incomplete. These findings reinforce the importance of long-term cardiovascular monitoring beyond the immediate postpartum period.

In parallel, the gut environment exhibited notable shifts. Although FC levels remained low, excluding overt intestinal inflammation, serum zonulin levels were markedly elevated at both time points, indicating increased intestinal permeability. Notably, zonulin levels correlated positively with postpartum weight and cardiovascular parameters such as cardiac mass and blood pressure, suggesting a possible role of gut barrier dysfunction in modulating systemic inflammation and maternal metabolic status after delivery.

A. muciniphila, a mucin-degrading bacterium known for its beneficial effects on gut barrier integrity and metabolic regulation, significantly declined from 3T to PP2. *A. muciniphila* levels did not show direct correlations with cardiovascular or weight-related parameters in this cohort.

Despite these promising findings, the impact of *A. muciniphila* supplementation during pregnancy remains unknown. Given the distinct physiological and immunological conditions of gestation and the unique composition of the maternal microbiome, extrapolation from non-pregnant populations should be made with caution. Further clinical trials in pregnant populations are needed to evaluate not only the efficacy of supplementation on maternal weight regulation, but also its safety for both mother and foetus, and its potential effects on postpartum recovery and infant outcomes. Therefore, the results underscore the need for further longitudinal and mechanistic studies to elucidate the role of *A.*

muciniphila in pregnancy-associated metabolic adaptations. It is also important to consider a broader range of microbial and host factors when assessing the complex interactions influencing gestational weight dynamics and postpartum recovery.

Diet emerged as a modifiable factor with important implications. While overall MED adherence showed a tendency to decrease at postpartum, specific dietary components have cardioprotective role, notably reduced consumption of processed and sugary foods, and increased consumption of olive oil, nuts, and wine in moderation. These dietary habits were associated with improvements in blood pressure and cardiac structure. Targeted nutritional strategies may therefore play a valuable role in cardiovascular prevention. These associations suggest that even in the absence of weight loss, diet quality can influence cardiovascular RR postpartum. Interestingly, while MED adherence was not significantly associated with *A. muciniphila* levels or intestinal inflammation markers, the negative trend observed between diet quality and *A. muciniphila* levels during pregnancy may reflect complex microbiota–host interactions unique to gestation.

Overall, while diet remains a key modulator of *A. muciniphila*, these findings highlight the complexity of microbiota–diet interactions in the context of pregnancy and underscore the need for more granular dietary and microbial analyses to elucidate the specific factors that influence its abundance during this physiological state.

In conclusion, the results of this thesis support the emerging concept of a gut–heart axis during the pregnancy-postpartum transition. Intestinal health, including permeability, appears to interact with cardiovascular and metabolic recovery following childbirth. These findings contribute to novel avenues for maternal care, including personalised nutrition, gut-targeted therapies, and extended monitoring strategies. Future research should focus on larger, longitudinal studies to confirm these associations and evaluate potential interventions aimed at improving maternal outcomes through gut-cardiometabolic modulation.

7. References

1. Gangakhedkar GR, Kulkarni AP. Physiological Changes in Pregnancy. Indian J Crit Care Med. 2021; 25(Suppl 3):S189-s92.

2. Ferreira AF, Araújo J, Azevedo MJ, Saraiva F, Diaz SO, Sousa C, et al. Cardiovascular remodelling and reverse remodelling during pregnancy and postpartum: Looking at the right side. Pregnancy Hypertension. 2024; 38:101171.

3. Ferreira AF, Azevedo MJ, Morais J, Trindade F, Saraiva F, Diaz SO, et al. Cardiovascular risk factors during pregnancy impact the postpartum cardiac and vascular reverse remodeling. Am J Physiol Heart Circ Physiol. 2023; 325(4):H774-h89.

4. Meah VL, Cockcroft JR, Backx K, Shave R, Stöhr EJ. Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. Heart. 2016; 102(7):518-26.

5. Bollen IA, Van Deel ED, Kuster DW, Van Der Velden J. Peripartum cardiomyopathy and dilated cardiomyopathy: different at heart. Front Physiol. 2014; 5:531.

6. de Haas S, Spaanderman MEA, van Kuijk SMJ, van Drongelen J, Mohseni Z, Jorissen L, Ghossein-Doha C. Adaptation of left ventricular diastolic function to pregnancy: a systematic review and meta-analysis. J Hypertens. 2021; 39(10):1934-41.

7. Cong J, Fan T, Yang X, Squires JW, Cheng G, Zhang L, Zhang Z. Structural and functional changes in maternal left ventricle during pregnancy: a three-dimensional speckle-tracking echocardiography study. Cardiovasc Ultrasound. 2015; 13:6.

8. Ying W, Catov JM, Ouyang P. Hypertensive Disorders of Pregnancy and Future Maternal Cardiovascular Risk. J Am Heart Assoc. 2018; 7(17):e009382.

9. Burden SJ, Alshehri R, Lamata P, Poston L, Taylor PD. Maternal obesity and offspring cardiovascular remodelling - the effect of preconception and antenatal lifestyle interventions: a systematic review. Int J Obes (Lond). 2024; 48(8):1045-64.

10. Cochrane ALK, Murphy MP, Ozanne SE, Giussani DA. Pregnancy in obese women and mechanisms of increased cardiovascular risk in offspring. European Heart Journal. 2024; 45(48):5127-45.

11. Behrens I, Basit S, Lykke JA, Ranthe MF, Wohlfahrt J, Bundgaard H, et al. Association Between Hypertensive Disorders of Pregnancy and Later Risk of Cardiomyopathy. Jama. 2016; 315(10):1026-33.

12. Minhas AS, Countouris M, Ndumele CE, Selvin E, Vaught AJ, Gandley R, et al. Association of Gestational Diabetes With Subclinical Cardiovascular Disease. JACC: Advances. 2024; 3(8):101111.

13. Eghan P, Folson AA, Donkor A, Kokuro C, Amo Wiafe Y. Relationship between hypertensive disorders of pregnancy (HDP) and cardiac remodeling during pregnancy: Systematic review and meta-analysis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2024; 298:108-15.

14. Bui TVA, Hwangbo H, Lai Y, Hong SB, Choi YJ, Park HJ, Ban K. The Gut-Heart Axis: Updated Review for The Roles of Microbiome in Cardiovascular Health. Korean Circ J. 2023; 53(8):499-518.

15. Ogunrinola GA, Oyewale JO, Oshamika OO, Olasehinde GI. The Human Microbiome and Its Impacts on Health. Int J Microbiol. 2020; 2020:8045646.

16. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. World J Gastroenterol. 2015; 21(29):8787-803.

17. Edwards SM, Cunningham SA, Dunlop AL, Corwin EJ. The Maternal Gut Microbiome During Pregnancy. MCN Am J Matern Child Nurs. 2017; 42(6):310-17.

18. Nuriel-Ohayon M, Neuman H, Koren O. Microbial Changes during Pregnancy, Birth, and Infancy. Front Microbiol. 2016; 7:1031.

19. Zakaria ZZ, Al-Rumaihi S, Al-Absi RS, Farah H, Elamin M, Nader R, et al. Physiological Changes and Interactions Between Microbiome and the Host During Pregnancy. Front Cell Infect Microbiol. 2022; 12:824925.

20. Gorczyca K, Obuchowska A, Kimber-Trojnar Ż, Wierzchowska-Opoka M, Leszczyńska-Gorzelak B. Changes in the Gut Microbiome and Pathologies in Pregnancy. Int J Environ Res Public Health. 2022; 19(16)

21. Violi F, Cammisotto V, Bartimoccia S, Pignatelli P, Carnevale R, Nocella C. Gut-derived low-grade endotoxaemia, atherothrombosis and cardiovascular disease. Nat Rev Cardiol. 2023; 20(1):24-37.

22. Yan J, Sheng L, Li H. Akkermansia muciniphila: is it the Holy Grail for ameliorating metabolic diseases? Gut Microbes. 2021; 13(1):1984104.

23. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A. 2013; 110(22):9066-71.

24. Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, et al. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. Nat Med. 2017; 23(1):107-13.

25. Abuqwider JN, Mauriello G, Altamimi M. Akkermansia muciniphila, a New Generation of Beneficial Microbiota in Modulating Obesity: A Systematic Review. Microorganisms. 2021; 9(5)

26. Lakshmanan AP, Murugesan S, Al Khodor S, Terranegra A. The potential impact of a probiotic: Akkermansia muciniphila in the regulation of blood pressure-the current facts and evidence. J Transl Med. 2022; 20(1):430.

27. Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, et al. Safety of pasteurised Akkermansia muciniphila as a novel food pursuant to Regulation (EU) 2015/2283. Efsa j. 2021; 19(9):e06780.

28. Niu H, Zhou M, Zogona D, Xing Z, Wu T, Chen R, et al. Akkermansia muciniphila: a potential candidate for ameliorating metabolic diseases. Front Immunol. 2024; 15:1370658.

29. Wade H, Pan K, Duan Q, Kaluzny S, Pandey E, Fatumoju L, et al. Akkermansia muciniphila and its membrane protein ameliorates intestinal inflammatory stress and promotes epithelial wound healing via CREBH and miR-143/145. Journal of Biomedical Science. 2023; 30(1):38.

30. Kim ES, Tarassishin L, Eisele C, Barre A, Nair N, Rendon A, et al. Longitudinal Changes in Fecal Calprotectin Levels Among Pregnant Women With and Without Inflammatory Bowel Disease and Their Babies. Gastroenterology. 2021; 160(4):1118-30.e3.

31. Kunutsor SK, Flores-Guerrero JL, Kieneker LM, Nilsen T, Hidden C, Sundrehagen E, et al. Plasma calprotectin and risk of cardiovascular disease: Findings from the PREVEND prospective cohort study. Atherosclerosis. 2018; 275:205-13.

32. Ortega FJ, Sabater M, Moreno-Navarrete JM, Pueyo N, Botas P, Delgado E, et al. Serum and urinary concentrations of calprotectin as markers of insulin resistance and type 2 diabetes. Eur J Endocrinol. 2012; 167(4):569-78.

33. Mortensen OH, Nielsen AR, Erikstrup C, Plomgaard P, Fischer CP, Krogh-Madsen R, et al. Calprotectin--a novel marker of obesity. PLoS One. 2009; 4(10):e7419.

34. Doherty J, Moore RL, Kivlehan C, Byrne DF, Yelverton CA, Twomey PJ, et al. Trends in Faecal Calprotectin Levels During Pregnancy in Non-IBD Patients. Ir Med J. 2022; 115(No.10):692.

35. Bálint A, Berényi A, Farkas K, Pallagi Kunstár É, Altorjay Á, Csonka A, et al. Pregnancy does not affect fecal calprotectin concentration in healthy women. Turk J Gastroenterol. 2017; 28(3):171-75.

36. Rezniczek GA, Förster C, Hilal Z, Westhoff T, Tempfer CB. Calprotectin in pregnancy and pregnancy-associated diseases: a systematic review and prospective cohort study. Arch Gynecol Obstet. 2019; 299(6):1567-77.

37. Veres-Székely A, Szász C, Pap D, Szebeni B, Bokrossy P, Vannay Á. Zonulin as a Potential Therapeutic Target in Microbiota-Gut-Brain Axis Disorders: Encouraging Results and Emerging Questions. Int J Mol Sci. 2023; 24(8)

38. Daneshvar M, Yadegari A, Ribaldone DG, Hasanzadeh M, Djafarian K. Zonulin levels in complicated pregnancy: a systematic review and meta-analysis. J Obstet Gynaecol. 2022; 42(7):2621-28.

39. Camilleri M, Vella A. What to do about the leaky gut. Gut. 2022; 71(2):424-35.

40. Bawah AT, Tornyi H, Seini MM, Ngambire LT, Yeboah FA. Zonulin as marker of pregnancy induced hypertension: a case control study. Clin Hypertens. 2020; 26:7.

41. Morales Suárez-Varela M, Peraita-Costa I, Marín AP, Marcos Puig B, Llopis-Morales A, Soriano JM. Mediterranean Dietary Pattern and Cardiovascular Risk in Pregnant Women. Life (Basel). 2023; 13(1)

42. Flor-Alemany M, Acosta P, Marín-Jiménez N, Baena-García L, Aranda P, Aparicio VA. Influence of the degree of adherence to the mediterranean diet and its components on cardiometabolic risk during pregnancy. The GESTAFIT project. Nutrition, Metabolism and Cardiovascular Diseases. 2021; 31(8):2311-18.

43. Flor-Alemany M, Migueles JH, Acosta-Manzano P, Marín-Jiménez N, Baena-García L, Aparicio VA. Assessing the Mediterranean diet adherence during pregnancy: Practical considerations based on the associations with cardiometabolic risk. Pregnancy Hypertens. 2023; 31:17-24.

44. Flor-Alemany M, Acosta-Manzano P, Migueles JH, Baena-García L, Aranda P, Aparicio VA. Association of Mediterranean diet adherence during pregnancy with maternal and neonatal lipid, glycemic and inflammatory markers: The GESTAFIT project. Matern Child Nutr. 2023; 19(2):e13454.

45. Miller CB, Benny P, Riel J, Boushey C, Perez R, Khadka V, et al. Adherence to Mediterranean diet impacts gastrointestinal microbial diversity throughout pregnancy. BMC Pregnancy Childbirth. 2021; 21(1):558.

46. Zaragoza-Martí A, Ruiz-Ródenas N, Herranz-Chofre I, Sánchez-SanSegundo M, Serrano Delgado VC, Hurtado-Sánchez JA. Adherence to the Mediterranean Diet in Pregnancy and Its Benefits on Maternal-Fetal Health: A Systematic Review of the Literature. Front Nutr. 2022; 9:813942.

47. Cortez-Ribeiro AC, Meireles M, Ferro-Lebres V, Almeida-de-Souza J. Olive oil consumption confers protective effects on maternal-fetal outcomes: A systematic review of the evidence. Nutrition Research. 2023; 110:87-95.

48. Amati F, Hassounah S, Swaka A. The Impact of Mediterranean Dietary Patterns During Pregnancy on Maternal and Offspring Health. Nutrients. 2019; 11(5)

49. Zhou L, Ding C, Wu J, Chen X, Ng DM, Wang H, et al. Probiotics and synbiotics show clinical efficacy in treating gestational diabetes mellitus: A meta-analysis. Prim Care Diabetes. 2021; 15(6):937-47.

50. Movaghar R, Farshbaf-Khalili A, Hajizade K, MirzaRezaei ME, Shahnazi M. The Effect of Probiotics or Synbiotics on the Hypertensive Disorders of Pregnant Women with Gestational Diabetes: A Systematic Review and Meta-analysis. J Caring Sci. 2022; 11(2):94-104.

51. Ma L, Tu H, Chen T. Postbiotics in Human Health: A Narrative Review. Nutrients. 2023; 15(2)

52. Rodrigues VF, Elias-Oliveira J, Pereira Í S, Pereira JA, Barbosa SC, Machado MSG, Carlos D. Akkermansia muciniphila and Gut Immune System: A Good

Friendship That Attenuates Inflammatory Bowel Disease, Obesity, and Diabetes. Front Immunol. 2022; 13:934695.

53. Gascoigne EL, Webster CM, Honart AW, Wang P, Smith-Ryan A, Manuck TA. Physical activity and pregnancy outcomes: an expert review. Am J Obstet Gynecol MFM. 2023; 5(1):100758.

54. Zhang L, Liu Y, Wang X, Zhang X. Physical Exercise and Diet: Regulation of Gut Microbiota to Prevent and Treat Metabolic Disorders to Maintain Health. Nutrients. 2023; 15(6)

55. Duong QA, Curtis N, Zimmermann P. The association between prenatal antibiotic exposure and adverse long-term health outcomes in children: A systematic review and meta-analysis. Journal of Infection. 2025; 90(1):106377.

56. Gestels T, Vandenplas Y. Prenatal and Perinatal Antibiotic Exposure and Long-Term Outcome. Pediatr Gastroenterol Hepatol Nutr. 2023; 26(3):135-45.

57. Bianchi F, Cappella A, Gagliano N, Sfondrini L, Stacchiotti A. Polyphenols-Gut-Heart: An Impactful Relationship to Improve Cardiovascular Diseases. Antioxidants (Basel). 2022; 11(9)

58. Ferreira AF, Azevedo MJ, Saraiva FA, Trindade F, Barros A, Leite S, et al. The PERInatal MYocardial Remodeling (PERIMYR) cohort study protocol: A prospective study of cardiac remodeling and "recovery" in pregnancy as a model to understand the impact of comorbidities in cardiac remodeling and reverse remodeling. Rev Port Cardiol. 2023; 42(6):585-96.

59. Regitz-Zagrosek V, Roos-Hesselink JW, Bauersachs J, Blomström-Lundqvist C, Cífková R, De Bonis M, et al. 2018 ESC Guidelines for the management of cardiovascular diseases during pregnancy. Eur Heart J. 2018; 39(34):3165-241.

60. Gregório M, Rodrigues A, Salvador C, Dias S, de Sousa R, Mendes J, et al. Validation of the Telephone-Administered Version of the Mediterranean Diet Adherence Screener (MEDAS) Questionnaire. Nutrients. 2020; 12:1511.

61. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, 3rd, Dokainish H, Edvardsen T, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2016; 29(4):277-314.

62. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr. 2005; 18(12):1440-63.

63. Keshavarz Azizi Raftar S, Hoseini Tavassol Z, Amiri M, Ejtahed HS, Zangeneh M, Sadeghi S, et al. Assessment of fecal Akkermansia muciniphila in patients with osteoporosis and osteopenia: a pilot study. J Diabetes Metab Disord. 2021; 20(1):279-84.

64. Martínez-González MA, Gea A, Ruiz-Canela M. The Mediterranean Diet and Cardiovascular Health. Circ Res. 2019; 124(5):779-98.

65. Conrad KP. Emerging role of relaxin in the maternal adaptations to normal pregnancy: implications for preeclampsia. Semin Nephrol. 2011; 31(1):15-32.

66. De Haas S, Ghossein-Doha C, Geerts L, van Kuijk SMJ, van Drongelen J, Spaanderman MEA. Cardiac remodeling in normotensive pregnancy and in pregnancy complicated by hypertension: systematic review and meta-analysis. Ultrasound in Obstetrics & Gynecology. 2017; 50(6):683-96.

67. Kitt J, Krasner S, Barr L, Frost A, Tucker K, Bateman PA, et al. Cardiac Remodeling After Hypertensive Pregnancy Following Physician-Optimized Blood Pressure Self-Management: The POP-HT Randomized Clinical Trial Imaging Substudy. Circulation. 2024; 149(7):529-41.

68. Kapel N, Ouni H, Benahmed NA, Barbot-Trystram L. Fecal Calprotectin for the Diagnosis and Management of Inflammatory Bowel Diseases. Clin Transl Gastroenterol. 2023; 14(9):e00617.

69. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 2011; 91(1):151-75.

70. Tripathi A, Lammers KM, Goldblum S, Shea-Donohue T, Netzel-Arnett S, Buzza MS, et al. Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. Proc Natl Acad Sci U S A. 2009; 106(39):16799-804.

71. Güvey H, Çelik S, Çalışkan CS, Yılmaz Z, Yılmaz M, Erten Ö, Tinelli A. How Do Serum Zonulin Levels Change in Gestational Diabetes Mellitus, Pregnancy

Cholestasis, and the Coexistence of Both Diseases? International Journal of Environmental Research and Public Health. 2021; 18(23):12555.

72. Baioumy SA, Elgendy A, Ibrahim SM, Taha SI, Fouad SH. Association between serum zonulin level and severity of house dust mite allergic asthma. Allergy Asthma Clin Immunol. 2021; 17(1):86.

73. Reshadmanesh T, Behnoush AH, Farajollahi M, Khalaji A, Ghondaghsaz E, Ahangar H. Circulating Levels of Calprotectin as a Biomarker in Patients With Coronary Artery Disease: A Systematic Review and Meta-Analysis. Clin Cardiol. 2024; 47(7):e24315.

74. Xiao JH, Wang Y, Zhang XM, Wang WX, Zhang Q, Tang YP, Yue SJ. Intestinal permeability in human cardiovascular diseases: a systematic review and meta-analysis. Front Nutr. 2024; 11:1361126.

75. Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, et al. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med. 2019; 25(7):1096-103.

76. Frederico V, Rui P, Cláudia A. Estado nutricional pré-concecional e aumento ponderal na gravidez: estudo de acompanhamento na ilha do Faial. Acta Portuguesa de Nutrição. 2021(23):14-17.

77. Alves P, Malheiro MF, Gomes JC, Ferraz T, Montenegro N. Risks of Maternal Obesity in Pregnancy: A Case-control Study in a Portuguese Obstetrical Population. Rev Bras Ginecol Obstet. 2019; 41(12):682-87.

78. Grieger JA, Hutchesson MJ, Cooray SD, Bahri Khomami M, Zaman S, Segan L, et al. A review of maternal overweight and obesity and its impact on cardiometabolic outcomes during pregnancy and postpartum. Ther Adv Reprod Health. 2021; 15:2633494120986544.

79. Endres LK, Straub H, McKinney C, Plunkett B, Minkovitz CS, Schetter CD, et al. Postpartum weight retention risk factors and relationship to obesity at 1 year. Obstet Gynecol. 2015; 125(1):144-52.

80. Portincasa P, Bonfrate L, Khalil M, Angelis MD, Calabrese FM, D'Amato M, et al. Intestinal Barrier and Permeability in Health, Obesity and NAFLD. Biomedicines. 2022; 10(1):83.

81. Moreno-Navarrete JM, Sabater M, Ortega F, Ricart W, Fernández-Real JM. Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. PLoS One. 2012; 7(5):e37160.

82. Mokkala K, Tertti K, Rönnemaa T, Vahlberg T, Laitinen K. Evaluation of serum zonulin for use as an early predictor for gestational diabetes. Nutr Diabetes. 2017; 7(3):e253.

83. Demir E, Ozkan H, Seckin KD, Sahtiyancı B, Demir B, Tabak O, et al. Plasma Zonulin Levels as a Non-Invasive Biomarker of Intestinal Permeability in Women with Gestational Diabetes Mellitus. Biomolecules. 2019; 9(1)

84. Liu E, Ji X, Zhou K. Akkermansia muciniphila for the Prevention of Type 2 Diabetes and Obesity: A Meta-Analysis of Animal Studies. Nutrients. 2024; 16(20):3440.

85. Amabebe E, Anumba DO. Diabetogenically beneficial gut microbiota alterations in third trimester of pregnancy. Reprod Fertil. 2021; 2(1):R1-r12.

86. Lv LJ, Li SH, Li SC, Zhong ZC, Duan HL, Tian C, et al. Early-Onset Preeclampsia Is Associated With Gut Microbial Alterations in Antepartum and Postpartum Women. Front Cell Infect Microbiol. 2019; 9:224.

87. Richardson LA, Izuora K, Basu A. Mediterranean Diet and Its Association with Cardiovascular Disease Risk Factors: A Scoping Review. Int J Environ Res Public Health. 2022; 19(19)

88. Fowles ER, Bryant M, Kim S, Walker LO, Ruiz RJ, Timmerman GM, Brown A. Predictors of dietary quality in low-income pregnant women: a path analysis. Nurs Res. 2011; 60(5):286-94.

89. Martínez-González MA. Benefits of the Mediterranean diet beyond the Mediterranean Sea and beyond food patterns. BMC Medicine. 2016; 14(1):157.

90. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. N Engl J Med. 2018; 378(25):e34.

91. Hernáez Á, Castañer O, Elosua R, Pintó X, Estruch R, Salas-Salvadó J, et al. Mediterranean Diet Improves High-Density Lipoprotein Function in High-Cardiovascular-Risk Individuals: A Randomized Controlled Trial. Circulation. 2017; 135(7):633-43.

92. Sureda A, Bibiloni MDM, Julibert A, Bouzas C, Argelich E, Llompart I, et al. Adherence to the Mediterranean Diet and Inflammatory Markers. Nutrients. 2018; 10(1)

93. Liang L, Saunders C, Sanossian N. Food, gut barrier dysfunction, and related diseases: A new target for future individualized disease prevention and management. Food Sci Nutr. 2023; 11(4):1671-704.

94. Diakité MT, Diakité B, Koné A, Balam S, Fofana D, Diallo D, et al. Relationships between gut microbiota, red meat consumption and colorectal cancer. J Carcinog Mutagen. 2022; 13(3)

95. Astudillo-López CC, Castro-Alarcón N, Ariza AC, Muñoz-Valle JF, de la Cruz-Mosso U, Flores-Alfaro E, et al. Influence of Diet and Levels of Zonulin, Lipopolysaccharide and C-Reactive Protein on Cardiometabolic Risk Factors in Young Subjects. Nutrients. 2021; 13(12)

96. Lucerón-Lucas-Torres M, Saz-Lara A, Díez-Fernández A, Martínez-García I, Martínez-Vizcaíno V, Cavero-Redondo I, Álvarez-Bueno C. Association between Wine Consumption with Cardiovascular Disease and Cardiovascular Mortality: A Systematic Review and Meta-Analysis. Nutrients. 2023; 15(12)

Impact of intestinal biomarkers in cardiac reverse remodelling induced by pregnancy

Maria Adelina Azevedo Almeida

FACULDADE DE CIÊNCIAS DA NUTRIÇÃO E ALIMENTAÇÃO