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PERInatal MYocardial Remodelling (PERIMYR)

Cardiac Remodelling and "*Recovery*" in Pregnancy as a Model to Understand the Mechanisms of Cardiovascular Diseases

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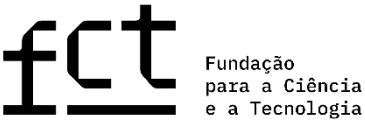

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Under the terms of the Degree-Law n° 388/70, article n° 8, 7th August, it is stated that the following articles are an integral part of this thesis.

1. Falcão-Pires I*, **Ferreira AF***, Trindade F*, Bertrand L, Ciccarelli M, Visco V, Dawson D, Hamdani N, Laake L, Lezoualc'h F, Linke W, Lunde I, Rainer P, Velden J, Consentino N, Paldino A, Pompilio G, Zacchigna S, Heymans S, Thum T, Tocchetti C. **Mechanisms of myocardial reverse remodelling and its clinical significance: a scientific statement of the ESC Working Group on Myocardial Function**. (* among authors contributed equally to this work). Submitted to European Heart Journal of Heart Failure.

2. **Ferreira AF**, Azevedo MJ, Saraiva FA, Trindade F, Barros A, Leite S, Proença T, Sousa C, Machado AP, Leite-Moreira A, Sampaio-Maia B, Ramalho C, Falcão-Pires I. **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 Jun;42(6):585-596. English, Portuguese. doi: 10.1016/j.repc.2022.08.015. Epub 2023 Mar 21. PMID: 36958578.

3. **Ferreira AF**, Azevedo MJ, Morais J, Trindade F, Saraiva F, Diaz SO, Alves IN, Fragão-Marques M, Sousa C, Machado AP, Leite-Moreira A, Sampaio-Maia B, Ramalho C, Barros AS, Falcão-Marques I. **Cardiovascular risk factors during pregnancy impact the postpartum cardiac and vascular reverse remodeling**. Am J Physiol Heart Circ Physiol. 2023 Oct 1;325(4):H774-H789. doi: 10.1152/ajpheart.00200.2023. Epub 2023 Jul 21. PMID: 37477690.

4. **Ferreira AF**, Trindade F, Azevedo MJ, Morais J, Douché T, Diaz SO, Saraiva FA, Sousa C, Machado AP, Matondo M, Leite-Moreira A, Ramalho C, Vitorino R, Falcão-Pires I, Barros AS. **The extent of postpartum cardiac reverse remodelling is reflected in urine proteome.** Under second review at Journal of Proteome Research.

5. **Ferreira AF**, Azevedo MJ, Morais J, Almeida-Coelho J, Leite-Moreira A, Lourenço AP, Saraiva FA, Diaz SO, Amador AF, Sousa C, Machado AP, Sampaio-Maia B, Ramalho C, Leite-Moreira A, Barros AS, Falcão-Pires I. **Stretch-Induced Compliance mechanism in pregnancy-induced cardiac hypertrophy and the impact of cardiovascular risk factors.** Submitted to Acta Physiologica.

6. **Ferreira AF***, Araújo J*, Azevedo MJ, Saraiva FA, Diaz SO, Sousa C, Machado AP, Sampaio-Maia B, Ramalho C, Leite-Moreira A, Barros AS, Santos M, Falcão-Pires I. **Cardiovascular remodelling and reverse remodelling during pregnancy and postpartum: looking at the right side of the heart** (* both authors contributed equally to this work). Ready to submit.

7. **Ferreira AF**, Saraiva FA, Diaz SO, Azevedo MJ, Sousa C, Leite-Moreira A, Sampaio-Maia B, Ramalho C, Barros AS, Falcão-Pires I. **The Impact of Echocardiographic Indexation to Evaluate Cardiac Reverse Remodelling throughout Pregnancy and Postpartum.** Rev Port Cardiol. 2023 Jul 24:S0870-2551(23)00387-6. English, Portuguese. doi: 10.1016/j.repc.2023.04.014. Epub ahead of print. PMID: 37495102.

Although not an integral part of this thesis, the following works were carried out and published during the PhD period and contributed to the development of this thesis:

1. Azevedo MJ, Garcia A, Costa CFFA, **Ferreira AF**, Falcão-Pires I, Brandt BW, et al. The contribution of maternal factors to the oral microbiota of the child: Influence from early life and clinical relevance. Japanese Dental Science Review. 2023;59:191-202. https://doi.org/10.1016/j.jdsr.2023.06.002

2. Azevedo MJ, Araujo R, Campos J, Campos C, **Ferreira AF**, Falcão-Pires I, Ramalho C, Zaura E, Pinto E, Sampaio-Maia B. Vertical Transmission and Antifungal Susceptibility Profile of Yeast Isolates from the Oral Cavity, Gut, and Breastmilk of Mother–Child Pairs in Early Life. International Journal of Molecular Sciences. 2023; 24(2):1449. https://doi.org/10.3390/ijms24021449.

3. Fernandes M, Azevedo MJ, Campos C, **Ferreira AF**, Azevedo Á, Falcão-Pires I, Zaura E, Ramalho C, Campos J, Sampaio-Maia B. Potential Pathogenic and Opportunistic Oral Bacteria in Early Life: The Role of Maternal Factors in a Portuguese Population. Pathogens. 2023; 12(1):80. https://doi.org/10.3390/pathogens12010080.

4. Trindade F, **Ferreira AF**, Saraiva F, Martins D, Mendes VM, Sousa C, Gavina C, Leite-Moreira A, Manadas B, Falcão-Pires I, Vitorino R. Optimization of a Protocol for Protein Extraction from Calcified Aortic Valves for Proteomics Applications: Development of a Standard Operating Procedure. Proteomes. 2022; 10(3):30. https://doi.org/10.3390/proteomes10030030.

5. Raimundo R, Saraiva F, Moreira R, Moreira S, **Ferreira AF**, Cerqueira RJ, Amorim MJ, Pinho P, Barros AS, Lourenço AP, Leite-Moreira A. Arterial Stiffness Changes in Severe Aortic Stenosis Patients Submitted to Valve Replacement Surgery. Arq Bras Cardiol. 2021 Mar;116(3):475-482. English, Portuguese. doi: 10.36660/abc.20190577. PMID: 33909777; PMCID: PMC8159560.

6. Rocha-Gomes JN, Saraiva FA, Cerqueira RJ, Moreira R, **Ferreira AF**, Barros AS, Amorim MJ, Pinho P, Lourenço AP, Leite-Moreira AF. Early dual antiplatelet therapy versus aspirin monotherapy after coronary artery bypass surgery: survival and safety outcomes. J

Cardiovasc Surg (Torino). 2020 Oct;61(5):662-672. doi: 10.23736/S0021-9509.20.11306-5. Epub 2020 May 19. PMID: 32431135.

Content

Abstract

Thesis outline: This thesis includes six chapters organized in an introduction (Chapter I), aims (Chapter II), methodologic considerations (Chapter III), description of the original results (Chapters IV) and the discussion, where the thesis findings are contextualized with the literature followed by major conclusions, limitations, and futures perspectives (Chapter V). These chapters are ensued by thesis references (Chapter VI).

Aims: The first aim of this thesis was to evaluate cardiovascular remodelling during pregnancy and to identify cardiovascular risk (CVR) factors and plasma biomarkers that might predict cardiac and vascular reverse remodelling (RR) after delivery (Results, subchapter i). The second aim was to profile the urinary proteome in pregnant women with or without CVR factors and identify putative proteins associated with cardiac postpartum RR (Results, subchapter ii). Thirdly, we aim to characterize the response to stretch induce-compliance mechanism in the pregnancy context and the impact of CVR factors, maternal age and parity in this process (Results, subchapter iii). For a complete characterization of cardiac remodelling and RR, we also aim to characterize the right cardiac changes, including the comparison of pregnant women with and without CVR factors (Results, subchapter iv). Lastly, we identified and compared the most common indexing methodologies in the literature of pregnant populations by carrying out a systematic review and using PERIMYR cohort sample (Results, subchapter v).

Methods: The PERIMYR project is a prospective cohort study, which includes volunteer pregnant women (healthy, obese and/or hypertensive and/or with type 2 diabetes or gestational diabetes) recruited in two tertiary centres between 2019 and 2021. Women were evaluated by transthoracic echocardiography and pulse wave velocity (PWV) at the 1st trimester [10-15 weeks, beginning of cardiovascular remodelling] and 3rd trimester [30-35 weeks, peak of cardiovascular remodelling] of pregnancy as well as at the 1st/6th/12th month after delivery (during RR). The blood and urine samples were also collected. Plasma biomarkers were quantified through ELISA, while urinary proteins were profiled by shotgun high-performance liquid chromatography-mass spectrometry (except for IGF-1 quantification done in urine samples using ELISA kits). The LVM and PWV were defined as primary outcomes for assessing cardiac and vascular remodelling/RR, respectively. Generalized linear mixed-effects models evaluated the extent of RR and its potential predictors. To study the LV response to stretch-induced compliance (SIC) mechanism, pregnant women underwent an echocardiographic assessment before (baseline), immediately after and 15 minutes after passive leg elevation. Blood samples were collected before and 15 minutes after this acute volume overload manoeuvre.

Results: Cardiac reverse remodelling was characterized by significant regression of LV hypertrophy and improvement of diastolic function associated with reduced left atrial volume as soon as 1 month after delivery that progressed until baseline values for the following 6-12 months. In parallel, we observed a significant increase in PWV and systemic vascular resistance. We evidenced that, among the CVR factors analyzed, arterial hypertension was a common predictor of cardiac and vascular remodelling and RR, evidenced by the lower regression of LV hypertrophy and higher arterial stiffness after delivery. Additionally, we showed a distinct functional LV response to stretch-induced compliance (SIC) imposed by passive leg elevation between 1st and 3rd trimesters, being influenced by the presence of CVR factors. Interestingly, the right ventricle is not sensitive to the impact of risk factors in cardiovascular remodelling and reverse remodelling (revealing some degree of deterioration of right ventricular diastolic function and myocardial deformation).

Among the plasma biomarkers evaluated, low ST2/IL33 receptor and high C-reactive protein levels were associated with worse regression of LV mass. The urinary proteins Glutathione S-transferase P (GSTP1), ADP-ribosylation factor 1 (ARF1), Fibronectin (FN1), Sortilin (SORT1), Sialate O-acetylesterase (SIAE), Alpha-galactosidase A (GLA), Inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) and Desmoglein-2 (DGS2) were involved in pathways that were independently associated with LV mass regression, such as regulation of insulin-like growth factor (IGF-1) transport and uptake by IGF binding proteins, platelet activation, signalling and aggregation and immune system. Indeed, IGF-1 concentration in urine samples was associated with low LVM regression after delivery.

Finally, considering that echocardiography guidelines suggest normalizing LVM to body surface area (BSA) and that body size varies significantly during pregnancy, we explored the best indexation method and concluded that normalising echocardiographic parameters to pre-pregnancy BSA or an allometric indexation yielded a higher magnitude of effect compared to the most frequently used indexation method (BSA measured at each evaluation) and showed equal effect to not indexing using a within-subjects design.

Conclusion: The CVR factors revealed a significant impact in LV and vascular remodelling and RR induced by pregnancy, as well as in LV response to SIC-mechanism. From all CVR, arterial hypertension significantly impacts RR, evidencing lower LV hypertrophy regression associated with a high arterial stiffness after delivery. Additionally, low ST2/IL33 receptor and high C-reactive protein levels were also associated with worse LV mass regression. We can conclude that postpartum cardiovascular RR may provide important clues that can be translated to the context of heart failure and allow us to assess the women's risk of developing this syndrome at the long-term.

Resumo

Estrutura da tese: Esta tese inclui seis capítulos, organizados numa introdução (Capítulo I), objetivos (Capítulo II), considerações metodológicas (Capítulo III), descrição dos resultados originais (Capítulo IV, subcapítulos i-v), e a discussão, onde os resultados da tese são contextualizados com a bibliografia existente, e as principais conclusões, limitações e perspetivas futuras são fornecidas (Capítulo V). Finalizando com as referências bibliográficas da tese (Capítulo VI).

Objetivos: O primeiro objetivo da tese foi avaliar a remodelagem cardiovascular decorrente da gravidez e a identificação dos fatores de risco e biomarcadores plasmáticos que poderão prever a remodelagem reversa cardíaca e vascular após o parto (Resultados, subcapítulo i). O segundo objetivo foi identificar o perfil do proteoma da urina em participantes com e sem fatores de risco cardiovascular que se associa à remodelagem reversa cardíaca no pós-parto (Resultados, subcapítulo ii). O terceiro capítulo foi caracterizar a resposta aguda ao estiramento no contexto da gravidez, bem como avaliar o impacto dos fatores de risco cardiovascular, paridade e idade materna neste processo (Resultados, subcapítulo iii). Para uma caracterização da remodelagem cardíaca e remodelagem reversa mais completa, foi realizada a avaliação cardiovascular direita, incluindo a comparação entre grávidas com e sem fatores de risco cardiovascular (Resultados, subcapítulo iv). Por fim, procuramos identificar a metodologia de indexação mais aplicada na população grávida descrita na literatura através de uma revisão sistemática; e, comparar 4 métodos de indexação usando uma amostra da coorte PERIMYR (Resultados, subcapítulo v).

Métodos: O projeto PERIMYR é um estudo de coorte prospetivo, que incluiu grávidas voluntárias (saudáveis, obesas e/ou hipertensas e/ou com diabetes tipo II ou gestacional) recrutadas em dois centros terciários entre 2019 e 2021. As participantes foram avaliada por ecocardiografia transtorácica e velocidade de onda de pulso (VOP) no 1º [10-15 semanas, início da remodelagem cardiovascular] e 3º trimestres [30-35 semanas, pico da remodelagem cardiovascular] de gravidez, bem como no 1º/6º/12º mês após o parto (durante a remodelagem reversa). Foram também realizadas colheitas de sangue e urina. Os biomarcadores plasmáticos foram quantificados através de kits de ELISA, enquanto o perfil das proteínas da urina foi adquirido por cromatografia líquida acoplada à espectrometria de massa em tandem (com a excepção da quantificação do IGF-1 na urina através de kits de ELISA). A massa do ventrículo esquerdo e a VOP foram definidas como indicadores da remodelagem cardíaca e vascular/remodelagem reversa, respetivamente. Os modelos lineares generalizados de efeitos mistos foram utilizados para avaliar a extensão da remodelagem reversa, bem como identificar os seus preditores. Para

o estudo da resposta aguda do ventrículo esquerdo ao estiramento, grávidas realizaram uma avaliação ecocardiográfica antes, imediatamente após a elevação passiva dos membros inferiores e nos seguintes 15 minutos nesta posição. Neste contexto procedeu-se à colheita de sangue antes e 15 minutos após a sobrecarga aguda de volume.

Resultados: A remodelagem reversa cardíaca foi caracterizada por uma regressão significativa da hipertrofia ventricular esquerda e melhoria da função diastólica associada à redução da aurícula esquerda logo após o 1º mês após o parto, progredindo para valores basais entre os 6-12 meses. Concomitantemente, foi observado um aumento significativo da velocidade de onda de pulso e das resistências vasculares periféricas. De todos os fatores de risco analisados, a hipertensão arterial foi identificada como preditor da remodelagem cardíaca e vascular, e remodelagem reversa, evidenciando uma menor regressão da hipertrofia ventricular esquerda e maior rigidez arterial após o parto. Adicionalmente, demonstramos que a resposta aguda ao estiramento causada pela elevação passiva dos membros inferiores é distinta entre o 1º e 3º trimestres, sendo influenciada pela presença dos fatores de risco cardiovascular. Curiosamente, o ventrículo direito não é afetado significativamente pelos fatores de risco cardiovascular (induzindo apenas um ligeiro grau de deterioração da função diastólica e da deformação miocárdica). Relativamente aos biomarcadores plasmáticos, baixos níveis do recetor ST2/IL33 e elevados da proteína C reativa foram associados a pior regressão de massa do ventrículo esquerdo. As proteínas urinárias Glutationa S-transferase P (GSTP1), ADP-ribosilação fator 1 (ARF1), Fibronetina (FN1), Sortilina (SORT1), Sialato O-acetilesterase (SIAE), Alfa-galactosidase A (GLA), Inter-alfa-tripsina inibidor cadeia pesada H4 (ITIH4) e Desmogleína-2 (DGS2) estão envolvidas em vias de sinalização que foram associadas de forma independente à regressão da massa do ventrículo esquerdo, tais como a regulação do transporte do fator de crescimento semelhante à insulina e a captação pelas proteínas de ligação do IGF, a ativação, sinalização e agregação plaquetária e o sistema imunológico. De facto, a concentração urinária de IGF-1 associou-se a uma menor regressão de massa no pós-parto. Por fim, atendendo que as recomendações de ecocardiografia transtorácica sugerem a indexação da massa do ventrículo esquerdo à área de superfície corporal e que esta varia significativamente durante a gravidez, nós pesquisamos qual seria o melhor método de indexação e concluímos que a indexação dos parâmetros ecocardiográficos à área de superfície corporal prévia à gestação ou uso de coeficientes alométricos têm um tamanho de efeito superior à indexação à ASC calculada em cada momento de avaliação, e igual à ausência de indexação, na análise intra-sujeito.

Conclusão: Os fatores de risco cardiovascular revelaram um impacto significativo na remodelagem do ventrículo esquerdo e vascular e remodelagem reversa induzida pela gravidez, bem como na resposta aguda ao estiramento do ventrículo esquerdo. De todos os fatores de

risco cardiovascular, destaca-se a hipertensão arterial evidenciando uma menor regressão da hipertrofia ventricular esquerda associada a uma elevada rigidez arterial após o parto. Adicionalmente, baixos níveis do recetor ST2/IL33 e elevados de proteína C reativa foram associados a pior regressão de massa do ventrículo esquerdo. Podemos concluir que a remodelagem cardiovascular reversa após o parto poderá revelar importantes indicadores/pistas que poderão ser translacionadas para o contexto da insuficiência cardíaca e permitir-nos avaliar o risco destas mulheres desenvolverem esta patologia a longo-prazo.

Chapter I. Introduction

This chapter integrates parts of the submitted review paper:

Falcão-Pires I*, **Ferreira AF***, Trindade F*, Bertrand L, Ciccarelli M, Visco V, Dawson D, Hamdani N, Laake L, Lezoualc'h F, Linke W, Lunde I, Rainer P, Velden J, Consentino N, Paldino A, Pompilio G, Zacchigna S, Heymans S, Thum T, Tocchetti C. **Mechanisms of myocardial reverse remodelling and its clinical significance: a scientific statement of the ESC Working Group on Myocardial Function.** (* among authors contributed equally to this work). Submitted for European Heart Journal of Heart Failure. (Supplement I)

I.I. Pregnancy as a "human model" to study cardiovascular diseases

According to CardioRenal and Metabolic disease (CaReMe) Heart Failure Study, Portugal has the highest prevalence of heart failure (HF) (2.9%), with 75% of patients presenting with preserved ejection fraction (1). Women have revealed a higher propensity to develop HF with preserved ejection fraction (HFpEF), anticipating a rise in HF incidence in females (2).

Recently, pregnancy was suggested to have prognostic relevance for HFpEF development. For instance, formerly preeclamptic women show an increased risk of developing HFpEF and subsequent HFpEF hospitalization (2). Also, women with a history of pregnancy hypertensive diseases, including pre-existing hypertension, gestational hypertension and pre-eclampsia, presented a higher probability of having concentric left ventricular (LV) remodelling accompanied by LV diastolic dysfunction later in life. This geometric and functional pattern may have persisted since gestation, corroborated by HFpEF echocardiographic findings (2). Indeed, advanced age, pregnancy hypertensive disorders and indexed left ventricular mass (LVM) arose as potential predictors for impaired LV relaxation after delivery (3). Accordingly, the new recommendations of the American Heart Association suggest extending the follow-up period up to one year after delivery for women with pregnancy-hypertensive diseases to control blood pressure, glucose levels and lipid profile, preventing the development of cardiovascular diseases (4). Thus, pregnancy might represent a unique opportunity to predict and prevent HFpEF progression in women with traditional risk factors, such as arterial hypertension (HTA), diabetes mellitus (DM) and obesity, which impose a greater attributable risk for HFpEF development (2).

Considering the above, a prospective cohort study was designed to recruit pregnant women with and without cardiovascular risk factors (chronic hypertension or gestational hypertension and/or gestational diabetes and/or type 2 diabetes mellitus and/or obesity). This study is described in the methods section and comprises five evaluation moments, from 1st trimester to one year after delivery.

In addition, pregnant women represent an excellent and unique human model to investigate cardiovascular remodelling and reverse remodelling (RR) under physiological (pregnancy-induced remodelling and postpartum associated RR) and pathological conditions, i.e., in the presence of risk factors. Some of the advantages provided by pregnant women include: 1) presenting fewer confounding factors than HF patients (comorbidities and medication), 2) outperforming rodents in terms of translation potential, 3) comprising metabolic adaptations to cardiac (reverse) remodelling that may elucidate important mechanisms of HF

progression and 4) representing an excellent model to clarify the stretch-induced-compliance (SIC)-mechanism following acute myocardial stretch in physiological and hypertrophic conditions (5).

I.II. Cardiac Remodelling and RR induced by Pregnancy and Postpartum

Pregnancy is characterized by a hemodynamic overload that leads to significant physiological multisystemic changes to meet the mother's and foetus's rising metabolic demands, such as adequate uteroplacental circulation for foetal development.

The elevated oestrogen levels induce renin substrate production and, consequently, an increment of angiotensin concentration during gestation (6). Relaxin also leads to water retention through vasopressin secretion and drinking stimulation (6). The consequent increased activation of rennin-angiotensin-aldosterone system triggers plasma volume to rise from 6-8 until 28-30 weeks of pregnancy (6, 7), maintaining blood pressure and causing salt and water retention during pregnancy (6). This continuous augmentation of plasmatic volume throughout gestation causes the cardiac output to progressively rise during pregnancy until the end of 2nd trimester, revealing a constant or descendent trajectory up to the end of gestation (6, 8). This cardiac output variation is influenced by increased stroke volume in early pregnancy and heart rate later in gestation (10 to 20 bpm) (6, 8-11).

The changes in ventricular geometry described in the literature are not consensual. While some authors report an enlargement of cardiac volumes associated with LV physiological hypertrophy and a proportional change in wall thickness (eccentric remodelling) in response to the rise of cardiac output rise (3, 6, 9, 10, 12-15). Others describe a significant increase in LVM (from 22nd weeks of pregnancy until early postpartum) (8, 16, 17) and wall thickness consistent with concentric remodelling, as assessed by cardiac magnetic resonance imaging (18) or other imaging techniques (17, 19). The significant augmentation of left atrium (LA) and LV dimensions starts around the 22th week of gestation, culminating at pregnancy term (17, 20) or even later, in the first 48 hours postpartum, as described by Burlingame *et al.* (16). These conformational alterations are accompanied by a reduction of diastolic function within the normal range, being more pronounced in the first 48 hours after childbirth (3). In early postpartum, BNP and NT-proBNP levels are also reported to be elevated, returning to non-pregnant levels within 6-12 weeks postpartum (15, 16). This may be associated with reduced haemoglobin levels or additional dilation of cardiac chambers immediately after delivery induced by myocardial stretch

due to an increment of cardiac preload (16, 21). In addition, elevated cardiac troponin I (TnI) levels were also documented during the peripartum period, indicating myocardial injury during labour and delivery (15, 21).

As mentioned before, an important aspect of cardiac (reverse) remodelling is hypertrophy. Several signalling pathways are critically involved in pregnancy-induced hypertrophy. Such is the case of pathways highly responsive to changes of the hormonal millieau and volemia, i.e., the extracellular signal-regulated kinase ½ (ERK1/2), the phosphatidylinositol 3'-kinase(PI3K)-Akt and calcineurin (Figure 1) (11, 22). During gestation, the raised levels of progesterone increase ERK1/2 phosphorylation, independently of JNK and p38 activation, resulting in physiologic hypertrophy (11, 23, 24). Late in pregnancy and postpartum (11, 23), hemodynamic overload triggers increased phosphorylation of Akt, which inhibits ERK1/2 phosphorylation (23) independently of the hormonal milieu (23). Activation of calcineurin by increased calcium levels also (24) induces pregnancy cardiac hypertrophy under the influence of progesterone (24). Calcineurin dephosphorylates the cytoplasmic nuclear factor of activated T cells (NFAT), leading to the translocation of NFAT to the nucleus and, consequently, to the activation of pro-hypertrophic target genes (24). Calcineurin also upregulates ERK1/2 signalling in myocytes, suggesting an interdependence between these pathways (24). In late pregnancy, as oestradiol rises and counterbalances progesterone levels, calcineurin activity decreases significantly (24) and other hypertrophic pathways are activated, namely neuregulin-1 (NRG1)/ErbB system (25), c-Src/Ras/MAPK(ERK) pathway and Kv4.3 gene remodelling (22, 26). In addition, other hypertrophic signalling pathways are activated independently of hormonal influence, such as ACE2/Ang-(1–7)/Mas receptor axis (27) and STAT3 signalling (7).

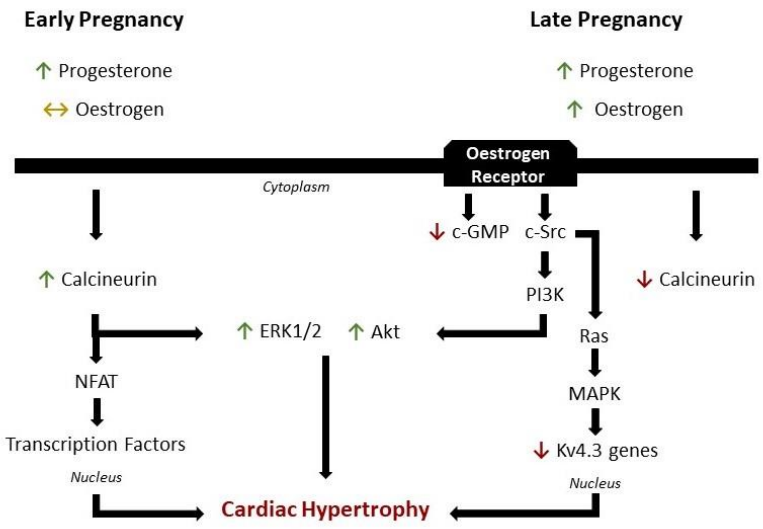

Figure 1: Scheme of signalling pathways involved in pregnancy-induced hypertrophy. Legend: Akt: serine/threonine kinase; c-GMP: cyclic guanosine monophosphate; c-Src: stretch-responsive kinase; ERK1/2: extracellular signal-regulated kinase ½; MAPK: mitogen-activated protein kinases; NFAT: nuclear factor of activated T cells; PI3K: phosphatidylinositol-3-kinase.

Moreover, at the molecular level, the vascular endothelial growth factor B (VEGF-B), insulin, growth hormone (GH) and insulin-like growth factor 1 (IGF1), as well as the thyroid hormone triiodothyronine (T3) have also reported in control of cardiac myocyte contractility, sarcomere remodelling, cell survival, metabolic and mitochondrial adaptations, electrical remodelling and angiogenesis needed to physiological cardiac remodelling (12). During this process, the fibroblast growth factor 21 (FGF21) plays also a critical role against oxidative stress, promoting a physiological cardiac hypertrophy (28, 29).

Another key player of cardiac (reverse) remodelling is myocardial fibrosis. P38 plays an essential role in cardiac remodelling by attenuating fibrosis and apoptosis (23), as confirmed by the absence/minimal fibrosis in overloaded hearts from pregnant rodents (7, 11). An animal study found higher pericardial and vascular fibrosis in late pregnancy compared to the non-pregnant group, which reversed in postpartum (30). These were associated with the downregulation of metalloproteinases (MMP-1, MMP-2, and MMP-9) counterbalancing the upregulation of tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-4) in late pregnancy (30). MMP-1, MMP-9 and TIMP-1 expression in LV reversed seven days after delivery (30). In addition, the reduction of MMP-2 expression was correlated with increased TIMP-4 levels during postpartum (30). Indeed, the metalloproteinase system revealed an essential role in keeping the structural integrity of the cardiac extracellular matrix (30). Collagens are the main component of the cardiac extracellular matrix, which contributes to passive tension in myocardial walls during diastolic filling, determining LV stiffness (31). Collagen I and collagen III were upregulated in LV during pregnancy, reversing during postpartum (31). In this context, fibrosis arises as a compensatory effect in response to pregnancy's hemodynamic overload (30, 31), tightly regulated by hormones (31). Indeed, expression of collagen III protein (more compliant isoform) and oestrogens levels have been reported to augment in late pregnancy, while collagen I and progesterone have decreased (31). Relaxin also regulates the cardiac extracellular matrix, downregulating fibroblast activation and proliferation as well as collagen secretion, evidencing antifibrotic effects (11, 32).

Considering it is more uniform than heart failure cohorts, blood samples collected at different time points of this cohort will be used to assess potential biomarkers of myocardial injury, fibrosis and extracellular matrix turnover, as well as vasoactive peptides to characterize cardiac (reverse) remodelling (subchapter I of the Results section i).

Cardiac extracellular matrix can determine LV stiffness and consequently influence diastolic function. The mitral inflow A wave velocity increases progressively throughout gestation

with a consequent decrease of the E/A ratio, revealing the importance of atrial contraction for LV filling during cardiac remodelling (LVM augmentation and reduced ventricular compliance) (9, 33). Concomitantly and following the Frank-Starling mechanism, the left atrial (LA) volume increases as an attempt to increase atrial stroke volume and its contribution to LV filling (33, 34) (20, 35). In addition, a rise of E/e' was documented throughout pregnancy, suggesting increased ventricular filling pressures (20, 33, 36) toward the 3rd trimester, recovering 6 months after delivery (37). This has been corroborated by more recent parameters, such as LA strain that correlates independently with LV diastolic dysfunction and inversely with stroke volume (37).

Regarding systolic function, several authors report substantial enhancement of intrinsic myocardial contractility during pregnancy that contributes to cardiac output increase without repercussions in ejection fraction, independently of loading conditions (38). Nonetheless, Geva *et al.* demonstrated a transient reduction of the contractile index in the 2nd trimester, which may be caused by a decrease of end-systolic stress without the expected increase in velocity of fibre shortening, returning toward nonpregnant values in the early postpartum period (39). More recent observations derived from myocardial deformation are not consensual. Some studies report a significant reduction of LV segmental longitudinal systolic deformation throughout pregnancy, which recovers four to seven months postpartum (10, 14, 40). The late pregnancy reduction of LV deformation may reflect physiological adaptation to a persistent increase in volume load combined with increasing afterload and heart rate in the 3rd trimester (14, 41). The circumferential and radial strain did not show significant alterations during similar follow-up (10, 40). Contrarily, Sengupta *et al.* documented increased global radial strain during gestation, peaking at the 2nd trimester, suggesting a compensatory mechanism for the loss of long-axis function (20). Indeed, longitudinal strain is determined by the vertical arrangement of subendocardial fibres, which are more susceptible to alterations in loading conditions, whereas circumferential and radial strain is related to the radial arrangement of mid-myocardial and subepicardial fibres, which may be the latest to become affected (20).

Myocardial mechano-energetic efficiency (calculated by stroke volume/[heart rate/60]), remains unchanged throughout gestation, despite rapid augmentation of LVM, heart rate, and stroke volume. This confirms myocardial efficiency even in 3rd trimester, when structural changes and cardiac function demands are exacerbated (33). In addition, ventricular-arterial coupling was unchanged during pregnancy due to the reduction of LV end-systolic elastance and effective arterial elastance, evidencing the greatest efficiency (33).

The duration of reverse remodelling is not consensual. Left ventricle volumes, stroke volume, cardiac output and systemic vascular resistances return gradually to pre-pregnancy values after parturition, despite remaining significantly above pre-gravid values throughout the first year postpartum (37, 42). Notwithstanding, 23% of women present increased cardiac output and 30% present decreased systemic vascular resistance one year after delivery (42). In contrast, LV hypertrophy and increased wall thickness seem to resolve earlier, as soon as three months postpartum (18, 43). Interestingly, Iacobaeus *et al.* proposed that nine months after delivery would represent the time for all cardiovascular parameters to return to baseline (33).

Women's parity and breastfeeding status are critical for postpartum reverse remodelling (11). Although parity did not significantly affect the time course or magnitude of the pregnancy-associated changes in heart rate, arterial blood pressure, LV volume, and stroke volume (42), the multiparous women revealed a greater magnitude of absolute change in these parameters from 24 pregnancy weeks to 52 postpartum weeks compared to primiparous women (42). Higher parity was also associated with increased cardiac volumes, LVM and diastolic dysfunction (44). In addition, parity arose as a determinant of cardiovascular risk later in life (42, 44) and higher parity was defined as a novel risk factor for adverse LV remodelling, diastolic dysfunction, and possibly future HFpEF (44).

Regarding right ventricle remodelling during pregnancy, healthy primiparous women have shown a significant increase of right ventricular diameters and areas from 2nd to 3rd trimester, accompanied by a reduction of functional parameters, such as right ventricular fractional area change, tricuspid annular plane systolic excursion and right ventricular longitudinal strain (9, 10, 34, 45, 46). All these variables seem to normalise between 3-12 months after delivery (45, 46).

Information about right cardiovascular remodelling and reverse remodelling in pregnancy is scarce in scientific literature. For this reason, subchapter iv in the Results section is dedicated to its characterization in the PERIMYR cohort sample.

Breastfeeding duration has been inversely correlated with a lower risk for preeclampsia, cardiovascular disease, coronary heart disease, stroke, metabolic syndrome, fatal cardiovascular disease and type II diabetes mellitus in subsequent pregnancies (47-50), with additional gain beyond 12 months (49). The effect of oxytocin, a lactational hormone, has arisen as the most consensual explanation for the beneficial impact of breastfeeding on cardiovascular health, revealing blood pressure-lowering effects, vasodilatation, antidiabetic actions, antioxidant effects, inhibition of inflammation, and lowering of fat mass (48, 49). Indeed, oxytocin synthesis

and receptors are found in the heart and vascular tissue (48). The rapid weight loss after delivery induced by breastfeeding may also justify the reduced cardiovascular risk because the increased weight is a risk factor for future cardiovascular events (49). In addition, Stuebe and Rich-Edwards suggested that breastfeeding may re-establish maternal metabolism, namely glucose and lipid homeostasis, after delivery, reducing long-term cardiovascular risk (51), and reestablishing metabolic disturbances such as diabetes or hyperlipidaemia (51). Breastfeeding may also increment vagal tone, which may cause the reduction of systolic blood pressure and heart rate in parturients (48).

I.III. Impact of Cardiovascular Risk Factors in Cardiac Remodelling and RR induced by Pregnancy and Delivery

Pregnant women represent a unique human model to investigate cardiovascular remodelling and reverse remodelling (RR) under physiological (pregnancy-induced remodelling and postpartum associated RR) and pathological conditions, i.e., in the presence of risk factors. As such, the impact of several comorbidities will be detailedly described in the following sections.

Obesity

Morbidly obese pregnant women showed higher LVM with lower stroke volume index compared to normal-weight women in 3rd trimester, suggesting a limited adaptative reserve for a significant increase of cardiac output and systolic blood pressure rising of obese women (52) (53, 54). These women present with a higher relative wall thickness (RWT) and LV internal diameter in diastole (53), prolonged isovolumetric relaxation time and smaller septal e' without major impact on E/e' (53). Diastolic and systolic dysfunction was subsequently confirmed by speckle-tracking methodology, highlighting a potential maladaptation to volume overload in obese pregnant women at term (52). Similar findings were found in overweight pregnant women (55-58). Concerning the right ventricle, no functional differences (TAPSE and S' tricuspid velocity) were noted between obese and non-obese pregnant women in 3rd trimester, except higher E'/A' in the obese group (53).

Regarding cardiac structure, most women with class III obesity showed normal geometry during pregnancy. However, 32%, presenting concomitantly pregnancy hypertensive diseases, developed mostly concentric remodelling after adjusting for nulliparity, chronic hypertension and smoking habits (59). Additionally, pre-pregnancy body mass index and excessive gestational

weight gain were considered modifiable factors associated with the incidence of pregnancy hypertensive diseases. Indeed, women with excessive gestational weight gain above the values recommended (60) presented a nearly 1.8 times higher risk of developing pregnancy hypertensive diseases (61).

Moreover, maternal obesity was associated with reduced cardiac recovery (defined as ejection fraction ≥50%) at 6 and 12 months after delivery in women with peripartum cardiomyopathy (62). In multivariate analysis, LV end-diastolic diameter arose as the strongest predictor of cardiac recovery and seemed to mediate the impact of body surface index on recovery (62).

Gestational Diabetes

Pregnant women with gestational diabetes showed elevated total vascular resistances and mean arterial pressure, which triggers LV hypertrophy and remodelling, persisting 2 to 4 days postpartum (63, 64). However, the results about that were not concordant. While some authors characterize cardiac remodelling through increased RWT without proportional LV mass increment (65), others revealed a significant LV mass gain without a proportional increase of RWT (66). Indeed, higher prevalence of concentric remodelling, eccentric hypertrophy and concentric hypertrophy has been reported in pregnant women with gestational diabetes in the 3rd trimester (63). The activation of calcineurin/NFAT and CaMKII/HDAC hypertrophic pathways under the influence of progesterone (67) has been ascribed to concentric remodelling in rodents with gestational diabetes mellitus and persisting up to two months after delivery (67). Besides LV hypertrophy, impaired diastolic (augmented E/e') and systolic function (decreased global longitudinal strain, despite preserved ejection fraction) was also described in gestational diabetes in the 3rd trimester (64, 65, 68).

Regarding the impact of gestational diabetes in RR, it is important to notice that hyperglycaemic phenotype and LV hypertrophy resolve immediately after and 6 months after delivery (68), respectively. However, certain diastolic (E/A ratio, E mitral inflow velocity, and e' septal velocity) and systolic variables (global longitudinal strain) take longer to normalize, even with tight anti-diabetic treatments (68). Increasing the follow-up time for 20 years, CARDIA study demonstrated a significant association between gestational diabetes and LV mass increased, impaired systolic (measured by longitudinal and circumferential strain) and diastolic function in a pattern similar to diabetic cardiomyopathy (65). In fact, gestational diabetes is associated with

higher cardiovascular risk after delivery, including the development of type 2 diabetes mellitus (69-71).

Hypertensive Diseases of Pregnancy

Cardiac remodelling in pregnancy hypertensive disease was characterized by increased LV dimensions, mass and RWT (17, 72, 73). Increased prevalence of concentric LV remodelling (17, 74-76) and concentric LV hypertrophy was reported in pregnant women with gestational hypertension, often persisting postpartum despite antihypertensive treatment (63, 74, 76, 77). This LV pattern was associated with adverse pregnancy outcomes and adverse hypertensive LV remodelling (74, 77). Vasapollo *et al.* determined pre-gravid higher E/e' and concentric hypertrophy as the strongest predictors of complications in early or long-term postpartum of patients with pre-existing arterial hypertension diagnosed (78). Arterial hypertension also imposes a major toll on the heart by inducing an earlier stroke volume reduction (from the 2nd trimester onwards) (79) and subclinical cardiac dysfunction, which persists 12 months after delivery (80).

Mesquita *et al.* also evidenced that women with hypertensive pregnancy disorders may exhibit persistent abnormalities in LV structure independent of blood pressure levels (77), which impact subsequent pregnancies. Women with a history of pregnancy hypertensive diseases present increased LV volumes, LVM index and impaired LV relaxation in following gestations compared to women with previous normotensive pregnancies (72, 81). Moreover, E/e' assessed during pregnancy was demonstrated to be an independent predictor for gestational hypertension development in subsequent pregnancies (82) and confirming the long-term persistence of these postpartum changes (82, 83). Impaired LV relaxation seems to persist postpartum (84), being peripartum hs-TnI a good predictor of poor LV relaxation 1 month after delivery in women with hypertensive disorders of pregnancy (84).

Women with other concomitant hypertensive conditions, such as arterial hypertension, have a more pronounced increase of LV mass and abnormal cardiac remodelling, which can persist up to 2 years postpartum (85). Also, pregnancies complicated by preeclampsia revealed a more pronounced increase in LV mass, indexed volumes and RWT, significantly increasing the propensity to develop concentric LV remodelling (43, 74, 86-88). Subendocardial fibrosis may lead to LV relaxation impairment, regional longitudinal systolic dysfunction and circumferential strain, despite preserved ejection fraction (86, 89). Diastolic dysfunction can correlate with preeclampsia severity (74), being indexed LA volume and E/e' increased in preeclamptic

pregnancies (86). Indeed, the recognition of impairment of cardiac function is critical in the contemporary management of preeclampsia to improve pregnancy outcomes and long-term cardiovascular health (74).

During the first year after a pregnancy complicated by preeclampsia, the low plasma volume and increased pressure load were reported to be associated with a higher risk of developing later concentric cardiac remodelling (90). Subclinical systolic dysfunction and increased cardiac volumes and interventricular septum thickness persisted for three months postpartum after preeclampsia when their blood pressure returned to normal range and ejection fraction was kept preserved (91). Interestingly, these signs of abnormal reverse remodelling correlated positively with NT-proBNP (91). At long-term, formerly women who have had preeclampsia displayed a three-fold higher prevalence of significant maladaptation of cardiac reverse remodelling (including LV hypertrophy, concentric remodelling, mildly impaired LV ejection fraction or asymptomatic valvular disease) at least four years postpartum (92). Accordingly, women with a history of preeclampsia 14 months earlier showed increased LV mass index and RWT associated with higher filling pressures (measured by E/e') compared to normotensive pregnancies, despite preserved ejection fraction (93). Ghossein-Doha *et al.* showed that women with former preeclampsia develop LV concentric remodelling more rapidly and presented a higher incidence of chronic hypertension over a period of 14 years postpartum (94). Indeed, these women presented a positive correlation between chronic hypertension and higher LVM index and diastolic blood pressure (95).

Regarding right cardiac remodelling, pregnant women with gestational hypertension showed higher right ventricular diameter and right atrial volume associated with lower fractional area change and global and free right ventricular longitudinal strain (96). Preeclampsia induced right ventricle enlargement with impaired diastolic and systolic function (96-98). Indeed, increased pulmonary resistance was also observed in gestational hypertension and preeclampsia, leading to reduced LV compliance, increased LV diastolic filling pressures and decreased RV longitudinal strain (96, 98).

The cardiac remodelling developed throughout pregnancy reveals an important window to predict and prevent future cardiovascular events and HF progression in women with traditional risk factors.

I.IV. Vascular Remodelling and RR induced by Pregnancy and Delivery and the Impact of Cardiovascular Risk Factors

Elevated levels of oestrogen, progesterone and relaxin during pregnancy induce systemic vasodilatation as soon as five weeks of gestation (6, 11). This vasodilation decreases to a nadir at the 2nd trimester and stabilizes until the end of pregnancy when it suffers a slight increment (6, 8, 10). Accordingly, peripheral vascular resistance decreases approximately 35% to 40% of baseline during gestation, returning to non-pregnant levels during the first two weeks after delivery (3, 6, 13). Arterial pressure shows similar variation, dropping 5 to 10 mmHg below preconception values until the 2nd trimester with a more notorious reduction of diastolic and mean arterial pressure (6, 8, 10, 99). In obese pregnant women, the changes in blood pressure and systemic vascular resistance are attenuated due to insufficient nitric oxide bioavailability, leading to vascular dysfunction (54, 55) and thereby interfering in cardiac remodelling (55).

The aortic stiffness, assessed by carotid-femoral pulse wave velocity and augmentation index, are reported to decrease until the 2nd trimester, normalising after that (99). Accordingly, common carotid artery diameter increases continuously during gestation, which, supported by increased microvascular reactivity, increases vasodilator capacity until the 3rd trimester (99, 100). However, this vascular homeostasis can be disrupted by arterial hypertension. Women with gestational hypertension are reported to have higher systemic vascular resistance than normotensive pregnant women but lower than those with preeclampsia (74). Interestingly, pulse wave velocity in the 3rd trimester was not significantly higher in pregnant women with preeclampsia than in healthy pregnant women, suggesting minor effects of preeclampsia on arterial stiffness (101).

Interstingly, there seems to be an imprinting of these vascular changes since, pregnant women with a history of hypertensive diseases in previous gestation showed higher arterial stiffness and total vascular resistance in following pregnancies than women with previous normotensive gestation (81). Higher pulse wave velocities were also documented 5 years after delivery in women with a history of gestational diabetes, being this complication an independent predictor of increased arterial stiffness in the same follow-up (102). In addition, as gestation advances, the common carotid artery intima-media thickness decreases progressively until the 3rd trimester due to its passive enlargement, normalizing 9 months after delivery (99). Neutrophil to lymphocyte ratio, an inflammatory biomarker, correlated positively with carotid artery wall thickness in multivariable analysis in the 3rd trimester (36). Accordingly, pregnancy is chronic low-

grade inflammation state inherent to continuous adaptation of the maternal immune systems to protect the mother against the environment and to ensure foetus development (36).

Regarding endothelial function, the postischemic hyperemia-induced flow-mediated dilation augments during pregnancy due to increased vascular nitric oxide availability, returning to baseline values 9 months after delivery (99). In gestational diabetes, endothelial dysfunction persists even after hyperglycemia normalization, up to 8 weeks postpartum (103). Interestingly, 4-10 years after delivery, these women show similar vascular function (measured by pulse pressure, mean arterial pressure, large and small artery elasticity index as well as systemic vascular resistance) to age-matched controls despite the higher incidence of obesity, dyslipidaemia and abnormal glucose metabolism (104). Contrarily, women who have had preeclampsia maintain endothelial dysfunction 6 months to 4 years postpartum, which is ascribed to impaired nitric oxide bioavailability (92, 105).

Morris *et al.* recruited forty-five nulliparous women who underwent vascular evaluation in preconception moment and repeated an average of 14 months postpartum. Interestingly, even after this period, these women showed decreased arterial stiffness and reduced blood pressure compared to their pre-pregnancy assessment, suggesting a beneficial effect of pregnancy in cardiovascular remodelling (106).

I.V. Stretch-Induce Compliance (SIC) mechanism during pregnancy

Considering the progressive preload increment throughout gestation, we hypothesised that pregnant women would represent an interesting model to clarify the mechanism of stretch-induced compliance (SIC) in a physiologic hypertrophy context. We have previously shown that a sudden increase in volume overload (induced by 45º passive leg-elevation manoeuvre) triggers an acute myocardial stretch characterised by an immediate rise in contractility (the Frank-Starling mechanism), followed by a progressive increase known as the slow force response (5). The myocardial compliance also increases in response to SIC-mechanism due to cGMP-PKG pathway activation and titin phosphorylation (5). Importantly, we have shown that under pathologic conditions, such as chronically pressure-overloaded hearts with hypertrophic remodelling and ischemia, this mechanism is compromised (5).

Previous studies have already used passive leg elevation with various durations (from 90-180 seconds) in pregnant women to compare their hemodynamic response in different

trimesters of pregnancy (107) and to evaluate their preload reserve/fluid responsiveness (defined as the variation in LV stroke volume ≥15%) (41, 108). The transient volume overload caused by passive leg elevation should increase stroke volume according to Frank-Starling law in preload responsive participants (107). Interestingly, healthy pregnant women (beyond thirty-two weeks of gestation or with preeclampsia) were not able to significantly increase stroke volume (41, 107). These women displayed reduced cardiac contractility as gestation advances (107). This reduced contractility may limit the heart's capacity to increase heart rate and stroke volume in response to transient fluid challenge. Alternatively, leg elevation may also increase systemic vascular resistance, despite reduced blood pressure, causing a lower capacity to increase cardiac output in late pregnancy (107). Interestingly, passive leg elevation has been useful for predicting preload responsiveness and guiding fluid therapy (107); nevertheless, the usefulness of pregnant women to explore the acute response to SIC has never been detailedly characterized.

Chapter II. Aims of the Thesis

This chapter integrates parts of published protocol:

Ferreira AF, Azevedo MJ, Saraiva FA, Trindade F, Barros A, Leite S, Proença T, Sousa C, Machado AP, Leite-Moreira A, Sampaio-Maia B, Ramalho C, Falcão-Pires I. **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 Jun;42(6):585-596. English, Portuguese. doi: 10.1016/j.repc.2022.08.015. Epub 2023 Mar 21. PMID: 36958578.

Pregnancy induces cardiac remodelling that rapidly normalises in the postpartum. Postpartum cardiac recovery might provide important new insights into the process and extent of RR. Therefore, our goal is to evaluate cardiovascular functional and structural adaptations during pregnancy and meticulously characterise changes in the postpartum through innovative echocardiographic methods, urinary proteome analysis and plasma biomarkers assessment. Moreover, we aim to characterise these adaptations in women with and without cardiovascular risk (CVR) factors (chronic hypertension and/or gestational hypertension and/or type II diabetes mellitus or gestational diabetes and/or obesity) as well as explore 3rd trimester pregnancy response to acute myocardial overload/stretch and to clarify the underlying mechanisms (SIC mechanism). To achieve these endpoints, our specific goals are to:

1. Functionally and structurally characterise cardiovascular remodelling (during pregnancy) and RR (at the postpartum) and assess the impact of CVR factors (chronic hypertension and/or gestational hypertension and/or gestational diabetes and/or obesity) on these cardiac adaptations through innovative echocardiographic methods and quantification of plasma biomarkers (Results, subchapter i).

2. Profile the urinary proteome by liquid chromatography with tandem mass spectrometry in pregnant women with or without CVR factors (chronic hypertension and/or gestational hypertension and/or gestational diabetes and/or obesity) and identify putative proteins associated with cardiac postpartum RR (Results, subchapter ii).

3. Clarify the SIC mechanism in pregnancy with or without CVR factors (chronic hypertension and/or gestational hypertension and/or gestational diabetes and/or obesity) (Results, subchapter iii).

4. Characterise the right cardiovascular remodelling and RR induced by pregnancy and explore the impact of CVR factors (chronic hypertension and/or gestational hypertension and/or gestational diabetes and/or obesity and/or smoking) in these processes (Results, subchapter iv).

5. Identify the most appropriate indexing methodology to evaluate the cardiac remodelling and RR in pregnant women population (Results, subchapter v).

Chapter III. Methodological Considerations

This chapter integrates parts of published protocol:

Ferreira AF, Azevedo MJ, Saraiva FA, Trindade F, Barros A, Leite S, Proença T, Sousa C, Machado AP, Leite-Moreira A, Sampaio-Maia B, Ramalho C, Falcão-Pires I. **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 Jun;42(6):585-596. English, Portuguese. doi: 10.1016/j.repc.2022.08.015. Epub 2023 Mar 21. PMID: 36958578.

Considering the limitations of comparing pregnant women with HF patients (sex- and age-associated differences), we trust we can find valuable clues in pregnancy and post-partum remodelling, and so we hypothesise that:

1. Pregnant women represent a valuable model to study the impact of comorbidities and lifestyle in the process of cardiac and vascular remodelling (during pregnancy) and RR (during post-partum), considering that they represent a more uniform population compared to HF patients (fewer medication and less confounding factors).
2. Including subgroups of pregnant women with such comorbidities offers a platform to uncover potential biomarkers and important mechanisms underlying cardiac remodelling and RR that can be subsequently translated/validated in the context of HF.
3. Lastly, pregnant women represent a tool to study the SIC mechanism, which will likely be exacerbated considering the cardiac adaptations to blood volume during pregnancy and post-partum.

To investigate previous hypothesis, we developed the followed study protocol.

Study design and participants

This is a prospective cohort study. Adult pregnant women (≥18 years-old) were recruited during their first medical appointment (in the 1st or 3rd trimester of pregnancy) at the Obstetrics Departments of *Centro Hospitalar Universitário São João* (CHUSJ), and *Unidade Local de Saúde de Matosinhos- Hospital Pedro Hispano* (ULSM-HPH). or may register themselves through online forms available on https://perimyrobb.wordpress.com/. Exclusion criteria were twin pregnancy, pre-existing cardiomyopathy, renal disease, chronic obstructive airway disease, active systemic infection, genetic syndromes or type-1 DM.

Subgroups

The study groups were as follows:

1) Healthy pregnant women (without cardiovascular risk factors);
2) Hypertensive pregnant women (if systolic blood pressure (SBP) ≥140mmHg and/or diastolic blood pressure (DBP) ≥90mmHg measured in office or in-hospital, which precedes pregnancy or developed before 20 weeks of gestation) or gestational hypertensive (if SBP ≥140mmHg and/or DBP ≥90mmHg measured in office or in-hospital, which develops after 20 weeks of gestation and usually resolves within 42 days postpartum);

3) Gestational diabetic women (if 92≤fasting-glucose≥126mg/dL at 1st trimester or fasting glucose ≥92 mg/dL or ≥180mg/dL or ≥153mg/dL 1 or 2 hours after an oral glucose tolerance test (75g oral glucose load) performed at 24-28 pregnancy weeks;
4) Obese pregnant women (if body mass index ≥30kg/m^2 before pregnancy).

Procedures

A qualified and trained multidisciplinary clinical research team conducted all study procedures. Participants underwent echocardiographic evaluation, pulse wave velocity, EndoPat®, blood and urine sample collection at the Department of Surgery and Physiology of the Faculty of Medicine of the University of Porto, during the following time-points (Table 1):

1. First trimester of pregnancy – 1T (11-14 weeks), before cardiac remodelling (baseline conditions).
2. Third trimester – 3T (30-35 weeks), at peak of cardiac remodelling when cardiovascular adaptations are expected to be most prominent.
3. Post parturition (PP1, 6-8 weeks; PP2, 6-7 months; and PP3, 1 year after delivery) – during RR to assess cardiovascular recovery.

Table 2: Planned follow-up for all participants

	1st Trimester	3rd Trimester	6-8 Weeks after Delivery	6-7 Months after Delivery	1 Year after Delivery
Informed Consent	✓	✓			
Blood Sample Collection	✓	✓	✓	✓	✓
Urine Sample Collection	✓	✓	✓	✓	✓
Clinical Characterisation through Questionnaires	✓	✓	✓	✓	✓
Echocardiography Evaluation	✓	✓	✓	✓	✓
Arterial Stiffness Assessment (Pulse Wave Velocity)	✓	✓	✓	✓	✓
Endothelial Function Assessment (EndoPAT)	✓	✓	✓	✓	✓
Lifestyle Characterisation		✓		✓	
Maternal Chronic Stress Quantification		✓		✓	
Stretch-induced Compliance Mechanism		✓		✓	

Echocardiographic assessment of cardiac function and structure during RR in baseline and response to the SIC mechanism

Conventional transthoracic echocardiography evaluation with a 3 MHz phased-array probe (ACUSON SC2000 PRIME^{TM}) was performed by a single operator and measurements were obtained from standard views according to European Society of Cardiology recommendations for chamber quantification and diastolic function evaluation (109, 110). At least three cardiac cycle images were acquired for data analysis. Two certified cardiologists independently analysed, interpreted and harmonised the results. Appendix 1 shows the detailed protocol of transthoracic echocardiogram image acquisition and measurements.

Myocardial deformation will be assessed in LA and LV through strain and strain rate analysis by Syngo Velocity Vector Imaging software, version 3.5 (Siemens Healthcare, Erlangen, Germany). The images were stored in digital imaging and communications in medicine format at 30 frames/second and analysed offline posteriorly. The endocardium was tracked manually using a point-and-click approach while the system automatically traces the epicardium and generates six segments. The tracing was readjusted manually to increase tracking accuracy, and strain curves for each segment was generated. Longitudinal strain was calculated using apical two-, three-, and four-chamber views and averaged. Radial and circumferential strain were be assessed using the parasternal short-axis view at the papillary muscle level. Left atrial strain and strain rate curves were measured from a standard non-foreshortened apical 4-chamber view, whose border was outlined manually. Regional peak strain and time to peak strain were averaged to obtain global peak strain, strain rate, and time to peak strain. Time to peak strain and strain rate in systole and diastole were recorded for each strain measurement and then adjusted for heart rate.

Lastly, a specific echocardiographic protocol was performed during 1T and 3T visits to evaluate the response to an acute myocardial stretch. This protocol consists of performing a brief echocardiography exam in left lateral decubitus at baseline (T0), immediately after leg elevation to 45° (T1, passive leg raising) and after 15 minutes in this position (T2). This manoeuvre increases cardiac venous return, triggering myocardial stretch and the SIC mechanism (111). Blood samples were collected in T0 and T2.

Vascular stiffness evaluation

Brachial blood pressure was measured in the non-dominant arm after 10 minutes of resting. Complior® (Alam Medical, France) device was used to quantify the arterial stiffness by carotid-femoral pulse wave velocity (PWV), calculated from carotid-femoral distance/transit time, and peripheral augmentation index, defined as the ratio of late systolic pressure to early

systolic pressure. In this context, the participant lies supine on the exam bed and relaxed to stabilise her heart rate and blood pressure. One sensor was placed at the carotid artery level and another at the femoral artery level until the software stabilises. Each participant underwent at least two PWV measurements (whose difference between them should be ≤0.5m/s) in each session. The Complior Analyse software displays the pulse wave velocity and the central pressure waveform analysis.

Determination of potential plasma biomarkers

We assessed plasma levels of circulating TMAO and relaxin-2, markers of cardiac injury, markers of cardiac repair, markers of neurohormonal activity, markers of endothelial function and markers of fibrosis and extracellular matrix turnover (Table 2) using ELISA kits following manufacturer instructions. Exceptionally, C-Reactive Protein (marker of inflammation) was quantified by immunoturbidimetry in the analyser Beckman Coulter Olympus AU5800 and high-sensitivity Troponin I and BNP (markers of cardiac injury) by chemiluminescence immunoassay in the analyser Abbott Architect i2000SR.

Urinary proteomic profile

Fifty milliliters of first-morning urine samples were collected, centrifuged (2370g, 15 minutes, 4°C), and stored at -80ºC in each visit (1T, 3T, PP1, PP2 and PP3). Urine protein was concentrated using centrifugal concentrators (cutoff-10kDa) and quantified by a standard bicinchoninic acid assay.

Proteome was analysed following a label-free shotgun approach to disclose the urinary protein profile changes induced by each cardiovascular risk factor. Briefly, for each sample, 100µg of protein was reduced by incubation with dithiothreitol and alkylated in the dark. The sample was diluted with ammonium bicarbonate and the proteins trypsin-digested overnight (16h, 37°C). After sample clean-up (C18-resins), peptides were analysed using high-performance liquid chromatography (LC) coupled to a high-resolution mass spectrometer operating in tandem (MS/MS).

Raw data was processed, and quantitative label-free analysis of the LC-MS/MS data was performed with MaxQuant or similar algorithms. The data was searched against a database containing all proteins corresponding to humans in the Swissprot database, plus a list of common contaminants and all the corresponding decoy entries. No more than three missed cleavages were allowed for trypsin. Peptides and proteins were filtered out using a 1% false discovery rate.

Ethical Considerations

All eligible participants were able and willing to provide written informed consent to be included in this study. The Ethics Committees of CHUSJ and ULSM-HPH approved the study (ID 201/18 and ID 154/20/RS, respectively). The confidentiality and data anonymity comply with the Declaration of Helsinki of 1964, revised in Fortaleza, in 2013. Participant's recruitment began in February 2019.

Data collection and storage

The clinical characterisation of the study sample included maternal cardiovascular health, obstetric and perinatal outcomes, maternal health-related habits, maternal smoking habits, drinking and medical history. In addition, obstetric (gestational hypertension, gestational diabetes, type of delivery, gestational age at delivery) and perinatal (foetal death, birth weight, Apgar score) outcomes were also registered. These data were collected through the application of questionnaires and collected from medical records whenever necessary.

Clinical parameters, echocardiographic and plasma determinations and pulse wave velocity values were gathered on a database in IBM-SPSS-Statistics, version 25.0 and subsequently analysed using R programming language**.**

All data management was done under professional confidentiality and using pseudonymisation. Only PERIMYR team members had access to the data. An exclusively dedicated server was used to store study information and its access was restricted to the study team. Data collection and management comply with General Data Protection Regulation (GDPR) 2016/679 27th April 2016 and 58/2019 Portuguese Law. Biological samples will be kept throughout the project and destroyed 5 years after its conclusion.

Table 1: Primary and secondary endpoints

Primary endpoint
• LV mass (6-8 weeks, 6-7 months and 1 year after delivery) - RR
Secondary endpoints
• Echocardiographic characterisation of cardiac remodelling and RR: o LA volume, LV end-systolic and end-diastolic volumes; o Septum and posterior wall thickness; o Global longitudinal and circumferential strain; o LA longitudinal strain; o 3D LV volumes and ejection fraction quantification; o Systolic function: ejection fraction (Simpson Biplane); o Diastolic function: E and A waves, E/A ratio, E wave deceleration time, e' medial, e' lateral, E/e' ratio, isovolumetric relaxation time; o Right atrium volume; o Right ventricle assessment: right ventricular index of myocardial performance, fractional area change, tricuspid annular plane systolic excursion, systolic pulmonary artery pressure, pulsed doppler peak velocity at the annulus.
• Vascular stiffness assessment: o Pulse wave velocity
• Biomarkers of cardiac injury: o NT-proBNP; o BNP; o TnI.
• Biomarkers of cardiac repair: o ST2/IL33 receptor
• Biomarkers of inflammation: o C-reactive protein
• Biomarkers of neurohormonal activity: o Aldosterone; o Renin.
• Biomarkers of endothelial function: o Plasminogen-activator inhibitor type 1; o Homocysteine; o Relaxin-2.
• Biomarkers of fibrosis and extracellular matrix turnover: o Tissue-inhibitor-of-matrix-metalloproteinases-1; o Procollagen I C terminal propeptide; o Lysyl-oxidase; o MMPs expression.

- Urinary proteomic profiles using liquid chromatography with tandem mass spectrometry.

Statistical analysis

Sample Size Estimation

For sample size estimation, we considered the pre-specified analysis of LV mass index regression at 6-8 weeks after delivery. Thus, a sample size of 25 patients per group was estimated to allow the detection of a difference in means of 9.8g/m^2 (power of 90% and α=0.05), assuming a standard deviation of 12.3g/m^2 in each group and a conservative 3rd trimester/7 postpartum weeks measurement correlation of 0.2 to account for the intrasubject biological variation. Considering a potential drop-out rate of 20%, the final estimated sample size needed in each allocation group is 30 participants.

Characterization and descriptive analysis of the samples

Continuous variables will be expressed by means and standard deviation or by median, minimum and maximum as adequate. Data normality was checked after examining histograms and Q-Q plots. Absolute values and relative frequencies were presented for categorical variables.

One-way ANOVA or Kruskal-Wallis test was performed to evaluate differences among study groups for continuous variables and chi-square analysis will be used for categorical variables.

Inference analysis

Generalised linear mixed-effects models (GLMM) were used to explore the longitudinal nature of the data and consequently the cardiovascular reverse remodelling induced by pregnancy. The time-point variable 3T (peak of cardiovascular remodelling in the present study design) was used as a reference.

Missing Data

Missing data happened when participants fail the cardiovascular appointment without a rescheduling possibility, as occurred during COVID-19 pandemic (Appendix 2). The maternal lifestyle and clinical characterization were alternatively registered by phone call or e-mail. However, quantitative variables of pulse wave velocity, EndoPAT^{TM} and echocardiographic assessment cannot be measured without the participant's presence. In this case, we focused the

data analysis exclusively on the 3rd trimester and 6-7 months or applied the generalised linear mixed-effects models.

Appendix 1. Transthoracic Echocardiogram protocol

Patient positioning:

1. Left lateral position (PLAX; PSAX; Apical A4C, A5C, A2C, A3C)
2. Supine position (suprasternal, subcostal)

General recommendations:

1. Attention to the quality of the ECG;
2. Perform measurements in at least 3 cycles;
3. Attention to the quality and framing of the images - adjust gains, depth, focus, scale and baseline;
4. Doppler:

The Nyquist speed between 50-70 cm / s, (ideally, 64 cm/s);

B. Sample volume size: 5mm;

5. Frame rate: 50-80 IPS;
6. Sweep speed: 100 mm/s;
7. Tissue Doppler scale: 20 cm/s.

Check-list	
Parasternal Long Axis	
Acquisitions	**Recomendations**
2D	Measurements: IVS, PWT, LVEDD, LVESD, LAD, sinuses of valsalva diameter, sinotubular junction diameter
2D - focused on Ascending Ao	Measure the diameter of the ascending Ao
Color Doppler focused on Aortic valve	Evaluate coaptation and flow (type, direction)
Color Doppler focused on Mitral valve	Evaluate coaptation and flow (type, direction)
M Mode on Aortic Valve	Evaluate coaptation
M Mode on Mitral Valve	Evaluate coaptation
2D - focused on Aortic valve	Measurements: aortic root
Parasternal Short Axis	
Acquisitions	**Recommendations**
2D - Short axis of large vessels	Evaluate Aortic valve morphology
Color Doppler in the aortic valve	If aortic regurgitation: evaluate mechanism
Color Doppler in the tricuspid valve	Evaluate flow (type, direction)
CW Tricuspid valve	If tricuspid regurgitation: measure gradients
Color Doppler in the pulmonary valve	Evaluate flow (type, direction)
PW Pulmonary valve	Quantification of pulmonary acceleration time and ejection time
2D - Mitral Valve	Evaluate mitral valve morphology
Color Doppler Mitral Valve	Evaluate the coaptation of leaflets If mitral regurgitation: evaluate mechanism
2D - Papillary Muscles	Assess function and contractility Evaluate the segment (s) involved
2D - LV apex	Assess function and contractility Evaluate the segment (s) involved
Apical 4 Chambers View	
2D	Assess function and contractility, calculate EF (Simpson Method) Measure: LVEDV, LVESV, LA and RA volumes

LV apex plane	If poor function: assess the presence of thrombi
Color Doppler Mitral Valve	Evaluate coaptation and flow (type, direction)
PW Mitral valve	Measure E and A waves, E / A ratio, deceleration time, isovolumic relaxation time
CW Mitral valve	If regurgitation: calculate VTI If stenosis: calculate THP and measure gradients
Color Doppler Tricuspid valve	Evaluate flow (type, direction)
CW Tricuspid valve	If tricuspid regurgitation: measure gradients
Tissue doppler (PW) in the lateral mitral annulus	Measure lateral E' peak velocity
Tissue doppler (PW) in the medial mitral annulus	Measure medial E' peak velocity
Tissue doppler (PW) in the tricuspid ring	Measure E', A' and S' peak velocity;
M mode on the lateral tricuspid ring	TAPSE
2D focused on right ventricle	Measure: end-diastolic area and end-systolic area for FAC calculation; right ventricular basal and mid-cavity diameters and longitudinal diameter PW Tricuspid valve: Measure E and A waves, E / A ratio, deceleration time, isovolumic relaxation time
Apical 5 Chambers View	
Doppler (color) LVOT	Evaluate coaptation and flow (type, direction)
CW AoV	Measure gradients
PW in LVOT	If stenosis: calculate VTI
Apical 2 Chambers View	
2D	Assess function and contractility, calculate EF (Biplane Simpson Method) Measure: LVEDV, LVESV, LA volume
Doppler (color) Mitral valve	Evaluate coaptation and flow (type, direction)
Apical 3 Chambers View	
2D	Assess function and contractility
Doppler (color) in LVOT and Ao	Evaluate coaptation and flow (type, direction)
Doppler (color) in Mitral Valve	
Suprasternal	
2D	Evaluate Ascending Ao morphology (calculate aortic arch and descending aorta diameter)
Color Doppler in the Ao and Ao descending arc	Evaluate flow (type, direction) Measure velocity
Pulsed doppler in descending Ao	
Subcostal	
2D of right ventricular wall	Measure end-diastolic right ventricular wall thickness
Doppler (color) IA septum	Measurements: DV Assess the presence of shunts / PFO
IVC, inferior vena cava	Measure diameter Assess respiratory variability (<50% /> 50%)

Legend: IVSd - septal interventricular septum in diastole; PWd - posterior wall thickness in diastole; LVEDd - left ventricular internal diameter in diastole; LVESd - left ventricular internal diameter in systole; LA – left atrium; RA – Right atrium; LVEDV - left ventricular end-diastolic volume; LVESV - left ventricular end-systolic volume; EF – ejection fraction; VTI - velocity time integral; FAC - fractional area change; PFO - Patent Foramen Ovale

LV mass will be estimated using the following equation 0.8×(1.04×[(LVEDd+PWd+IVSd)^3–(LVEDd)^3]+0.6). In addition, the relative wall thickness will be calculated using the equation: 2×PWd/LVEDd. The biplane Simpson method will be used to estimate LV end-diastolic and end-systolic volumes, stroke volume and ejection fraction (calculated through [LVIDd]^3–[LVIDs]^3/[LVIDd]^3×100%). LV cardiac output(CO) will be calculated by $LV\ Stroke\ Volume \times Heart\ Rate$. Systemic vascular resistance will be determined using the equation: $80 \times {\text{mean arterial pressure}}/{\text{LV CO}}$ (dyn.s.cm^{-5}), and mean arterial pressure will be calculated by the formula: $SBP + {(2 \times DBP)}/{3}$. All cardiac volumes and LV mass will be indexed to the body surface area of the participants ($0.007184 \times height(cm)^{0.725} \times weight(kg)^{0.425}$). The right ventricular index of myocardial performance will also be calculated by the isovolumic time ratio divided by ejection time.

Appendix 2. COVID-19 Pandemic

The COVID-19 pandemic interfered in the progression of some PERIMYR tasks, namely the recruitment process and the cardiovascular evaluations scheduling. Following the Portuguese Government recommendations for the pandemic period, an addendum was added to the protocol of PERIMYR to cope with the updated national recommendations from the national health system, such as : 1) transfer the clinical evaluation room to a research building outside the hospitalar environment and thereby minimizing the risk of exposure of the participants to COVID-19 infection; 2) disinfect the room and all equipment and devices between participants; 3) use of personal protective equipment by the PERIMYR team and participants; 4) regular COVID-19 testing of the PERIMYR team members, and 5) suppress the leg elevation manoeuvre to assess SIC mechanism to shorten the duration of each evaluation. The reformulated PERIMYR protocol was approved by Ethics Committee of Centro Hospitalar Universitário São João and University of Porto Task-Force in May 2020.

Chapter IV: Results

The results will be presented in the form of published, submitted and in preparation articles. These articles have been organized into five subchapters:

1. **Cardiovascular risk factors during pregnancy impact the postpartum cardiac and vascular reverse remodeling**.

   Ferreira AF, Azevedo MJ, Morais J, Trindade F, Saraiva FA, Diaz SO, Alves I, Fragão-Marques M, Sousa C, Machado AP, Leite-Moreira A, Sampaio-Maia B, Ramalho C, Barros AS, Falcão-Pires I.

   Published in American Journal of Physiology-Heart and Circulatory Physiology

2. **The extent of postpartum cardiac reverse remodeling is reflected in urine proteome.**

   Ferreira AF, Trindade F, Azevedo MJ, Morais J, Douché T, Diaz SO, Saraiva FA, Sousa C, Machado AP, Matondo M, Leite-Moreira A, Ramalho C, Vitorino R, Falcão-Pires I, Barros AS.

   Under second review at Journal of Proteome Research

3. **Stretch-Induced Compliance mechanism in pregnancy-induced cardiac hypertrophy and the impact of cardiovascular risk factors.**

   Ferreira AF, Azevedo MJ, Morais J, Almeida-Coelho J, Leite-Moreira A, Lourenço AP, Saraiva FA, Diaz SO, Amador AF, Sousa C, Machado AP, Sampaio-Maia B, Ramalho C, Leite-Moreira A, Barros AS, Falcão-Pires I.

   Submitted to Acta Physiologica

4. **Cardiovascular remodelling and reverse remodelling during pregnancy and postpartum: looking at the right side of the heart**.

   Ferreira AF*,Araújo J*, Azevedo MJ, Saraiva FA, Diaz SO, Sousa C, Machado AP, Sampaio-Maia B, Ramalho C, Leite-Moreira A, Barros AS, Santos M, Falcão-Pires I. (* both authors contributed equally to this work).

   Ready to submit

5. **The Impact of Echocardiographic Indexation to Evaluate Cardiac Reverse Remodelling throughout Pregnancy and Postpartum.**

   Ferreira AF, Saraiva FA, Diaz SO, Azevedo MJ, Sousa C, Leite-Moreira A, Sampaio-Maia B, Ramalho C, Barros AS, Falcão-Pires I.

   Published in Revista Portuguesa de Cardiologia

Subchapter i. Cardiovascular risk factors during pregnancy impact the postpartum cardiac and vascular reverse remodeling

Based on: Ferreira AF, Azevedo MJ, Morais J, Trindade F, Saraiva F, Diaz SO, Alves IN, Fragão-Marques M, Sousa C, Machado AP, Leite-Moreira A, Sampaio-Maia B, Ramalho C, Barros AS, Falcão-Marques I. Cardiovascular risk factors during pregnancy impact the postpartum cardiac and vascular reverse remodeling. Am J Physiol Heart Circ Physiol. 2023 Oct 1;325(4):H774-H789. doi: 10.1152/ajpheart.00200.2023. Epub 2023 Jul 21. PMID: 37477690.

Title: Cardiovascular risk factors during pregnancy impact the postpartum cardiac and vascular reverse remodeling

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Abstract

Pregnant women with cardiovascular risk (CVR) factors are highly prone to develop cardiovascular disease later in life. Thus, recent guidelines suggest extending the follow-up period to 1-year after delivery. We aimed to evaluate cardiovascular remodeling during pregnancy and determine which CVR factors and potential biomarkers predict postpartum cardiac and vascular reverse remodeling (RR).

Our study included a prospective cohort of 76 healthy and 54 obese and/or hypertensive and/or with gestational diabetes pregnant women who underwent transthoracic echocardiography, pulse-wave velocity (PWV), and blood collection at the 1st (1T) and 3rd trimesters (3T) of pregnancy as well as at the 1st/6th/12th month after delivery. Generalized linear mixed-effects models was used to evaluate the extent of RR and its potential predictors.

Pregnant women develop cardiac hypertrophy, as confirmed by a significant increase in left ventricular mass (LVM). Moreover, ventricular filling pressure (E/e') and atrial volume increased significantly during gestation. Significant regression of left ventricular (LV) volume, LVM, and filling pressures was observed as soon as 1 month postpartum. The LV global longitudinal strain worsened slightly and recovered at 6 months postpartum. PWV decreased significantly from 1T to 3T and normalized 1 month postpartum.

We found that arterial hypertension, smoking habits, and obesity were independent predictors of increased LVM during pregnancy and postpartum. High C-reactive protein and low ST2/IL33-receptor levels are potential circulatory biomarkers of worse LVM regression. Arterial hypertension, age, and gestational diabetes positively correlated with PWV. Altogether, our findings pinpoint arterial hypertension as a critical risk factor for worse RR, CRP and ST2/IL33-receptor as potential biomarkers of postpartum hypertrophy reversal.

Words number of Abstract: 250 words

Keywords

Pregnancy

Postpartum

Cardiovascular Remodeling

Reverse Remodeling

Cardiovascular risk factors

Introduction

Pregnancy is a physiological condition characterized by volume overload that leads to adaptations in the circulatory system. Sympathetic and hormonal stimulation during pregnancy leads to increased preload due to blood volume expansion and reduced afterload due to decreased peripheral vascular stiffness (8, 20, 37). These physiological cardiovascular hemodynamic adaptations during pregnancy have been largely described (8), including left ventricle (LV) eccentric hypertrophy with preserved ejection fraction (20, 40) and impaired diastolic function with left atrial (LA) enlargement (20, 37, 40). Decreased arterial stiffness and vascular thickness have been ascribed to increased NO availability and vascular reactivity (99). Previous literature has focused mostly on peripartum cardiomyopathy and pre-eclampsia contexts (112, 113), while those addressing the postpartum period are scarce. Some authors reported that in the absence of pregnancy cardiovascular complications, the heart fully recovers during the postpartum period, and global and segmental myocardial performance normalizes to its pre-gravid structure and function in a process known as pregnancy-induced reverse remodeling (RR) (8, 37, 40). Cardiovascular risk factors, such as arterial hypertension, diabetes *mellitus* (DM), and overweight increase the risk of future cardiovascular disease and death (114-116). Women who develop hypertensive disease during their first pregnancy present an increased long-term risk for chronic arterial hypertension, hypercholesterolemia, type 2 DM, myocardial infarction, heart failure (HF), and stroke (4). Therefore, the 2021 recommendations of the American Heart Association underscored the importance of extending the follow-up to one year after delivery for women with pregnant hypertensive diseases to prevent the development of future cardiovascular diseases (4). Following this recommendation, we developed a prospective cohort study to recruit pregnant women with or without cardiovascular risk factors from two tertiary centers. We subjected the participants to 5 cardiovascular evaluations: at the 1st and 3rd trimesters of pregnancy (cardiovascular remodeling) and at the 1st, 6th and 12th months postpartum (cardiovascular RR). We aimed to evaluate cardiovascular remodeling during pregnancy and identify risk factors and plasma biomarkers that might predict cardiac and vascular RR after delivery. This was assessed by modelling the decrease in left ventricular mass (LVM) and the increase in pulse wave velocity (PWV) using a Generalised Linear Mixed-effects Model (GLMM).

Methods and Design

Study design and setting

This prospective cohort study was conducted at the Obstetrics Departments of *Centro Hospitalar Universitário São João* (CHUSJ) and *Unidade Local de Saúde de Matosinhos Hospital Pedro Hispano* (ULSM-HPH). The Ethics Committees of CHUSJ and ULSM-HPH approved this study (ID 201/18 and 154/20/RS, respectively). All participants were willing to provide written informed consent. The confidentiality and data anonymity complied with the Declaration of Helsinki of 1964, revised in Fortaleza, in 2013. The complete protocol of the present study has also been published (117).

Participants

Participants were recruited from February 2019 to July 2022, at their first medical appointment (in the 1st or 3rd trimester of pregnancy) or voluntarily through online forms available at https://perimyrobb.wordpress.com/ and followed up until 1 year after delivery.

Inclusion criteria were adult pregnant women (>18 years old) with or without cardiovascular risk factors, namely chronic and/or gestational hypertension, gestational diabetes mellitus (DM), and/or obesity. Arterial hypertension was defined as a systolic blood pressure[SBP] ≥ 140 mmHg and/or diastolic blood pressure [DBP] ≥ 90 mmHg measured in the office or in-hospital before 20 weeks of gestation (118). Gestational hypertension was defined as arterial hypertension diagnosed after 20 weeks of gestation that resolved within 42 days postpartum. Gestational DM was considered if fasting glucose was [92; 126] mg/dL at 1st 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 pregnancy weeks. Obesity was defined as a body mass index (BMI) ≥ 30 kg/m^2 before pregnancy. The participants were included irrespective of parity or gravidity.

Women with twin pregnancies, pre-existing cardiomyopathy, renal disease, chronic obstructive airway disease, active systemic infection, genetic syndromes or type-1 or type-2 DM were excluded. If pregnancy loss occurred throughout our follow-up period, the participant was also excluded from the present study.

Measurements

Included participants underwent the following evaluations: 1) clinical characterization, 2) transthoracic echocardiogram, 3) PWV 4) blood sample collection at the Department of Surgery and Physiology of the Faculty of Medicine of the University of Porto, during the following time-points: 1) 1st trimester of pregnancy – 1T [11-14 weeks], baseline conditions, before cardiac remodeling; 2) 3rd trimester – 3T [30-35 weeks], at the peak of cardiac remodeling when

cardiovascular adaptations should be most noticeable; 3) Postpartum (PP1, 6-8 weeks; PP2, 6-7 months; and PP3, 1 year after delivery) – during RR to assess cardiovascular recovery (Figure 1). In our study design, each pregnant woman was her own control compared to 1-year postpartum. Due to restrictions imposed by the COVID-19 pandemic, 8 participants skipped the 3T evaluation and 20 missed the PP1 evaluation.

Clinical characteristics included maternal cardiovascular health, maternal health-related habits, maternal smoking habits, parity, medical history and demographics, and obstetric and perinatal outcomes. Data were collected using questionnaires and electronic medical records. LVM and PWV were defined as primary outcomes for assessing cardiac and vascular remodeling and RR, respectively.

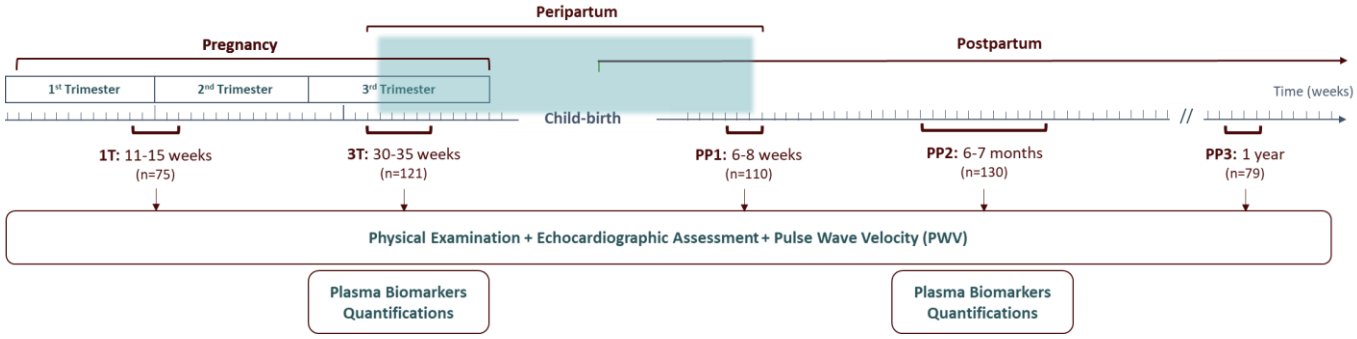

Figure 1: Project Scheme. Aiming to characterize the cardiovascular (reverse) remodeling induced by pregnancy, and the potential impact of cardiovascular risk factors, we enrolled 130 pregnant women. The participants were subjected to five cardiovascular assessments during the following time-points: 1) 1st trimester of pregnancy – 1T [11-14 weeks], before cardiovascular remodeling (n=75 subjects); 2) 3rd trimester – 3T [30-35 weeks], at the peak of cardiovascular remodeling when cardiovascular adaptations should be most noticeable (n=121 subjects); 3) Postpartum - PP1 [6-8 weeks] (n=110 subjects); PP2 [6-7 months] (n=130 subjects); and PP3 [1 year after delivery] (n=79 subjects) – during RR to assess cardiovascular recovery. In each visit, the participants performed a physical examination, transthoracic echocardiography and measurement of pulse wave velocity. Additionally, plasma biomarkers quantification was performed in 3T and PP2.

Echocardiographic assessment

Conventional transthoracic echocardiography evaluation with a 3 MHz phased-array probe (ACUSON SC2000 PRIME™) was performed by a single operator, and measurements were obtained from standard views according to the European Society of Cardiology recommendations for chamber quantification and diastolic function evaluation (109, 110). At least three cardiac cycle images were acquired for data analysis. Two certified cardiologists independently analyzed, interpreted, and harmonized the results. LV mass was estimated using the following equation 0.8 × (1.04 × [(LV end-diastolic diameter + LV posterior wall thickness in diastole + Interventricular septum thickness in diastole)^3 – (LV end-diastolic diameter)^3] + 0.6)g. The relative wall thickness was calculated using the following equation:2× LV posterior wall thickness in diastole / LV end-diastolic diameter. The biplane Simpson method was used to estimate LV end-diastolic and end-systolic volumes, stroke volume, and ejection fraction

(calculated using [LV internal end-diastolic dimension]^3−[LV internal end-systolic dimension]^3/[LV internal end-diastolic dimension]^3×100%). LV cardiac output (CO) was calculated as the product of the LV stroke volume and heart rate (L/min). Systemic vascular resistance was determined using the following equation:80 × mean arterial pressure/LV cardiac output (dyn.s.cm-5) and mean arterial pressure using the following formula: systolic blood pressure + (2 × diastolic blood pressure)/3. In addition, E/e' was based on the ratio between *E mitral inflow velocity to the average early diastolic septal velocity with lateral mitral annular velocity.* Myocardial deformation was assessed in the LV through strain and strain rate analysis using the Syngo Velocity Vector Imaging software, version 3.5 (Siemens Healthcare, Erlangen, Germany). The endocardium was tracked manually using a point-and-click approach, whereas the system automatically traced the epicardium and generated six segments. The tracing was manually readjusted to increase the tracking accuracy, and the strain curves for each segment were generated. The longitudinal strain was calculated using an apical four-chamber view.

Vascular stiffness evaluation

Brachial blood pressure was measured after 10 min of resting.

The Complior® device (Alam Medical, France) was used to quantify arterial stiffness by carotid-femoral pulse wave velocity, calculated from the carotid-femoral distance/transit time, and peripheral augmentation index, defined as the ratio of late systolic pressure to early systolic pressure. Each participant had at least two PWV measurements (the difference between them was ≤0.5 m/s) in each session, and their mean was calculated and used for further analysis.

Determination of potential plasma biomarkers

Blood samples were centrifuged for 15 min at 5000 rpm at 4°C. Plasma was aliquoted and stored at −80°C until analysis. We assessed cardiac biomarkers currently considered the gold-standard tools for diagnosing HF and myocardial ischemia, such as brain natriuretic peptide (BNP) and high-sensitivity cardiac troponin I (TnI). Other markers associated with extracellular matrix remodeling, such as procollagen I C terminal propeptide (PICP, E-EL-H6030, Elabscience), lysyl-oxidase (E-EL-H0174, Elabscience), and activin A (DY338, R&D Systems), a member of the transforming growth factor-ß family that is associated with cardiac remodeling and fibrosis, were also quantified. More recent potential biomarkers of myocardial repair have also been assessed, such as the ST2/IL33-receptor (DY523B-05, R&D Systems). Lectin-like oxidised low-density lipoprotein receptor 1 (DY1798, R&D Systems), plasminogen activator inhibitor type 1 (PAI-1, DY1786, R&D Systems) and vascular endothelial growth factor (VEGF, DY293B-05, R&D Systems) were selected for vascular and endothelial characterization. C- Reactive Protein (CRP) is a

valuable biomarker for monitoring the course of inflammatory diseases and is potentially useful for discriminating the impact of risk factors. In addition, we quantified relaxin-2 (DY2804-05, R&D Systems), a hormone known to promote angiogenesis and inhibit matrix metalloproteinases production and activity. Fibroblast growth factor 21 (FGF21, DY2539, R&D Systems), an endocrine hormone associated with glucose regulation and lipid metabolism, was quantified.

Three operators, blinded to the clinical and echocardiographic information, assessed most of these assays on each plasma sample using commercially available kits following the manufacturer's instructions. C-Reactive Protein was quantified by immunoturbidimetry in the analyser Beckman Coulter Olympus AU5800 and high-sensitivity Troponin I and BNP by chemiluminescence immunoassay in the analyser Abbott Architect i2000SR.

Statistical analysis

Clinical parameters, echocardiographic parameters, pulse wave velocity, and biochemical plasma measurements were collected in a database and analyzed using R statistical software version 4.2.1 (namely, lme4, gtsummary, and ggplot2 packages) (119)**.**

For descriptive statistics, continuous variables were expressed by means and standard deviation or by median, 1st and 3rd quartiles (Q1, Q3) as adequate. Data normality was assessed by inspecting histograms and Q-Q plots (not shown). Categorical variables were expressed as absolute values and relative frequencies.

Some plasma biomarkers (TnI, PICP, ST2/IL33 receptor, VEGF, and relaxin-2) were log-transformed owing to data skewness.

One-way ANOVA with random intercepts was applied to assess the plasma biomarker variation between 3T and PP2 moments.

Generalized linear mixed-effects models (GLMM) were used to explore pregnancy-induced cardiovascular reverse remodeling, namely hypertrophy reversal and PWV normalization. Univariate GLMM was used to evaluate the variation in parameters from physical, vascular, and echocardiographic assessments throughout the follow-up period. The time-point variable 3T (peak of cardiovascular remodeling in the present study design) was used as a reference. Two multivariate GLMM were built: Model 1) LVM progression during pregnancy and postpartum, adjusted for arterial hypertension (comprising chronic and gestational hypertension), gestational diabetes, obesity, maternal age at recruitment, smoking habits (excluding only non-smokers), parity, weight gain until the 3rd trimester, and each follow-up time (1T, PP1, PP2, and PP3); and Model 2) arterial stiffness modulation analysis during pregnancy and after delivery, adjusted for arterial hypertension (comprising chronic and gestational hypertension), gestational diabetes, obesity, maternal age at recruitment, smoking habits (excluding only the non-smokers), parity,

weight gain until 3rd trimester, the equivalent of mean arterial pressure in 1st trimester and each follow-up time (1T, PP1, PP2, and PP3). Then, on top of these clinical models, inflammatory plasma biomarkers and relaxin-2 combined with PAI-1 were added to assess their impact on LVM and arterial stiffness predictive models, respectively. A minimum significance level of 5% was considered to be statistically significant.

Results

Baseline demographic and clinical characterisation

We included 130 participants with a median age of 33 [21; 44] years, and 60.8% were nulliparous. The demographic and clinical characteristics of patients are shown in Table 1. Fifty-four (41.5%) women were diagnosed with at least one cardiovascular risk factor prior to or during gestation (7.7%, chronic arterial hypertension; 3.1%, gestational hypertension; 10.8%, gestational diabetes; and 8.5%, obesity), as shown in Table 1. Isolated insulin and metformin administration was reported in three and four participants, respectively, and their concomitant intake was performed by six pregnant women to manage gestational diabetes. The remaining women had controlled gestational diabetes through their diet. Only four women with chronic arterial hypertension were prescribed beta-blockers; among these, two also took nifedipine during pregnancy. Nifedipine was also indicated in another two hypertensive pregnant women. After delivery, 11 participants were diagnosed with arterial hypertension before pregnancy, and one woman with gestational hypertension took beta-blockers and/or diuretics and/or angiotensin-converting enzyme inhibitors and/or calcium antagonists. Eight (6.2%) participants were current smokers, 25 (16.9%) were former smokers, 9 (6.9%) had quit smoking before pregnancy, and 5 (3.8%) had quit smoking before pregnancy. Pregnant women increased their median weight by 10 (7, 12) kg until 3rd trimester and continuously reduced their weight until one year postpartum (Table 2). Two participants developed pre-eclampsia (one pregnant woman with chronic hypertension and another without any associated cardiovascular risk factor), and four developed cholestasis of pregnancy. Four women experienced other peripartum complications (arrhythmia and uncontrolled arterial hypertension). After delivery, 54 (41.5%) women breastfed exclusively, and 28 (21.5%) combined breastfeeding with formula milk. The remaining participants did not breastfeed (Table 1).

Table 1: Demographic and clinical characteristics [Data are presented as median (IQR)].

	n=130
Age, years	33 (30, 36)
Height, m	1.6 (1.6, 1.7)
Pre-pregnancy weight, kg	65 (57, 75)
Pre-pregnancy body mass index, kg/m^2	23.8 (21.1, 28.8)
Cardiovascular risk factors	
Chronic Arterial Hypertension n(%)	10 (7.7)
Gestational Hypertension n(%)	4 (3.1)
Obesity n(%)	11 (8.5)
Gestational Diabetes n(%)	14 (10.8)
Arterial Hypertension and Obesity n(%)	4 (3.1)
Arterial Hypertension and Gestational Diabetes n(%)	2 (1.5)
Arterial Hypertension and Gestational Diabetes and Obesity n(%)	4 (3.1)
Obesity and Gestational Diabetes n(%)	5 (3.8)
Pharmacological Therapy during Pregnancy	
Metformin	4 (3.1)
Insulin	3 (2.3)
Metformin and insulin	6 (4.6)
Beta-blockers	4 (3.1)
Nifedipine	2 (1.5)
Beta-blockers combined with nifedipine	2 (1.5)
Smoking habits	
Non-smoker n(%)	86 (66.2)
Smoker n(%)	8 (6.2)
Stopped during pregnancy n(%)	5 (3.8)
Stopped at pregnancy beginning n(%)	9 (6.9)
Ex-smoker n(%)	22 (16.9)
Primiparous women n(%)	79 (60.8)
No previous pregnancy n(%)	59 (45.4)
Gestational week at delivery	
Vaginal delivery n(%)	79 (60.8)
Caesarean delivery n(%)	51 (39.2)
Pregnancy weeks at Delivery	39 (38, 40)
Exclusively breastfeeding n(%)	54 (41.5)
Breastfeeding duration	
Until 4.5 months n(%)	32 (24.6)
Between 4.5 to 5 months n(%)	24 (18.5)
Between 5 to 5.5 months n(%)	31 (23.8)
After 5.5 months n(%)	28 (21.5)
Timing of the tests	
1st trimester, weeks	14 (13, 15)
3rd trimester, weeks	32 (31, 34)
PP1, weeks	7 (5, 8)
PP2, months	6 (6, 7)
PP3, months	13 (12, 13)

Table 2: Physical and vascular assessment.

Variables	N	Median (IQR)	p-value
Maternal body weight, kg			
3T	125	76 (68, 84)	
1T	124	67 (59, 77)	<0.001
PP1	113	69 (61, 79)	<0.001
PP2	130	66 (58, 77)	<0.001
PP3	77	65 (58, 78)	<0.001
Weight gain in pregnancy, kg			
3T	124	10 (7, 12)	
1T	124	2 (1, 3)	<0.001
PP1	109	-6 (-8, -4)	<0.001
PP2	125	-8 (-11, -5)	<0.001
PP3	72	-9 (-12, -5)	<0.001
Systolic blood pressure, mm Hg			
3T	122	110 (100, 120)	
1T	76	110 (102, 120)	0.084
PP1	110	111 (104, 121)	0.200
PP2	130	116 (108, 125)	0.008
PP3	79	118 (110, 124)	0.003
Diastolic blood pressure, mm Hg			
3T	122	70 (60, 80)	
1T	76	70 (60, 80)	0.900
PP1	110	78 (70, 80)	<0.001
PP2	130	80 (73, 86)	<0.001
PP3	79	79 (72, 86)	<0.001
Mean Arterial Pressure, mm Hg			
3T	122	83 (77, 91)	
1T	76	83 (77, 90)	0.600
PP1	110	88 (83, 93)	<0.001
PP2	130	90 (85, 97)	<0.001
PP3	79	90 (86, 98)	<0.001
Pulse wave velocity, m/s			
3T	122	6.00 (5.50, 6.70)	
1T	77	6.35 (5.95, 6.88)	<0.001
PP1	110	6.54 (6.04, 7.29)	<0.001
PP2	130	6.72 (6.20, 7.25)	<0.001
PP3	79	6.75 (6.30, 7.44)	<0.001
Augmentation index, %			
3T	122	-31 (-44, -14)	
1T	77	-21 (-40, -6)	0.005
PP1	110	-1 (-22, 11)	<0.001
PP2	130	-4 (-19, 5)	<0.001
PP3	79	-3 (-14, 9)	<0.001
Systemic vascular resistance, dyn· s/cm^5			
3T	107	1,476 (1,289, 1,694)	
1T	70	1,519 (1,327, 1,772)	0.084
PP1	102	2,096 (1,817, 2,332)	<0.001
PP2	120	2,045 (1,745, 2,341)	<0.001
PP3	73	2,015 (1,837, 2,232)	<0.001

3T settled as the reference category. N: number of participants. P-value determined with univariate generalized linear mixed-effects models.

Variation of Plasma Biomarkers during Pregnancy-induced Cardiovascular Reverse Remodeling

A multipanel of plasma biomarkers of myocardial injury (Figure 2), repair (Figure 3), fibrosis and extracellular matrix turnover (Figure 4), inflammation (Figure 5), metabolism (Figure 6), and vascular and endothelial function (Figure 7) were quantified in paired samples collected at 3rd trimester and six months after delivery to assess the molecular changes associated with postpartum cardiovascular RR.

B-type natriuretic peptide (BNP) and troponin I (TnI) levels were used to assess myocardial damage. Of these, only TnI levels were significantly reduced after delivery (P=0.045, Table 3). BNP levels were below 10pg/mL detection level in 79% and 72% of the 3rd trimester and 6 months after delivery, respectively. BNP and activin-A levels did not change significantly, which agrees with the literature on pregnant populations (15, 80). Regarding additional biomarkers of fibrosis and the extracellular matrix, the plasma levels of PICP decreased significantly after delivery (p<0.001), while lysyl oxidase increased significantly (p<0.001, Table 3). The graphical representation of PICP clearly separated the two groups above and below the cut-off value of 500pg/mL (Figure 4). Curiously, the highest values corresponded to 74% of participants with at least one cardiovascular risk factor (25 of 38 women with hypertension and/or gestational diabetes and/or obesity and plasma sample collected at 3rd trimester available). The ST2/IL33 receptor ratio decreased significantly postpartum (p<0.001, Table 3). A similar trend was observed for C-reactive levels (p<0.001, Table 3). No significant variation in FGF21 levels was observed from the 3rd trimester to 6 months after delivery (p=0.800, Table 3). All biomarkers of vascular and endothelial function assessed decreased significantly after delivery, namely relaxin-2 (p<0.001), lectin-like oxidized low-density lipoprotein receptor 1 (p<0.001), PAI-1 (p<0.001), and vascular endothelial growth factor (VEGF, p=0.058, Table 3).

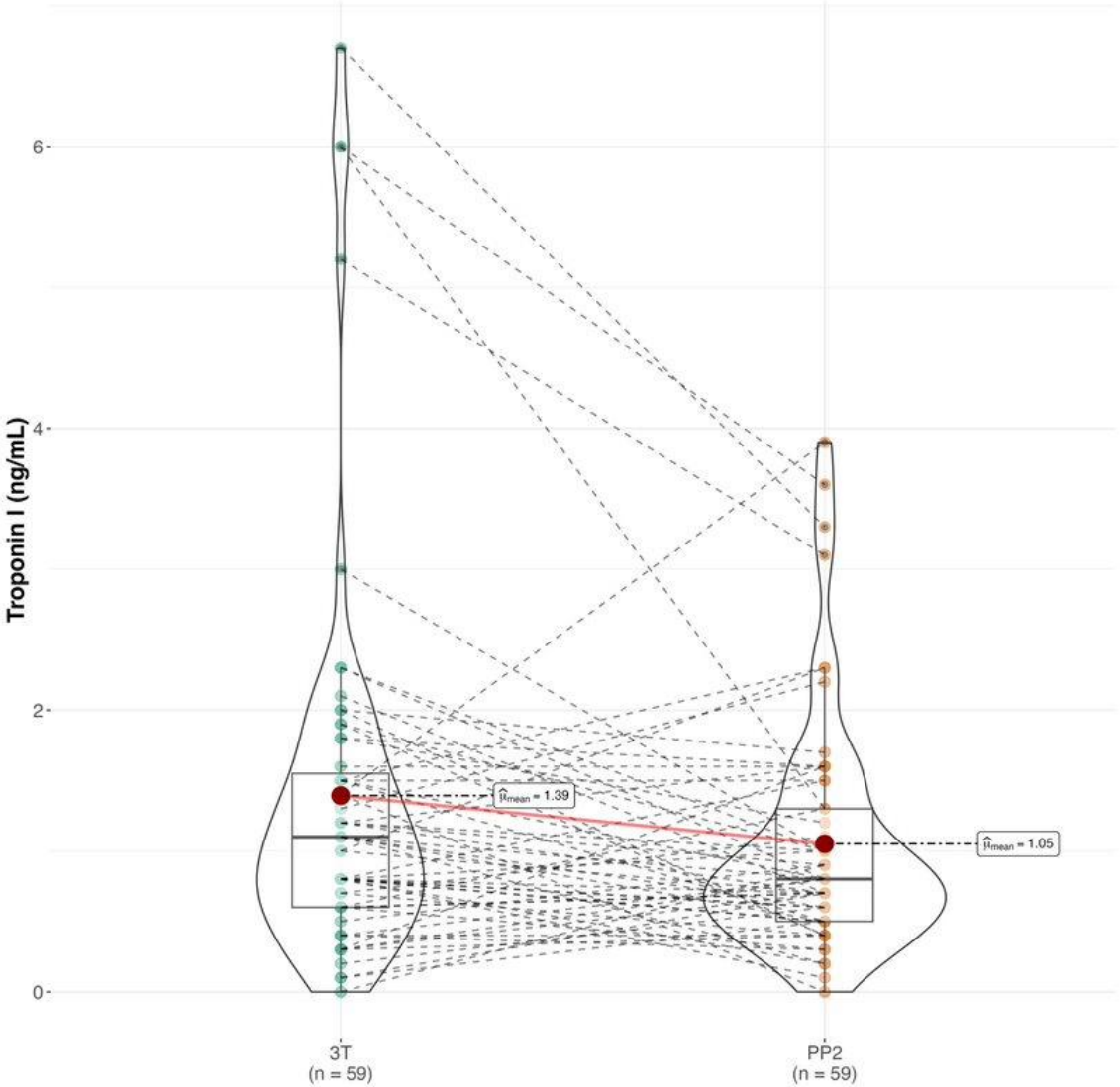

Figure 2: Troponin I, a marker of myocardial injury, was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=59 pairs). Troponin I progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 1.39ng/L and ûmean in 6 months after delivery: 1.05ng/L, p-value=0.020, one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

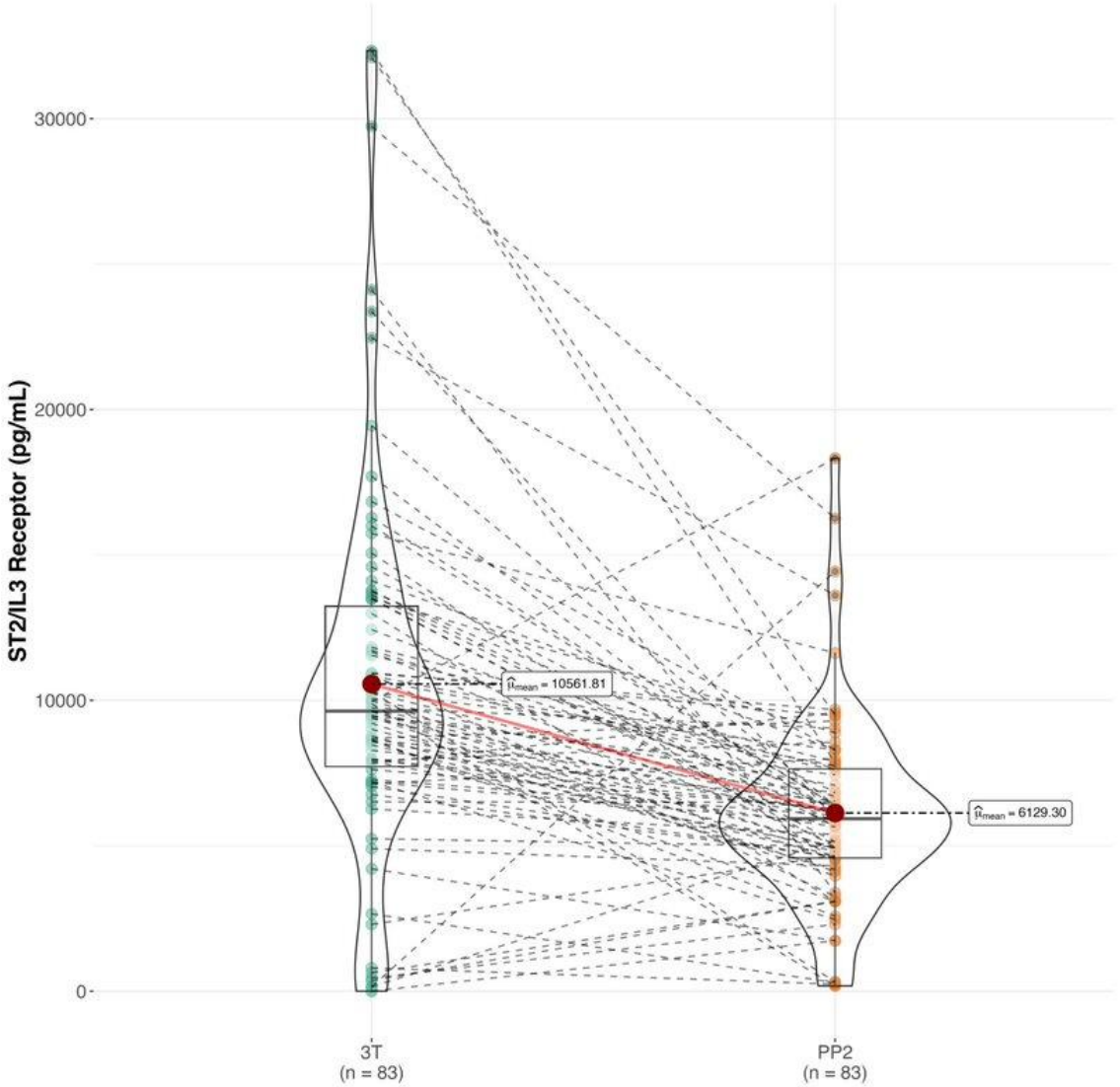

Figure 3: ST2/IL33 receptor was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=83 pairs). The variation of ST2/IL33 receptor levels for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 10561.81pg/mL and ûmean in 6 months after delivery: 6129.30pg/mL, p-value=1.31^{e-09} using one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

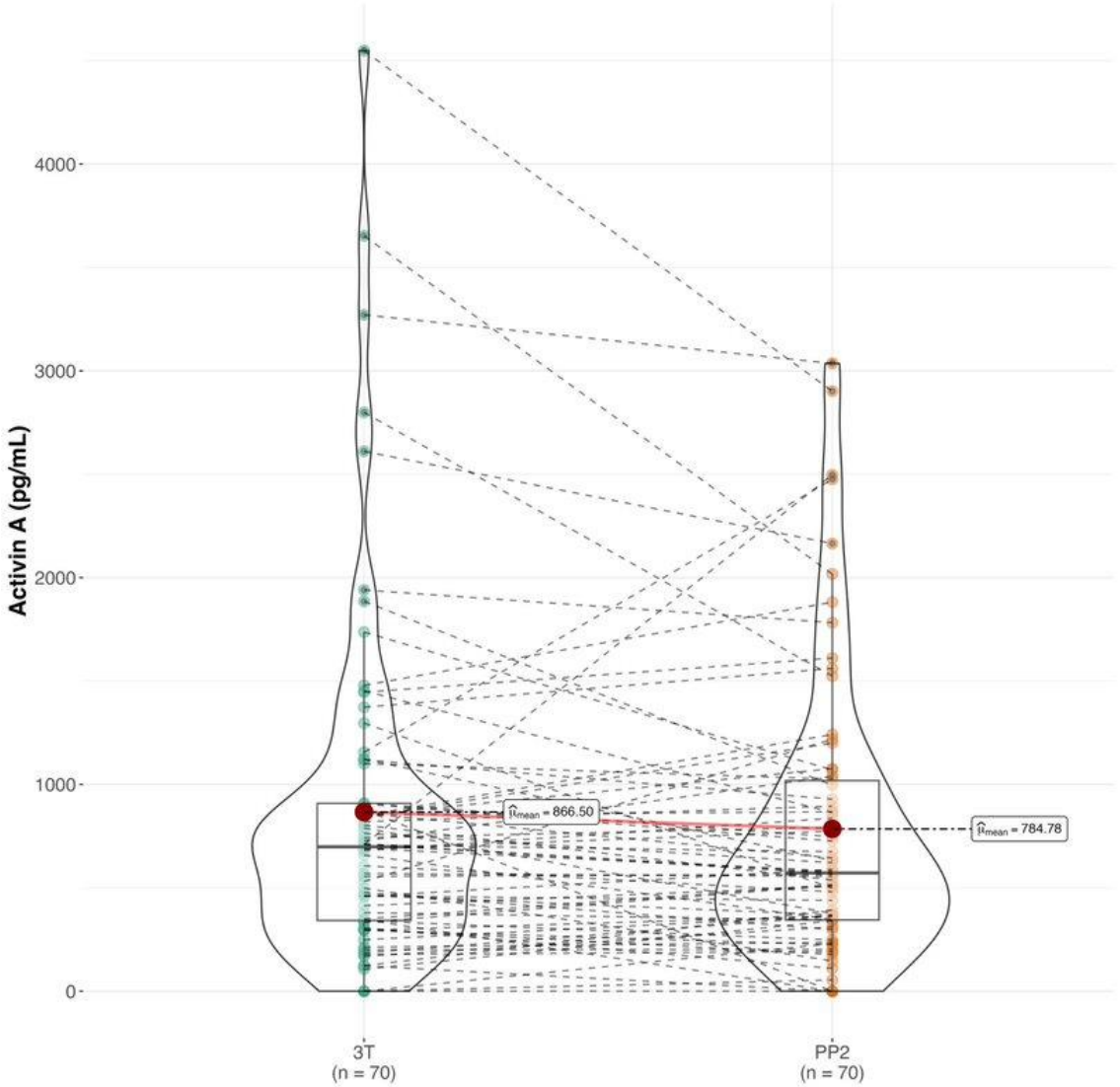

Figure 4 A: Activin A, a marker associated with fibrosis and extracellular matrix turnover, was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=70 pairs). Activin A progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 866.50pg/mL and ûmean in 6 months after delivery: 784.78pg/mL, p-value=0.180 using one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots

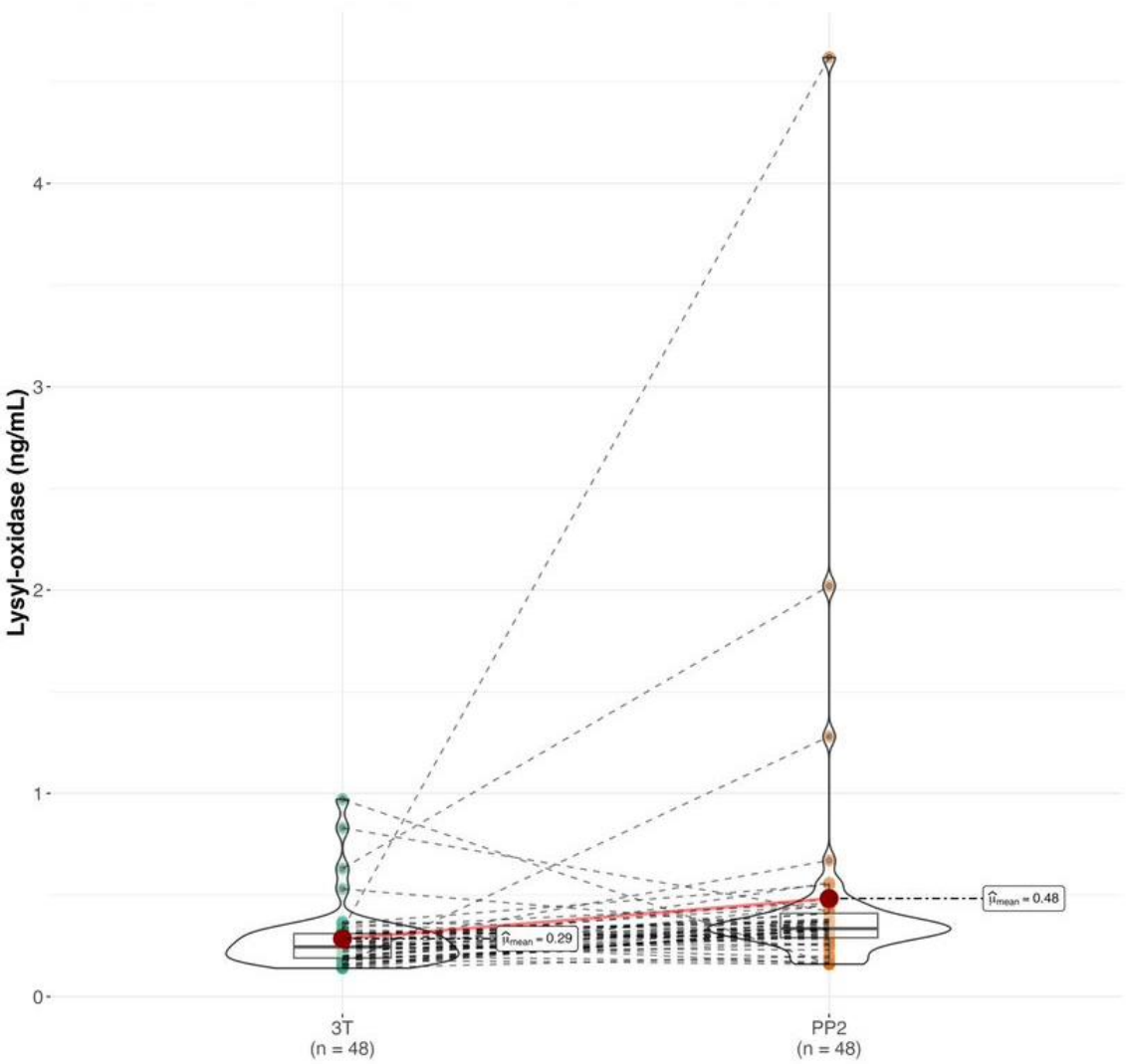

Figure 4 B: Lysyl-oxidase, a marker of fibrosis and extracellular matrix turnover, was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=48 pairs). Lysyl-oxidase progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 0.29ng/mL and ûmean in 6 months after delivery: 0.48ng/mL, p-value=0.030, one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

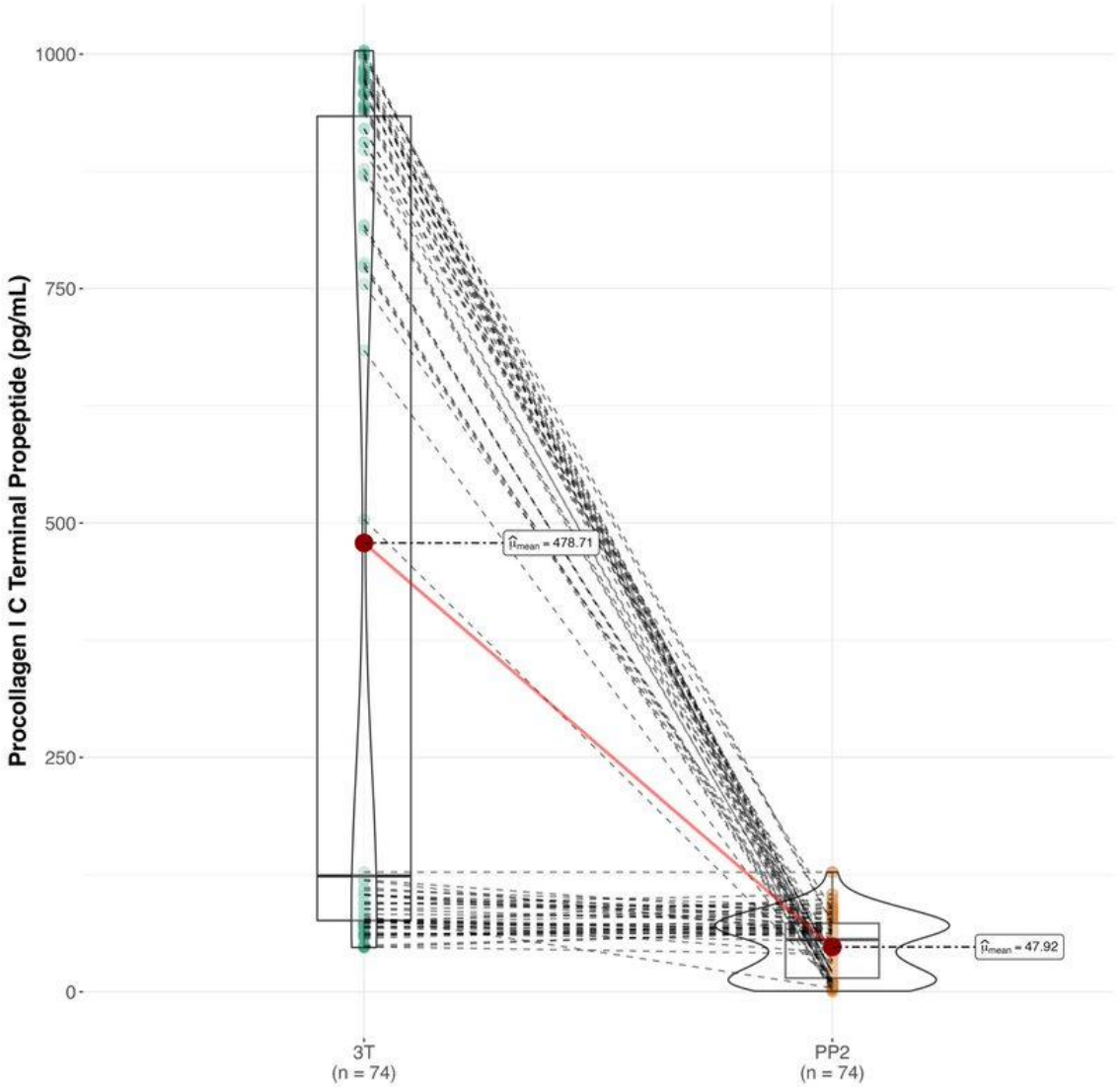

Figure 4 C: Procollagen I C Terminal Propeptide (PICP), a marker of fibrosis and extracellular matrix turnover, was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=74 pairs). PICP progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 478.71pg/mL and ûmean in 6 months after delivery: 47.92pg/mL, p-value= 2.67^{e-12}, one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

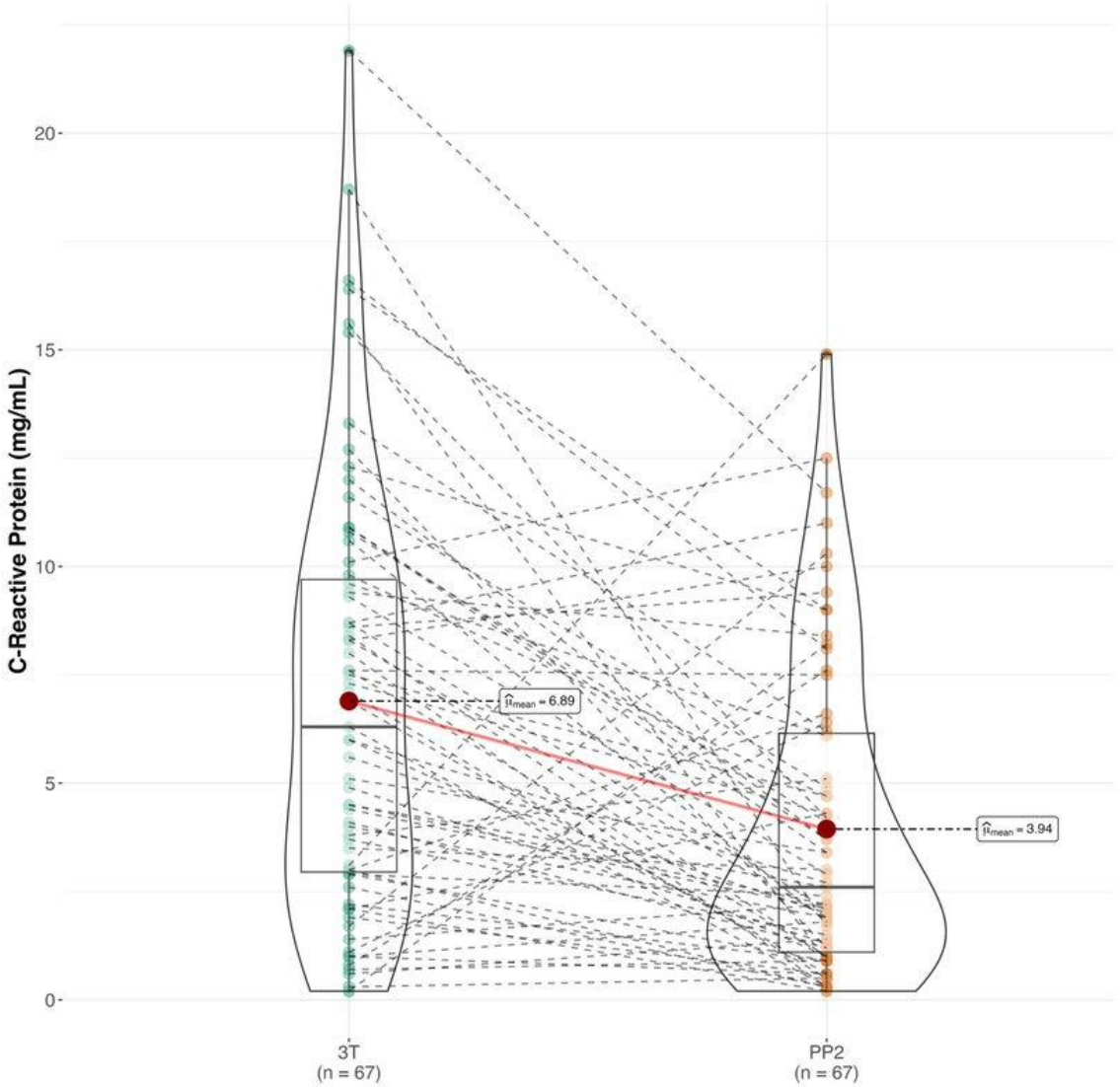

Figure 5: C-reactive protein (CRP) was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=67 pairs). CRP progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 6.89mg/L and ûmean in 6 months after delivery: 3.94mg/L, p-value= 1.64^{e-05}, one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

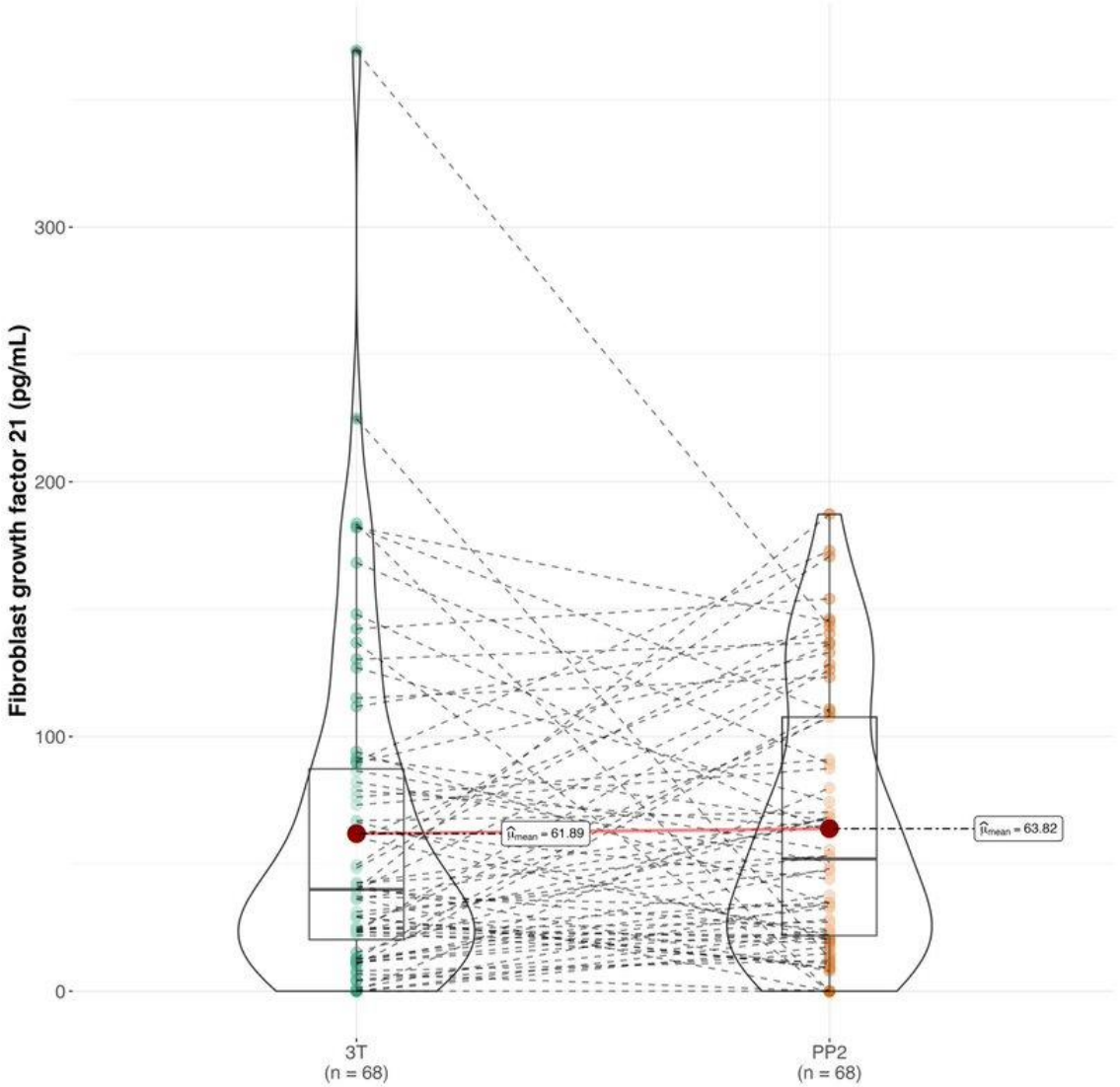

Figure 6: Fibroblast growth factor 21 (FGF21) was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=68 pairs). FGF21 progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 61.89pg/mL and ûmean in 6 months after delivery: 63.82pg/mL, p-value= 0.820, one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

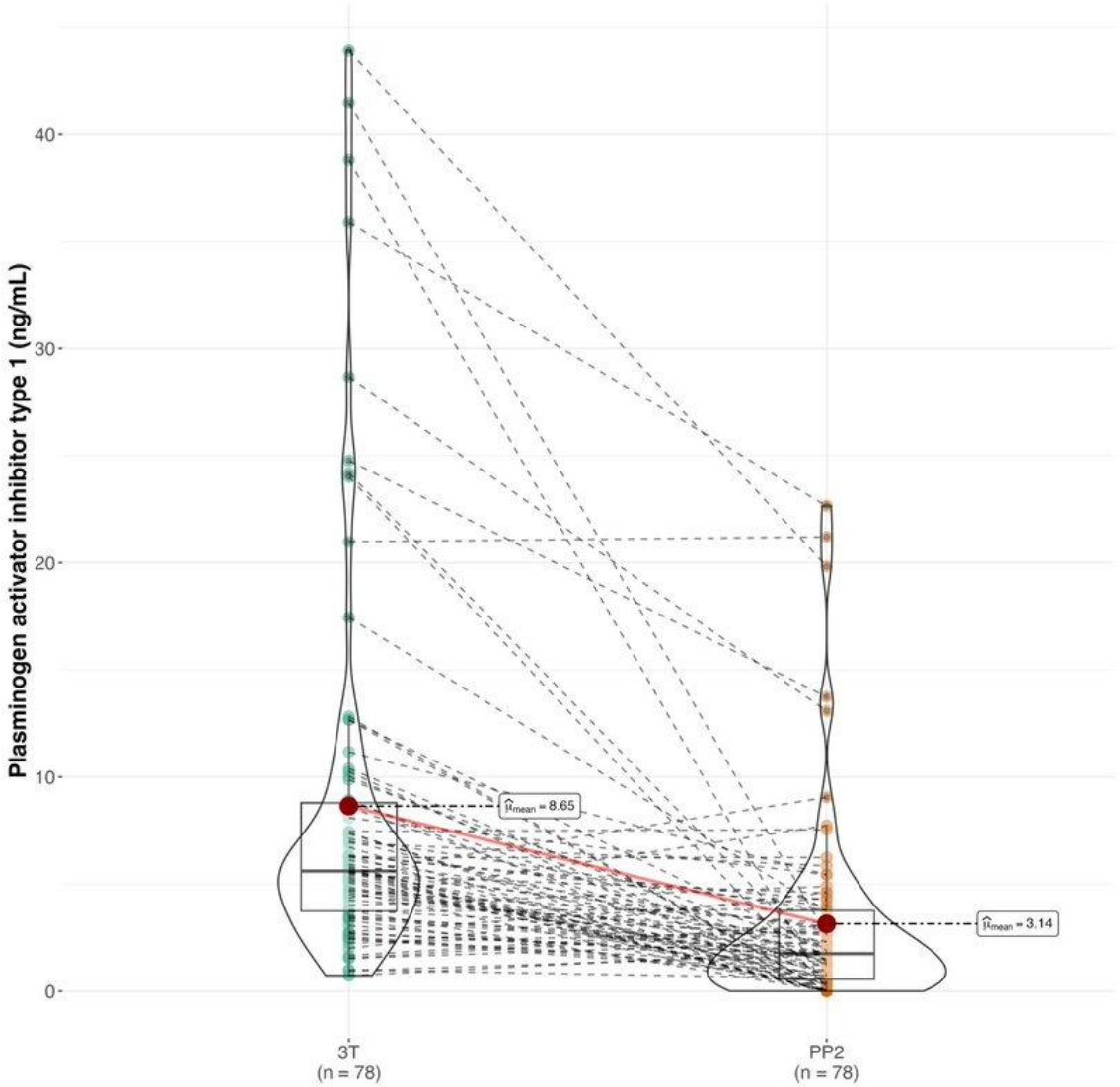

Figure 7 A: Plasminogen activator inhibitor type 1 (PAI-1) was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=78 pairs). PAI-1 progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 8.65ng/mL and ûmean in 6 months after delivery: 3.14ng/mL, p-value= 2.62^{e-08}, one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

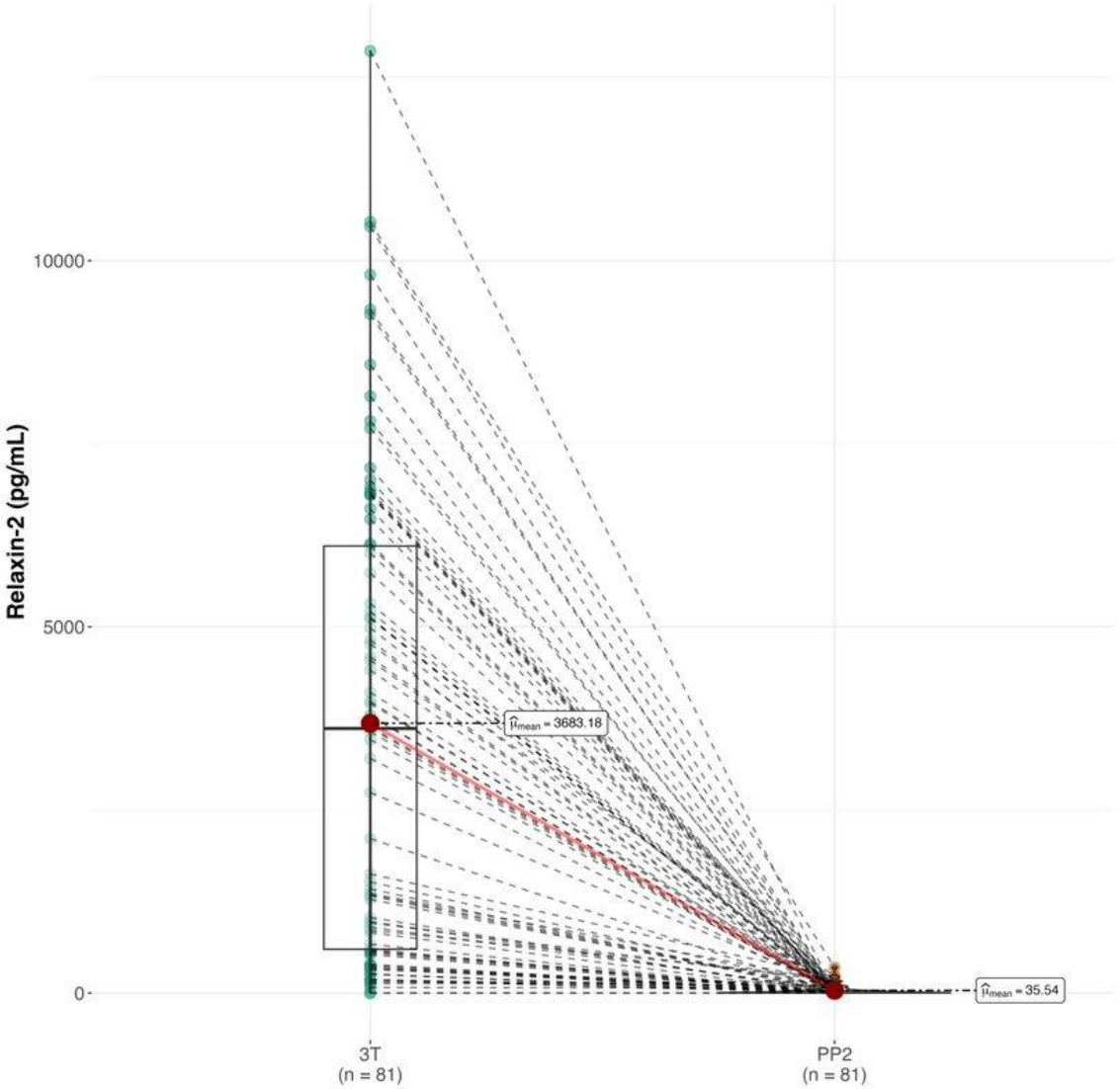

Figure 7 B: Relaxin-2 was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=81 pairs). Relaxin-2 progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 3683.18pg/mL and ûmean in 6 months after delivery: 35.54pg/mL, p-value= 3.11^{e-16}, one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

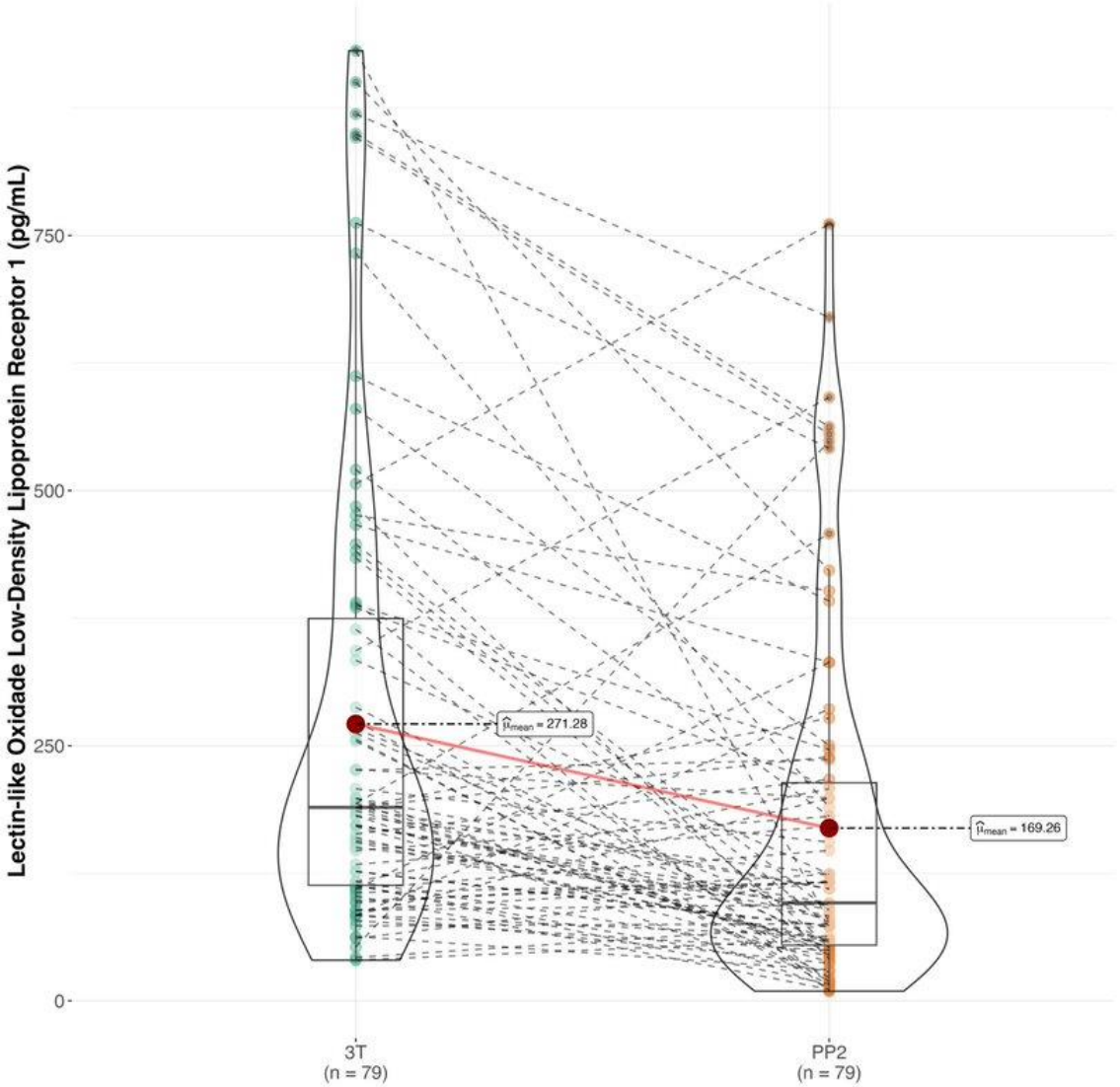

Figure 7 C: Leptin-like oxidized low density lipoprotein receptor (LOX-1) was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=79 pairs). LOX-1 progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 271.28pg/mL and ûmean in 6 months after delivery: 169.26pg/mL, p-value= 1.78^{e-05}, one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

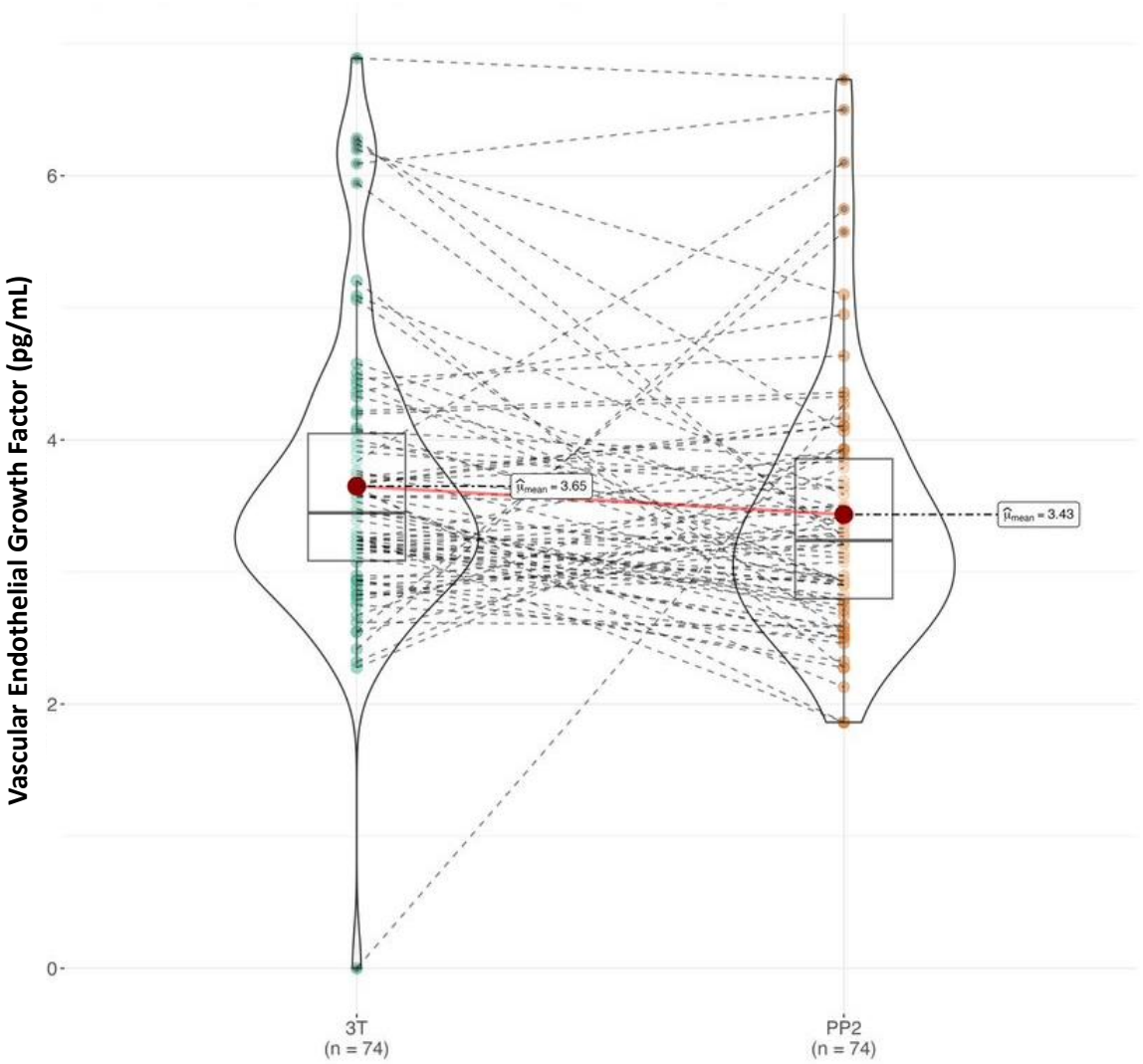

Figure 7 D: Vascular endothelial growth factor (VEGF) was measured in the 3rd trimester of pregnancy (green dots) and 6 months after delivery (orange dots), in each participant (n=74 pairs). VEGF progression for each woman is depicted by dashed grey lines. The global trend is shown by a red line, connecting average population means (ûmean in 3rd trimester: 3.65pg/mL and ûmean in 6 months after delivery: 3.43pg/mL, p-value= 0.140, one-way ANOVA with random intercept) in the two time-points. Data median and interquartile range are depicted through box plots and data distribution with violin plots.

Table 3: Quantification of plasma biomarkers

Variables	**3T**, N = 129[1]	**PP2**, N = 129[1]	**Difference**[2]	**95% CI**[23]	**p-value**[4]
Log-transformed Troponin I, ng/L	1.10 (0.60, 1.80)	0.80 (0.60, 1.30)	0.42	-0.67, 1.5	0.045
Activin A, pg/mL	688 (344, 957)	568 (344, 1,026)	133	-192, 457	0.600
Procollagen I C Terminal Propeptide, pg/mL	124 (76, 934)	56 (15, 73)	431	-174, 1,035	<0.001
Lysyl-oxidase, ng/mL	0.25 (0.19, 0.31)	0.33 (0.29, 0.41)	-0.19	-0.49, 0.11	<0.001
C-Reactive Protein, mg/mL	6.9 (3.0, 10.0)	2.4 (1.2, 6.2)	3.0	-4.4, 10	<0.001
ST2/IL33 receptor, pg/mL	9,548 (7,532, 12,572)	6,049 (4,609, 7,667)	4,174	-1,833, 10,182	<0.001
Log-transformed ST2/IL33 receptor	9.16 (8.93, 9.44)	8.71 (8.44, 8.94)	0.32	-0.24, 0.88	<0.001
Fibroblast growth factor 21, pg/mL	40 (23, 90)	51 (22, 91)	5.1	-16, 26	0.800
Relaxin-2, pg/mL	3,589 (650, 6,219)	11 (0, 36)	3,653	-542, 7,848	<0.001
Log-transformed Relaxin-2	8.19 (6.48, 8.74)	2.48 (0.00, 3.60)	5.5	4.9, 6.0	<0.001
Lectin-like Oxidised Low-Density Lipoprotein Receptor 1, pg/mL	190 (112, 348)	110 (56, 214)	99	-51, 248	<0.001
Plasminogen activator inhibitor type 1, ng/mL	6 (4, 9)	2 (1, 4)	5.6	-8.2, 19	<0.001
Log-transformed Plasminogen activator inhibitor type 1	4.82 (4.34, 6.84)	4.04 (2.74, 4.31)	2.0	1.2, 2.8	<0.001
Vascular Endothelial Growth Factor, pg/mL	31 (21, 58)	24 (17, 46)	19	-33, 72	0.058
BNP (>10pg/mL)	12 (21%)	11 (28%)			
(<10pg/mL)	44 (79%)	29 (72%)			

[1]Median (IQR)

Variables	**3T**, N = 129[1]	**PP2**, N = 129[1]	**Difference**[2]	**95% CI**[23]	**p-value**[4]

[2]One-way ANOVA with random intercept

[3]CI = Confidence Interval

[4]Wilcoxon rank sum test

Legend: BNP - B-type natriuretic peptide.

Pregnancy-induced Cardiac Morphofunctional Remodeling and Reverse Remodeling

Regarding cardiac remodeling, pregnant women developed cardiac hypertrophy, as shown by a significant increase in LVM ($p<0.001$) combined with a small relative wall thickness (RWT<0.42). This hypertrophy was associated with diastolic changes, reflected in LA enlargement ($p<0.001$), increased ventricular filling pressures (E/e', $p<0.001$), and the ratio of systolic pulmonary venous flow velocity to anterograde diastolic pulmonary venous flow velocity ($p=0.021$). Delivery triggered a significant regression of LV chamber dimensions (mass, RWT, and volumes; $p<0.001$) and recovery of diastolic function at 1st month postpartum [LA volume ($p<0.001$) and E/e' ($p<0.001$), Table 4)]. Systolic function, measured by global longitudinal strain (GLS), worsened slightly throughout pregnancy and recovered at 6 months postpartum (3T–6 months postpartum, $p=0.028$) despite preserved ejection fraction at all time points evaluated. These morpho-functional adaptations during postpartum were accompanied by a significant decrease in fibrosis and extracellular matrix turnover biomarkers (PICP, $p<0.001$, Table 3), inflammatory biomarkers (C-reactive protein and ST2/IL33 receptor, $p<0.001$, Table 3), and markers of myocardial damage (troponin I, $p=0.045$, Table 3) from 3rd trimester to 6 months postpartum. In contrast, a significant increase in lysyl oxidase ($p<0.001$, Table 3) was found between these two time points.

Table 4: Echocardiographic assessment

Variables	N	Median (IQR)	p-value
Heart rate, beats/min			
3T	121	80 (72, 85)	
1T	75	74 (68, 82)	<0.001
PP1	110	61 (55, 66)	<0.001
PP2	130	64 (60, 69)	<0.001
PP3	79	66 (61, 74)	<0.001
LVEF, %			
3T	116	60.5 (56.2, 63.2)	
1T	72	61.0 (57.7, 64.1)	0.300
PP1	104	60.8 (58.3, 64.1)	0.200
PP2	129	59.8 (56.9, 62.6)	0.600
PP3	77	60.5 (57.5, 64.1)	0.300
Global Longitudinal Strain, %			
3T	80	-21.60 (-23.83, -20.10)	
1T	39	-21.80 (-24.90, -20.70)	0.200
PP1	73	-22.50 (-24.10, -20.50)	0.300
PP2	103	-22.70 (-24.55, -20.90)	0.028
PP3	71	-23.30 (-24.95, -21.40)	0.002
E Mitral Inflow Velocity, cm/s			
3T	122	88 (78, 100)	
1T	75	89 (81, 99)	0.130
PP1	110	78 (68, 88)	<0.001
PP2	129	81 (72, 90)	<0.001
PP3	79	81 (69, 92)	<0.001
A Mitral Inflow Velocity, cm/s			
3T	122	61 (52, 70)	
1T	75	53 (46, 62)	<0.001
PP1	110	48 (44, 56)	<0.001
PP2	129	48 (42, 55)	<0.001
PP3	79	48 (42, 56)	<0.001
E/A			
3T	122	1.42 (1.20, 1.71)	
1T	75	1.69 (1.42, 2.00)	<0.001
PP1	110	1.62 (1.40, 1.87)	<0.001
PP2	129	1.64 (1.43, 1.91)	<0.001
PP3	79	1.61 (1.37, 1.97)	<0.001
S Inflow Pulmonary Veins Velocity, cm/s			
3T	103	54 (49, 61)	
1T	68	54 (47, 63)	0.700
PP1	100	50 (42, 58)	<0.001
PP2	124	50 (45, 59)	<0.001
PP3	78	50 (45, 59)	0.004
D Inflow Pulmonary Veins Velocity, cm/s			
3T	103	52 (44, 61)	
1T	68	58 (51, 64)	0.005
PP1	100	52 (46, 60)	0.200
PP2	124	52 (43, 59)	0.024
PP3	78	51 (43, 58)	0.043
S/D			
3T	103	1.08 (0.85, 1.23)	
1T	68	0.90 (0.78, 1.20)	0.021
PP1	100	0.98 (0.78, 1.20)	0.005
PP2	124	1.06 (0.80, 1.27)	0.200
PP3	78	1.08 (0.83, 1.24)	0.300
E' Mitral Annulus Septal, cm/s			
3T	122	10.30 (8.93, 11.67)	
1T	76	12.20 (10.57, 13.80)	<0.001
PP1	110	11.10 (9.90, 12.78)	<0.001
PP2	130	11.40 (10.12, 12.38)	<0.001
PP3	79	11.10 (9.90, 12.45)	0.008

Variables	N	Median (IQR)	p-value
E' Mitral Annulus Lateral, cm/s			
3T	121	16.1 (13.7, 18.3)	
1T	76	18.5 (16.5, 21.1)	<0.001
PP1	110	16.5 (15.0, 18.2)	0.400
PP2	129	16.6 (14.7, 18.2)	0.082
PP3	79	16.1 (14.3, 18.2)	0.300
E/E' average			
3T	121	6.70 (5.67, 7.82)	
1T	75	5.85 (5.08, 6.36)	<0.001
PP1	110	5.70 (4.82, 6.47)	<0.001
PP2	128	5.81 (5.26, 6.61)	<0.001
PP3	79	5.80 (5.13, 6.69)	<0.001
Stroke volume, mL			
3T	108	59 (52, 66)	
1T	71	58 (52, 67)	0.800
PP1	102	55 (49, 64)	0.004
PP2	124	58 (49, 64)	0.002
PP3	73	56 (50, 62)	<0.001
Cardiac output, L/min			
3T	108	4.68 (4.05, 5.25)	
1T	71	4.31 (3.78, 4.96)	0.002
PP1	102	3.38 (3.02, 3.82)	<0.001
PP2	120	3.61 (3.09, 4.03)	<0.001
PP3	73	3.70 (3.22, 4.14)	<0.001
Relative Wall Thickness			
3T	122	0.35 (0.31, 0.39)	
1T	76	0.32 (0.28, 0.35)	<0.001
PP1	110	0.33 (0.29, 0.36)	<0.001
PP2	130	0.32 (0.29, 0.36)	<0.001
PP3	79	0.34 (0.31, 0.37)	0.013
LV mass, g			
3T	122	127 (111, 145)	
1T	76	103 (93, 116)	<0.001
PP1	110	112 (100, 128)	<0.001
PP2	130	103 (92, 119)	<0.001
PP3	79	106 (94, 117)	<0.001
LV end-diastolic volume, mL			
3T	115	81 (71, 90)	
1T	72	82 (72, 90)	0.300
PP1	104	86 (77, 95)	<0.001
PP2	129	94 (81, 103)	<0.001
PP3	77	80 (72, 89)	0.900
Maximum LA volume, mL			
3T	118	50 (43, 56)	
1T	72	42 (36, 48)	<0.001
PP1	103	40 (34, 49)	<0.001
PP2	129	39 (33, 46)	<0.001
PP3	78	37 (33, 44)	<0.001

3T settled as the reference category. N: number of participants. P-value determined with univariate generalized linear mixed-effects models.

Legend: LA – Left atrium; LV – Left ventricle; LVEF - Left ventricular ejection fraction.

Predictors of Pregnancy-induced Cardiac Remodeling and Reverse Remodeling

To explore potential predictors of cardiac hypertrophy variation induced by pregnancy and delivery, LVM (Figure 8) was defined as the outcome in multivariable generalized linear mixed-effects models (GLMM). In the GLMM, arterial hypertension (β:14.34, 95% CI [6.02; 22.66], p<0.001), smoking habits (β:8.25, 95% CI [1.19; 15.31], p=0.022), and obesity (β:13.80, 95% CI [5.39; 22.21], p=0.001) were independent predictors of low LVM regression during the postpartum period, indicating that they are important modulators of worse hypertrophy reversal (Table 5). A significant effect of ST2/IL33 receptor (β: -3.59, 95% CI [-6.24;-0.94], p=0.008) and C-reactive protein (β:0.68, 95% CI [0.02;1.35], p=0.044) measured at 3rd trimester was also found when added to the clinical model. While higher levels of the ST2/IL33 receptor were associated with higher LVM regression, C-reactive protein levels showed an inverse relationship, highlighting the potential utility of these inflammatory biomarkers for assessing hypertrophy reversal (Table 5).

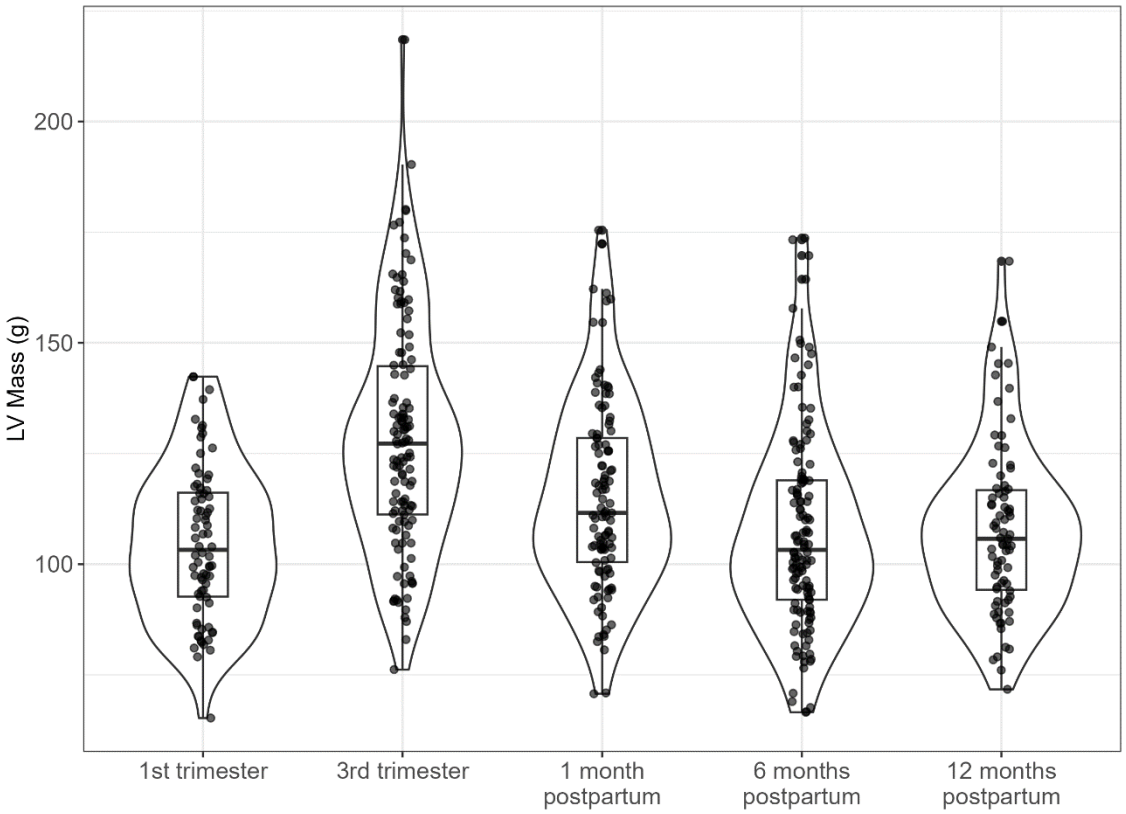

Figure 8: Left ventricular (LV) mass progression throughout the follow-up period. The black points represents LV mass in each evaluation moment (1st trimester n=76 subjects, 3rd trimester n=122 subjects, 1 month postpartum n=110 subjects, 6 months postpartum n=130 subjects and 12 months postpartum n=79 subjects). Data median and interquartile range are depicted through box plots and data distribution with violin plots.

Table 5: Multivariable generalised linear mixed model for LVM.

	Clinical Model		Clinical Model with C-Reactive Protein		Clinical Model with ST2/IL33 Receptor	
Parameter	**β, 95% CI**	**p-value**	**β, 95% CI**	**p-value**	**β, 95% CI**	**p-value**
Evaluation moment – 1T	-19.77 [-24.16;-15.37]	<0.001	-17.80 [-23.26;-12.33]	<0.001	-19.40 [-24.53;-14.28]	<0.001
Evaluation moment - PP1	-11.29 [-15.11;-7.46]	<0.001	-10.71 [-15.54;-5.87]	<0.001	-10.11 [-14.68;-5.54]	<0.001
Evaluation moment - PP2	-19.68 [-23.32;-16.05]	<0.001	-18.23 [-22.85;-13.61]	<0.001	-18.62 [-22.98;-14.27]	<0.001
Evaluation moment - PP3	-17.98 [-22.31;-13.65]	<0.001	-18.05 [-23.32;-12.79]	<0.001	-18.71 [-23.84;-13.59]	<0.001
Age	-0.50 [-1.31;0.31]	0.223	-0.59 [-1.72;0.54]	0.303	-0.44 [-1.46;0.58]	0.398
Arterial Hypertension	14.34 [6.02;22.66]	<0.001	12.88 [1.12;24.65]	0.032	9.64 [-1.54;20.83]	0.091
Gestational Diabetes	-4.30 [-12.40;3.81]	0.298	-1.39 [-11.43;8.66]	0.786	-2.58 [-11.66;6.49]	0.576
Obesity	13.80 [5.39;22.21]	0.001	10.99 [0.11;21.87]	0.048	12.50 [3.34;21.65]	0.008
Smoking Habits	8.25 [1.19;15.31]	0.022	4.43 [-4.11;12.97]	0.308	8.78 [0.80;16.76]	0.031
Primiparous	-3.69 [-10.75;3.36]	0.304	-3.04 [-11.97;5.88]	0.503	-4.05 [-12.49;4.39]	0.346
Weight Gain during Pregnancy	-0.45 [-1.18;0.27]	0.218	-0.04 [-0.91;0.83]	0.931	-0.44 [-1.26;0.39]	0.299
C-Reactive Protein			0.68 [0.02;1.35]	0.044		
ST2/IL33 Receptor					-3.59 [-6.24;-0.94]	0.008
R^2	0.646		0.627		0.639	

Characterisation of Vascular Remodeling and Reverse Remodeling

Although the mean arterial pressure was stable throughout pregnancy, a reduction in systemic vascular resistance (p=0.084), augmentation index (p=0.005), and arterial stiffness (p<0.001) were observed from 1st to 3rd trimester (Table 2). These three vascular parameters increased significantly as soon as 1 month after delivery, not only due to postpartum cardiac output reduction (Table 2), but also due to hormonal/vascular changes after pregnancy. These alterations were associated with a significant decrease in relaxin-2 (p<0.001) and PAI-1 (p<0.001) from the 3rd trimester to 6 months after delivery (Table 3).

Predictors of Vascular Remodeling and Reverse Remodeling

PWV (Figure 9), an indicator of vascular stiffness, was used as the dependent variable in the GLMM. Arterial hypertension (β =0.77, 95% CI [0.48; 1.07], p<0.001), age (β =0.05, 95% CI [0.02, 0.08], p=0.001), and gestational diabetes (β =0.37, 95% CI [0.06;0.68], p=0.018) were identified as potential indicators of vascular remodeling and RR (Table 6). Although relaxin-2 (β:0.04, 95% CI [-0.04;0.12], p=0.326) and PAI-1 (β:0.003, 95% CI [0.00, 0.01], p=0.446) were not statistically significant, both increased the robustness of the clinical model (r^2=0.726 to r^2=0.731) (Table 6).

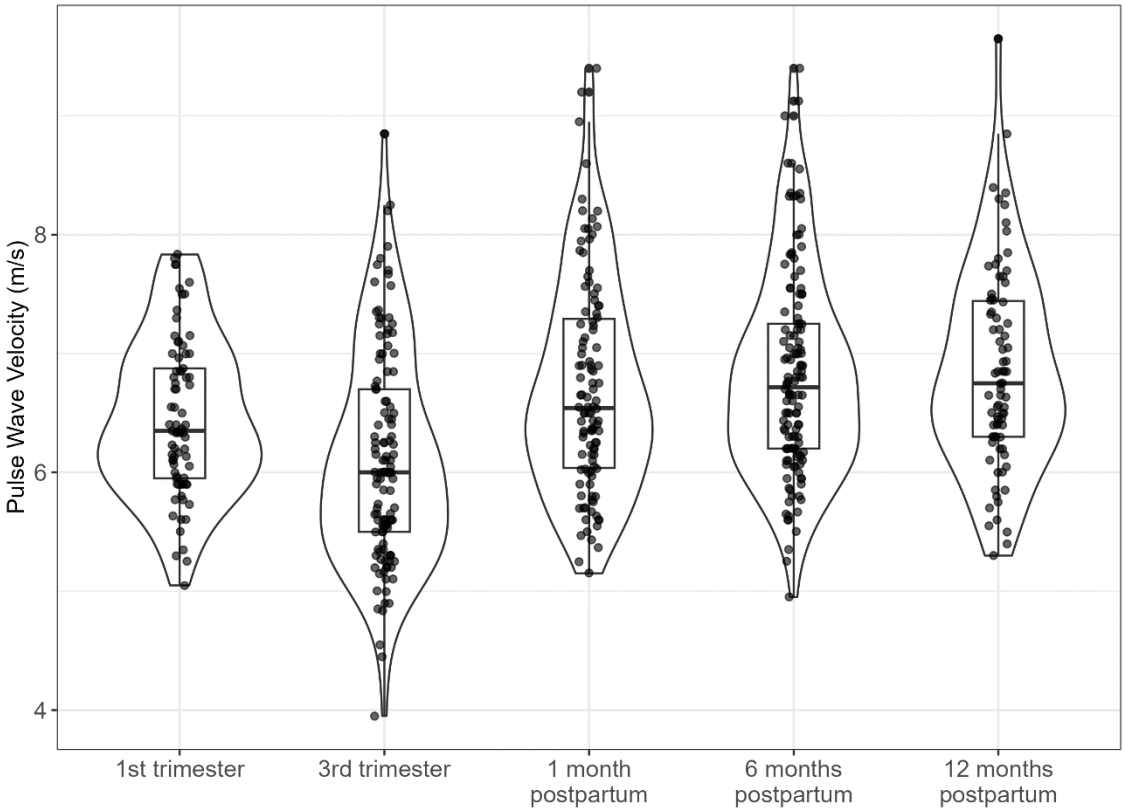

Figure 9: Progression of pulse wave velocity throughout the follow-up period. The black points represents pulse wave velocity in each evaluation moment (1st trimester n=77 subjects, 3rd trimester n=122 subjects, 1 month postpartum n=110 subjects, 6 months postpartum n=130 subjects and 12 months postpartum n=79 subjects). Data median and interquartile range are depicted through box plots and data distribution with violin plots.

Table 6: Multivariable generalised linear mixed model for arterial stiffness, measured by pulse wave velocity.

	Clinical Model		Clinical Model with Relaxin-2		Clinical Model with PAI-1	
Parameter	**β, 95% CI**	**p-value**	**β, 95% CI**	**p-value**	**β, 95% CI**	**p-value**
Evaluation moment – 1T	0.56 [0.41;0.71]	<0.001	0.59 [0.41;0.77]	<0.001	0.58 [0.40;0.75]	<0.001
Evaluation moment - PP1	0.65 [0.52;0.78]	<0.001	0.72 [0.53;0.88]	<0.001	0.70 [0.54;0.86]	<0.001
Evaluation moment - PP2	0.72 [0.59;0.84]	<0.001	0.77 [0.62;0.93]	<0.001	0.77 [0.62;0.92]	<0.001
Evaluation moment - PP3	0.71 [0.56;0.86]	<0.001	0.75 [0.57;0.93]	<0.001	0.74 [0.57;0.92]	<0.001
Age	0.05 [0.02;0.08]	0.001	0.06 [0.02;0.10]	0.002	0.06 [0.02;0.10]	0.005
Arterial Hypertension	0.77 [0.48;1.07]	<0.001	0.81 [0.44;1.19]	<0.001	0.84 [0.46;1.22]	<0.001
Gestational Diabetes	0.37 [0.06;0.68]	0.018	0.38 [0.01;0.74]	0.044	0.48 [0.11;0.85]	0.010
Obesity	-0.02 [-0.35;0.31]	0.899	-0.10 [-0.48;0.27]	0.585	-0.07 [-0.45;0.31]	0.711
Smoking Habits	0.20 [-0.06;0.47]	0.129	0.25 [-0.07;0.57]	0.132	0.21 [-0.11;0.53]	0.197
Primiparous	-0.11 [-0.37;0.15]	0.410	-0.22 [-0.53;0.10]	0.186	-0.16 [-0.49;0.17]	0.333
Equivalent of Mean Arterial Pressure at 1st Trimester	0.02 [-0.02;0.05]	0.286	0.02 [-0.02;0.06]	0.389	0.02 [-0.02;0.06]	0.350
Weight Gain during Pregnancy	-0.002 [-0.03;0.03]	0.867	0.007 [-0.04;0.03]	0.695	0.003 [-0.04;0.03]	0.871
Relaxin-2			0.04 [-0.04;0.12]	0.326		
Plasminogen activator inhibitor type 1 (PAI-1)					0.003 [0.00;0.01]	0.446
R^2	0.726		0.727		0.731	

Discussion

To our knowledge, this study is the first prospective cohort of pregnant women with or without risk factors to study cardiovascular remodeling (assessed at 2 time points during pregnancy) and RR (assessed at 3 time points after delivery).

In our cohort, we observed an increase in cardiac output from 1st to 3rd trimester linked to a progressive plasmatic volume increment throughout pregnancy. In agreement with previous studies (3, 6, 9, 10, 12-15), the increase in cardiac output triggered an enlargement of cardiac volumes and a proportional change in LV mass and wall thickness, consistent with LV physiological hypertrophy. We observed a significant regression of LV mass and of LV and LAenlargement, accompanied by an amelioration of diastolic function (E/e') as soon as 1 month after delivery. Likewise, we have shown a reduction of arterial stiffness during pregnancy and its early postpartum increase, previously attributed to, respectively, a rise or decline in pregnancy's hormones with vasodilatory effects (such as estrogen, progesterone and relaxin levels) (6, 11). Still, these cardiovascular changes may persist throughout the first year after delivery, as shown by us and supported by Tasar et al. (37) and Clapp et al. (42).

Determinants of hypertrophy reversal

Among the risk factors evaluated, arterial hypertension, smoking habits, and obesity were independent predictors of low LVM regression during the postpartum period. High C-reactive protein and low ST2/IL33 receptor levels were also showed to be independent predictors of hypertrophy reversal, holding potential to become biomarkers of worse LVM regression. Indeed, other authors have shown that pregnant women with hypertension had a significant increase in relative wall thickness without a proportional enlargement of the left ventricular end-diastolic diameter, resulting in concentric remodeling (17, 74, 77). Vasapollo *et al.* identified pre-gestational concentric hypertrophy (78) as the strongest predictor of early to long-term postpartum complications in women with chronic hypertension. In addition, women who developed hypertensive disorders during pregnancy revealed incomplete postpartum cardiovascular RR, which includes subclinical cardiac dysfunction, hypertrophy, impaired LV relaxation and increased peripheral vascular resistance (41, 72, 80, 84, 91). Our study also highlighted obesity as a strong predictor of worse hypertrophy reversal. We report, for the first time, postpartum cardiac mass regression in pregnant women with obesity without perinatal complications. The impact of obesity might be explained by a low-grade chronic inflammatory state, marked by an increase in CRP levels (120). We also documented smoking habits as a determinant of LVM adaptations during pregnancy and postpartum, which aligns with previous

studies that report an association between an extended duration of smoking and an increased number of daily cigarettes with increased LVM, relative wall thickness, and worse diastolic function (121, 122). This remodeling results from cardiac damage at the microcellular level, independently of atherosclerotic and thrombotic events (121), and is explained by an increase in reactive oxygen species production, endothelial dysfunction, and mitochondrial dysfunction (122).

Determinants of vascular reverse remodeling

Our analysis identified arterial hypertension, age, and gestational diabetes as determinants of vascular remodeling and RR, in line with most previous evidence. For instance, women with pre-eclampsia, a hypertensive disorder of pregnancy, showed elevated arterial stiffness up to 4 years after delivery (101, 105). Additionally, Castleman *et al.* reported increased arterial stiffness in normotensive pregnancies with a previous hypertensive disorder of pregnancy (81). Also, pregestational or gestational arterial hypertension has previously been found to impact the course of vascular RR extensively. Regarding the impact of maternal age on arterial stiffness, Graaf *et al.* reported a positive and significant correlation between PWV and age in healthy pregnant nulliparous women during 1st trimester (123). Regarding the postpartum period, Vilmi-Kerälä *et al.* reported age and gestational diabetes as determinants of PWV 3.7 years after delivery, using multiple linear regression analysis (124). Similarly, age and gestational diabetes were also predictors of PWV in a sub-analysis of the STORK study, including 284 women with and without a history of gestational diabetes 5 years after the index pregnancy (102). In contrast, other studies have shown that gestational diabetes was not associated with impaired arterial stiffness when assessed at 8 weeks (103) or 4–10 years postpartum (104). These discrepant results may be explained by the duration of hyperglycemia (103).

Potential plasma biomarkers

Postpartum cardiac remodeling and RR are complex processes associated with subclinical cardiac injury (herein evaluated with TnI), repair (ST2/IL33 receptor), fibrosis (PICP), and inflammation (C-reactive Protein). Thus, we and others have found that changes in plasma levels of the above mentioned markers parallel cardiac functional and structural adaptations, namely, in LV volume and mass (37, 40).

Our study found augmented LVM in the 3rd trimester accompanied by increased levels of high sensitivity Troponin I (hs-TnI) and fibrosis biomarkers, such as PICP. Indeed, hs-TnI had already been identified as the best predictor of LVM, reflecting LV fibrosis and cardiac myocyte damage (84). Complementary to this, we also assessed myocardial repair by ST2/IL33 receptor quantification, which we found to be elevated in the 3rd trimester compared to the postpartum

period. When IL-33 binds to ST2L, adverse cardiovascular remodeling is attenuated by activating signaling pathways such as ERK1/2, p38 MAPK, and NF-kB (125). Interestingly, although p38 activity does not directly mediate cardiac hypertrophy during pregnancy, it may play an essential role in cardiac remodeling, attenuating fibrosis and apoptosis (23), similar to what has been described for estrogen (30, 126). Progesterone, in turn, has been reported to regulate LV hypertrophy through ERK1/2 pathway activation early in pregnancy (23). The ST2/IL33 receptor has been reported to protect against atherosclerosis, obesity, diabetes, and cardiac fibrosis (127-129). Whether ST2/IL33 receptor is cardioprotective in the setting of pregnancy-induced cardiac (reverse) remodeling it remains to confirm. Although, for now, our study shows an association between plasmatic levels and LV mass throughout gestation.

We also observed diastolic deterioration in the 3rd trimester accompanied by increased levels of PICP, both of which resolved 6 months after delivery. The release of PICP is associated with mechanisms of tissue repair, which involve collagen synthesis and degradation, and subsequent fibrotic tissue deposition (130). Although the overloaded pregnant heart has reduced deposition of fibrotic tissue (7, 11), animal studies have shown higher pericardial and vascular fibrosis in late pregnancy compared to the non-pregnant group, which reversed in the postpartum period (30). These are associated with the downregulation of metalloproteinases (MMP-1, MMP-2, and MMP-9), counterbalancing the upregulation of tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-4) in late pregnancy (30). MMP-1, MMP-9, and TIMP-1 expression in the LV was reversed seven days after delivery (30). In addition, a reduction in MMP-2 expression was correlated with increased TIMP-4 levels during the postpartum period (30). In this context, fibrosis deposition could be a response to hemodynamic overload inherent to pregnancy development (30). Collagens are the main component of the cardiac extracellular matrix, which contribute to passive tension in the myocardial walls during diastolic filling, determining LV stiffness (31). In pregnancy, LV collagen I and III are upregulated, reversing during the postpartum period (31). This may be an adaptive mechanism to compensate for the volume overload inherent to fetal development (31). Accordingly, increased collagen III (a more compliant isoform) and estrogen levels have been reported to augment late in pregnancy, while collagen I and progesterone levels decrease (31). The late pregnancy predisposition to fibrosis may be counterbalanced by the pleiotropic hormone relaxin-2, which we found to be increasedin the 3rd trimester. Relaxin-2 protection extends to the postpartum, as previously documented in the Multicenter Investigation in Pregnancy Associate Cardiomyopathy (IPAC) Study, where high levels of relaxin-2 were reported to remain elevated for up to two months after delivery, associated with myocardial recovery through sodium and water excretion (131).

In contrast to the effects of PICP on the cardiac extracellular matrix, we believe that lysyl-oxidase levels have a higher impact on vascular remodeling (132). Lysyl-oxidase levels were decreased during pregnancy, possibly to support pregnancy-decreased arterial stiffness, and supposedly restrained by the protective effects of increased levels of relaxin-2 and ST2/IL33-receptor (133, 134). During the postpartum period, the increase in arterial stiffness imbalances extracellular matrix dynamics, favoring vascular fibrosis, explaining the high levels of lysyl-oxidase observed.

Regarding inflammation, we speculate that the higher CRP levels found late in pregnancy result from adaptations of the maternal immune system to preserve the fetoplacental unit, as supported by previous studies (135-137).

Similar to the literature, our participants revealed elevated levels, within the normal range (10-50 ng/mL) of PAI-1 in the 3rd trimester, followed by a significant postpartum fall (138, 139). We found raised levels of lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) in the 3rd trimester, which decreased in the postpartum period. In the atherosclerosis context, an increased LOX-1 is associated with higher oxidase low-density lipoprotein cholesterol and triglyceride levels (140, 141). However, it remains to elucidate if LOX 1 variation can be explained by changes in cholesterol levels during pregnancy and postpartum.

Methodologic considerations and study limitations

An important methodological aspect of our study was the longitudinal nature of the data and the application of an advanced statistical method (multivariate GLMM) to avoid the exclusion of participants with missing cardiovascular evaluations. This methodology allowed us to explore the impact of cardiovascular risk factors on LV hypertrophy reversal and arterial stiffness normalization without subgroup comparisons, which reduced the statistical power of the analysis. Another point worth mentioning is that this study was designed to explore pregnancy-induced remodeling and RR as a unique human model to investigate the cardiovascular impact of risk factors under more “uniform” conditions when compared to pathological conditions. Indeed, because pregnant women represent a more homogeneous population than heart failure patients (older, with many comorbidities and different medications), it becomes an invaluable “human model” to clarify the impact of hypertension, diabetes, and obesity without many confounding factors. We trust that this knowledge outperforms the information derived from animal models and can be cautiously translatable to the context of heart failure, even considering the limitations of pregnant women, who represent a younger and exclusively female population.

Our study had several limitations, such as a small sample size, which influenced the assessment of each risk factor impact, quantification of plasma biomarkers, and paired comparison.

Moreover, most participants with cardiovascular risk factors were enrolled in the study only in the 3rd trimester. These women had a higher incidence of non-attendance owing to duplication of evaluation complaints or professional reasons. enrolment bias may have affected our results. Accordingly, only fifty-five participants with cardiovascular risk factors agreed to participate and completed the follow-up evaluations, versus seventy-six healthy pregnant women. As already reported, the respondents in a voluntary study reported fewer risky behaviors (142). This precluded the subgroup analysis of each cardiovascular risk factor and their comparison. Indeed, a larger scale epidemiological study may be required to compare these groups, improving the assessment of the impact and severity of arterial hypertension, gestational diabetes and obesity; and a deeper molecular understanding of the changes observed in the most important predictors of hypertrophy reversal (CRP and ST2/IL33 receptor). With our study design, we can not exclude that the differences observed in plasma markers are the reflex of extra-cardiac gestation-induced adaptations. Finally, the COVID-19 pandemic compromised the cardiovascular follow-up evaluations of some participants owing to circulation restrictions and recommendations of the Directorate General of Health for pregnant women.

Conclusions

We found that cardiovascular pregnancy-induced reverse remodeling was characterized by significant regression of the LV chamber and improvement in diastolic function associated with a reduction in LA volume. In parallel, a significant increase in arterial stiffness and systemic vascular resistance was observed as soon as 1 month after delivery. Arterial hypertension is a common predictor of cardiac and vascular remodeling and RR, highlighting the importance of follow-up extension after delivery in women with cardiovascular risk factors following the recommendations of the American Heart Association 2021. In addition, CRP and ST2/IL33 receptors arise as potential predictive markers of postpartum hypertrophy reversal.

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Availability of data and materials

The data are available upon request.

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Subchapter ii. The extent of postpartum cardiac reverse remodeling is reflected in urine proteome

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Abstract

The impact of postpartum cardiac reverse remodeling (RR) on urinary proteome, particularly in pregnant women with cardiovascular risk (CVR) factors who show long-term increased risk of cardiovascular disease and mortality is unknown. We aim to profile the urinary proteome in pregnant women with/without CVR factors to identify proteins associated with postpartum RR.

Our study included a prospective cohort of 32 healthy and 27 obese and/or hypertensive and/or diabetic pregnant women that underwent transthoracic echocardiography, pulse-wave-velocity, and urine collection at the 3rd trimester and 6-months postpartum. Proteins were profiled by shotgun HPLC-MS/MS. Generalized linear mixed-effects models were used to identify associations between urinary proteins and left ventricle mass (LVM), a surrogate of RR.

The presence of at least one CVR factor was associated with worse LVM RR. We identify 5 and 11 proteins associated with high and low LVM regression, respectively. These proteins were functionally linked with insulin-like growth factor (IGF) transport and uptake regulation by IGF binding-proteins; platelet activation, signaling and aggregation and the immune system's activity.

Urine proteome reflects postpartum cardiac RR. Specifically, eight urine proteins (GSTP1, ARF1, FN1, SORT1, SIAE, GLA, ITIH4 and DGS2) show clinical value in identifying women at higher risk for incomplete postpartum RR.

Keywords

Pregnancy

Postpartum

Cardiovascular Reverse Remodeling

Cardiovascular Risk Factor

Hemodynamic overload

Urine

Proteome

Background

Pregnancy induces significant multisystemic changes to ensure a successful adaptation to fetus growth and development. Pregnancy's cardiovascular response to blood volume expansion triggers an increase in preload accompanied by a reduction of peripheral vascular stiffness and a subsequent compensatory decline in afterload (8, 20, 37). These circulatory adaptations induce cardiac remodeling, involving non-pathological left ventricle (LV) eccentric hypertrophy and slight diastolic dysfunction with left atrium (LA) enlargement (20, 37, 40) but with preserved ejection fraction (20, 40). This physiological cardiovascular remodeling may be influenced by cardiovascular risk (CVR) factors, such as arterial hypertension, gestational diabetes and obesity. Indeed, the cardiovascular remodeling in pregnant women with any of these CVR factors is characterized by diastolic dysfunction combined with an increased left ventricular mass (LVM) and increased systemic vascular resistances (64, 68, 74, 143, 144). Some authors suggest that women's heart fully recovers without cardiovascular complications during gestation and that global and segmental myocardial performances return to their pre-gravid state/condition in a process known as reverse remodeling (RR) (8, 37, 40). Although it has been reported that pregnant women with CVR factors have an increased risk for potential cardiovascular disease and mortality in the long term, few studies have explored their impact on cardiac RR during postpartum (114-116). We hypothesized that cardiac RR after delivery is associated with distinct proteomic traits in pregnant women with and without CVR factors and that these are mirrored in urine. As a plasma filtrate, urine can be viewed as a vast collection of products from cardiovascular activity. Therefore, it should harbor many proteins potentially differentiating physiological from pathological cardiac RR. Besides, urine analysis brings additional advantages when compared to plasma/serum, including sample stability (the bulk of proteolytic activity is completed in the bladder), lower matrix complexity (protein dynamic range in urine is about half of plasma's), and the non-invasive collection, excusing the need for health professionals (145, 146). Urine proteomics allows for uncovering potential markers and for developing predictive tools, such as the recently reported Heart Failure Predictor, a classifier based on 96 urinary peptides (147, 148). Identifying pregnant women with a higher risk of a pathological cardiac RR is important to establish a tight monitoring program and eventually extend the follow-up until full cardiac RR is attained. Thus, this study aimed to profile the urinary proteome in pregnant women with or without CVR factors and identify putative proteins associated with cardiac postpartum RR.

Methods and Design

Study design and participants recruitment

We designed a prospective cohort study, with participants being recruited at the Department of Obstetrics of *Centro Hospitalar Universitário São João (CHUSJ)* and *Unidade Local de Saúde de Matosinhos - Hospital Pedro Hispano (ULSM-HPH)*. The Ethics Committees of CHUSJ and ULSM-HPH approved the study (ID 201/18 and ID 154/20/RS, respectively). All included participants were willing and able to provide written informed consent. The confidentiality and data anonymity complied with the Declaration of Helsinki of 1964, revised in Fortaleza, in 2013. The full protocol of the present study has been published elsewhere (117).

Participants were recruited from March 2019 to March 2021 at their first medical appointment (in the 1st or 3rd trimester of pregnancy) or voluntarily through online forms available at https://perimyrobb.wordpress.com/.

The inclusion criteria were adult pregnant women (>18 years old) with or without CVR factors, namely chronic and/or gestational hypertension and/or gestational diabetes mellitus (DM) or type-2 DM, and/or obesity. Arterial hypertension was defined as systolic blood pressure (SBP) ≥140mmHg and/or diastolic blood pressure (DBP) ≥90mmHg measured in office or in-hospital before 20 weeks of gestation (118). Gestational hypertension was defined as arterial hypertension diagnosed after 20 weeks of gestation and resolving within 42 days postpartum. Gestational DM was diagnosed if 92≤fasting-glucose≥126mg/dL at 1st trimester or ≥180mg/dL or ≥153mg/dL, respectively, 1 or 2 hours after an oral glucose tolerance test (75g oral glucose load) performed at 24-28 pregnancy weeks. Type-2 DM was diagnosed through the following criteria: glycated haemoglobin≥6.5% or fasting plasma glucose≥126mg/dL or two-hour post-load venous plasma glucose≥200mg/dL after oral glucose tolerance test (149). Obesity was considered if the body mass index ≥30kg/m^2 before pregnancy.

Women with twin pregnancy, pre-existing cardiomyopathy, renal disease, chronic obstructive airway disease, active systemic infection, genetic syndromes or type-1 diabetes *mellitus* were excluded.

Two study groups were set according to the presence or absence of CVR factors defined above, resulting in the definition of a healthy group and an HDO group (which included any combination of hypertensive, diabetic and obese women).

Measurements

In the 3rd trimester (30-35 weeks), at the peak of cardiac remodeling (when cardiovascular adaptations are noticeable (8)) and 6-7 months after delivery (during RR), participants

underwent clinical characterization (maternal cardiovascular health, health-related habits, smoking habits, parity, medical history, demographics, obstetric and perinatal outcomes were obtained from questionnaires or electronic medical records), transthoracic echocardiography, pulse wave velocity (PWV) measurement and collection of urine samples at the Faculty of Medicine of the University of Porto. The LVM was defined as the surrogate for assessing cardiac RR.

Echocardiographic assessment

Conventional transthoracic echocardiography evaluation was performed with a 3 MHz phased-array probe (ACUSON SC2000 PRIME^{TM}) by a single operator and measurements obtained from standard views according to European Society of Cardiology recommendations for chamber quantification and diastolic function evaluation (Supplementary Table 1) (109, 110). At least three cardiac cycle images were acquired for data analysis. Two certified cardiologists independently analyzed, interpreted and harmonized the results. Myocardial deformation was assessed in LV through strain and strain rate analysis by Syngo Velocity Vector Imaging software, version 3.5 (Siemens Healthcare, Erlangen, Germany). The endocardium was tracked manually using a point-and-click approach, whereas the system automatically traced the epicardium and generated six segments. The tracing was manually readjusted to increase tracking accuracy, and strain curves for each segment were generated. The longitudinal strain was calculated using an apical four-chamber view.

Vascular stiffness evaluation

After 10 minutes of rest, brachial blood pressure was measured using Complior® device (Alam Medical, France). This device quantified arterial stiffness by carotid-femoral pulse wave velocity (cf-PWV), calculated from carotid-femoral distance/transit time, and peripheral augmentation index, defined as the ratio of late systolic pressure to early systolic pressure. The mean value of two cf-PWV measurements (whose difference was ≤0.5m/s) were performed in each session for each participant.

Urinary proteomic profiling

Fifty milliliters of first-morning urine samples were collected, centrifuged (2,370 × g, 15 minutes, 4□C), and stored at -80°C in each visit (3T and 6 months after delivery). Urine protein was concentrated using concentrators (cutoff-10kDa, Vivaspin 500, Sartorius Biotech, Göttingen, Germany) by centrifugation at 10,000 × g for 10 min at 10°C. The protein retentate was

solubilized in 100 µL of 0.3 M Tris pH 6.8 and 4% sodium dodecyl sulphate and quantified by a standard bicinchoninic acid assay (Pierce, ThermoFisher™, Rockford, IL, USA).

Proteome analysis was performed using a label-free shotgun approach to reveal the urinary protein profile changes from 3rd trimester to six months after delivery and the influence of the presence of at least one CVR. Briefly, for each sample, proteins were incubated 1h at RT in the dark for denaturation, reduction, and alkylation of the cysteines using a homogenization buffer composed of SDS 1%, TCEP 5 mM (646547 – Sigma-Aldrich), and chloroacetamide 20 mM (22790 – Sigma-Aldrich) in ammonium bicarbonate 50 mM (Sigma – 09830), pH8.

Then, auto Single-Pot Solid-Phase-enhanced Sample Preparation (auto-SP3) was performed on the Bravo (Agilent technologies) platform to isolate and digest proteins according to (150) with minor modifications. In brief, SP3 beads were prepared by mixing hydrophilic and hydrophobic Sera-Mag Speed-Beads (GE Healthcare (Chicago, IL, USA)) in a ratio of 1:1 (v/v), washed 3 times in water and reconstituted in water at a concentration of 25 µg bead/µL. After adding 6 µL of beads into 15 µg of proteins, 2 volumes of anhydrous acetonitrile (ACN) were added for a final concentration of 50%. Samples were agitated during 9 cycles at RT. Each cycle is divided in 2 steps of 800 rpm during 30 s and 200 rpm during 90 s. The supernatants were aspirated after 5 min on a magnet. The beads (with bound proteins) were washed 4 times with 80% ACN. For protein digestion, 75 µL of ammonium bicarbonate 50 mM, pH 8.0 containing 0.5 µg Sequencing Grade Modified Trypsin (V5111, Promega, Madison, Wisconsin, USA) were added in each samples. Following 12h digestion at 37°C, the samples were placed on a magnet for 2 min and the supernatants containing peptides were transferred in a new plate.

After sample clean up using C18 cartridge (Agilent Technologies, 5 µL bead volume, 5190-6532), the peptide solutions were speed-vac dried and resuspended in acetonitrile (ACN) 2%, formic acid (FA) 0.1% buffer.

A nanochromatographic system (Proxeon EASY-nLC 1200 - Thermo Fisher Scientific) was coupled on-line to a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific) using an integrated column oven (PRSO-V1 - Sonation). For each sample, peptides were loaded on a home-made 30 cm capillary column picotip silica emitter tip (75 µm inner diameter) with C18 resin (1.9 µm particles, 100 Å pore size, Reprosil-Pur Basic C18-HD resin, Dr. Maisch) after an equilibration step in 100 % solvent A (H_2O, FA 0.1 %). Peptides were eluted with a multi-step gradient using 5 to 25 % solvent B (ACN 80 %, FA 0.1 %) for 86 min, 25 to 40 % for 14 min and 40 to 95 % during 9 min at a flow rate of 250 nL/min over 118 min. The column temperature was set to 60°C.

Mass spectra were acquired using Xcalibur software using a data-dependent Top 10 method with a survey scan (300-1700 m/z) at a resolution of 70,000 and MS/MS scans (fixed first mass 100 m/z) at a resolution of 17,500. The automatic gain control (AGC) target and maximum injection

time for the survey scans and the MS/MS scans were set to 3.0E+06, 50ms and 1.0E+06, 80ms, respectively. The isolation window was set to 1.6 m/z and normalized collision energy fixed to 28 for HCD fragmentation. We used a minimum AGC target of 1.0E+04 for an intensity threshold of 1.3E+05. Unassigned precursor ion charge states as well as 1, 7, 8 and >8 charged states were rejected and peptide match was disabled. Exclusion of isotopes was enabled and selected ions were dynamically excluded for 45 seconds.

Raw data were analyzed using MaxQuant software version 2.0.3.0 (151) using the Andromeda search engine (152). The MS/MS spectra were searched against a UniProt *Homo sapiens* database (download in 13/12/2021). Usual known mass spectrometry contaminants and reversed sequences of all entries were included. Andromeda searches were performed, choosing trypsin as a specific enzyme with a maximum number of two missed cleavages. Possible modifications included carbamidomethylation (Cys, fixed), oxidation (Met, variable), Nter acetylation (variable) and deamidation (Asn, Gln, variable). The mass tolerance in MS was set to 20 ppm for the first search then 4.5 ppm for the main search and 20 ppm for the MS/MS. Maximum peptide charge was set to seven and seven amino acids were required as minimum peptide length. The “match between runs” feature was applied for all samples with a maximal retention time window of 0.7 minutes. One unique peptide to the protein group was required for the protein identification. For quantification, we only considered protein groups with two unique peptides. A false discovery rate (FDR) cutoff of 1 % was applied at the peptide and protein levels.

Proteins showing to be independently associated with cardiac RR were subjected to protein–protein interaction and functional enrichment analyses with STRING (version 11.5) webtool (accession date: 28th March 2023). A score of 0.4 was set as the minimum threshold for consideration of the validated and putative interactions.

Quantification of Insulin-like growth factor-1 through ELISA Assay Kit

Fifty milliliters of first-morning urine samples were collected, centrifuged (2,370 × g, 15 minutes, 4ºC), and stored at -80°C in each visit (3T and 6 months after delivery). We quantified Insulin-like growth factor-1 (IGF-1, DY291, R&D Systems) in pairwise urine samples collected 3T and 6 months after delivery.

Three operators, blinded to clinical and echocardiographic information, assessed this ELISA assay kit on each urine sample using a commercially available kit following the manufacturer's instructions.

Statistical analysis

Clinical parameters, physical assessment, echocardiographic and cf-PWV values, and urinary proteins detected and quantified were gathered on a database and analyzed using *lmer*, *ggplot2* and *gtsummary* R packages, in the R statistical software environment version 4.2.1. (119).

Continuous variables are expressed as median, minimum, and maximum, as appropriate. Data normality was checked after examining the histograms and Q-Q plots. Absolute values and relative frequencies are presented for categorical variables. Protein intensity and IGF-1 quantification were log2-transformed and scaled.

A multivariable generalized linear mixed-effects model (GLMM) was used to explore pregnancy-associated cardiac RR by including the 3rd trimester of pregnancy and 6 months after delivery. The analysis of LVM progression during pregnancy and postpartum was adjusted for CVR factors, age at recruitment moment, parity, weight gain until 3rd trimester and evaluation moments (3rd trimester and 6 months after delivery) due to their clinical relevance. Only adjusted statistically significant proteins with an absolute effect size (beta coefficient) higher than 6 standard deviations and IGF-1 quantification were considered for exploratory analysis. $P<0.05$ was deemed significant.

Results

Baseline demographic and clinical characterization

We included 59 participants that had paired urine samples at 32 [29; 39] weeks of gestation and 6 [4;9] months after delivery, with a median age of 34 [26; 44] years-old (50.8% nulliparous). The demographic and clinical characteristics of participants are shown in Table 1. Twenty-seven (45.8%) women were diagnosed with at least one CVR factor prior to or during gestation (Table 1). The study groups were matched for smoking habits (p=0.477), parity (p=0.604) and obstetric outcomes (type of delivery, p=0.279; gestational age at delivery, p=0.291) (Table 1). In addition, women with CVR factors were older (36 [28; 44] years *versus* 34 [26; 41] years, p=0.049) than healthy participants. Aspirin was the only drug prescribed to 4 healthy pregnant women. Other pharmacological classes were prescribed exclusively to the participants in the HDO group. Regarding vascular reverse remodeling, a significant increase in cf-PWV and, consequently, mean arterial pressure was noticed from 3rd trimester to 6 months after delivery in both study groups (Table 2). Women in the HDO group had higher blood pressure and arterial stiffness in both evaluation moments (Table 2). Three pregnancies developed pre-eclampsia (two hypertensive and one without any CVR factor), and one developed cholestasis of pregnancy. Two experienced other peripartum complications (arrhythmia and uncontrolled arterial hypertension).

Characterization of Cardiac Reverse Remodeling

Table 3 shows cardiac systolic and diastolic function indices. Both groups similarly decreased heart rate and cardiac output from the 3rd trimester to 6 months after delivery. Interestingly, global longitudinal strain was the only parameter significantly reduced in the 3rd trimester of HDO pregnant women (-23.3 [-17.9;-29.4]% versus -21.0 [-24.0;-15.3]%, p=0.019), indicating subclinical deterioration of systolic function. Pregnancy impaired diastolic function in both groups, as assessed by a significant decrease of E/A and an increase in E/e' and left atrial volume (LAV) compared to the postpartum period (Table 3). Again, pregnancy seems to mask the diastolic changes observed in the HDO group, as these differences only become evident and significantly different from those in the healthy group at 6 months postpartum (E/e', 6.1 [3.8;9.5] versus 5.6 [3.7;7.7], p=0.008; LAV, 43 [24;79]mL versus 38 [24;55]mL, p=0.045). At postpartum, pregnant women showed a significant reduction in LVM, volume and relative wall thickness, irrespective of the presence of CVR factors. However, this reduction was much less noticeable in the HDO group, which maintained values significantly higher than those in the healthy group (Table 3).

Table 1: Demographic and clinical baseline characteristics.

	Healthy Group n=32	**HDO Group n=27**	**p-value**
Age, years	34 [26;41]	36 [28;44]	**0.049**
Height, meters	1.64 [1.55;1.77]	1.66 [1.56;1.78]	0.492
Pre-pregnancy body mass index, kg/m^2	22.3 [17.9;29.7]	27.2 [19.8;38.8]	**0.001**
Cardiovascular risk factors			
Arterial hypertension n (%)	n/a	16 (59.3)	n/a
Obesity n (%)	n/a	11 (40.7)	n/a
Gestational diabetes n (%)	n/a	12 (44.4)	n/a
Type 2 Diabetes n (%)	n/a	1 (3.7)	n/a
Pharmacological therapy during pregnancy			
Aspirin	4 (12.5)	14 (51.9)	**0.002**
Metformin	0 (0.0)	11 (20.0)	**<0.001**
Insulin	0 (0.0)	10 (18.2)	**<0.001**
Beta-blockers	0 (0.0)	2 (7.4)	**0.205**
Nifedipine	0 (0.0)	1 (3.7)	**0.458**
Smoking habits			
Non-smoker n (%)	18 (56.3)	20 (74.1)	0.477
Smoker n (%)	3 (9.4)	2 (7.4)	
Stopped only during pregnancy n (%)	1 (3.1)	1 (3.7)	
Stopped at pregnancy beginning n (%)	2 (6.3)	2 (7.4)	
Ex-smoker n (%)	8 (25.0)	2 (7.4)	
Primiparous women n (%)	15 (46.9)	15 (55.6)	0.604
Pregnancy weeks at delivery	39 [33;41]	39 [35;40]	0.291
Type of delivery			
Vaginal delivery n (%)	19 (59.4)	20 (74.1)	0.279
Caesarean delivery n (%)	13 (40.6)	7 (25.9)	

Evaluation moment			
Pregnancy, weeks	31 [29;39]	32 [30;37]	0.146
Postpartum, months	7 [5;9]	6 [4;9]	**0.033**

Values expressed by median [minimum; maximum].

Table 2: Physical and vascular assessment

Group	**Healthy Group**			**HDO Group**			**Healthy Group vs HDO Group**	
Parameter / Moment	**3rd Trimester**	**6 Months Postpartum**	**p-value**	**3rd Trimester**	**6 Months Postpartum**	**p-value**	**3rd Trimester p-value**	**6 Months Postpartum p-value**
Maternal body weight, kg	70 [56;101]	61 [46;98]	**<0.001**	82 [59;116]	76 [50;113]	**<0.001**	**0.004**	**0.003**
Weight variation, kg	11 [-3;20]	-10 [-19;2]	**<0.001**	9 [-1;18]	-8 [-19;2]	**<0.001**	0.089	**0.043**
Systolic blood pressure, mmHg	110 [90;130]	110 [90;130]	0.753	110 [90;150]	120 [104;150]	**0.009**	0.160	**<0.001**
Diastolic blood pressure, mmHg	65 [60;80]	76 [60;90]	**<0.001**	80 [50;96]	82 [60;115]	**<0.001**	**<0.001**	**<0.001**
Mean arterial pressure, mmHg	80 [70;97]	88 [70;102]	**<0.001**	90 [67;114]	95 [77;124]	**<0.001**	**0.003**	**<0.001**
Pulse wave velocity, cm/s	6.0 [4.5;7.3]	6.7 [5.0;8.6]	**<0.001**	6.7 [5.3;8.9]	7.2 [5.5;9.4]	**<0.001**	**<0.001**	**0.021**

Values expressed by median [minimum; maximum].

Table 3: Echocardiographic assessments

	Healthy Group			HDO Group			Healthy Group vs HDO Group	
Parameter/ Evaluation moment	**3rd Trimester**	**6 Months Postpartum**	**p-value**	**3rd Trimester**	**6 Months Postpartum**	**p-value**	**3rd Trimester p-value**	**6 Months Postpartum p-value**
Heart rate, beats/min	78 [59;102]	62 [44;79]	**<0.001**	82 [64;101]	66 [47;82]	**<0.001**	0.364	0.027
LVEF, %	60 [51;72]	62 [54;73]	**0.008**	61 [53;74]	61 [53;72]	0.493	0.622	0.163
Global Longitudinal Strain, %	-23.3 [-17.9;-29.4]	-23.7 [-16.7;-28.8]	0.753	-21.0 [-24.0;-15.3]	-22.3 [-26.8;-17.5]	0.140	**0.019**	0.073
Mitral inflow parameters								
E, cm/s	88 [57;119]	82 [58;100]	**0.009**	92 [51;107]	80 [50;106]	0.061	0.867	0.690
A, cm/s	59 [40;93]	48 [27;79]	**<0.001**	66 [35;101]	52 [33;94]	**<0.001**	0.171	0.097
E/A	1.43 [0.84;2.13]	1.69 [1.00;3.00]	**<0.001**	1.33 [0.80;2.31]	1.47 [1.00;3.00]	**0.007**	0.161	0.062
Pulmonary vein inflow parameters								
S, cm/s	55 [37;83]	48 [36;69]	**0.028**	56 [38;75]	51 [35;77]	0.076	0.522	0.089
D, cm/s	50 [37;95]	52 [31;83]	0.637	52 [33;77]	49 [34;70]	0.088	0.089	0.402
S/D	0.93 [0.57;1.55]	1.08 [0.56;1.70]	0.122	1.17 [0.70;1.44]	1.15 [0.73;1.48]	0.557	0.332	0.152
TDI mitral annulus								
Septal								
e' sept, cm/s	10.8 [7.5;16.0]	12.2 [8.2;17.8]	**0.001**	10.1 [6.4;14.1]	10.8 [7.2;13.3]	0.088	0.103	**0.006**
Lateral								
e' lat, cm/s	17.2 [10.2;27.1]	17.9 [11.4;24.7]	0.079	15.5 [10.8;21.7]	15.3 [10.3;21.1]	0.732	**0.054**	**0.005**
E/e' average (E/e')	6.3 [4.0;8.4]	5.6 [3.7;7.7]	**<0.001**	7.0 [3.2;10.2]	6.1 [3.8;9.5]	**0.008**	0.122	**0.008**
Stroke volume, mL	62 [44;82]	60 [38;82]	0.131	60 [44;86]	60 [32;82]	0.166	0.486	0.847
Cardiac output, L/min	5.0 [3.1;6.2]	3.7 [2.4;5.5]	**<0.001**	4.9 [3.1;6.7]	3.8 [2.4;5.8]	**<0.001**	0.865	0.905
Relative wall thickness	0.34 [0.24;0.44]	0.31 [0.23;0.43]	**0.007**	0.37 [0.25;0.51]	0.34 [0.24;0.43]	**0.017**	0.061	0.093
LV mass, g	129 [83;166]	99 [78;133]	**<0.001**	128 [92;175]	112 [67;164]	**0.003**	0.475	**0.014**
Maximum LA volume, mL	49 [34;77]	38 [24;55]	**<0.001**	51 [31;73]	43 [24;79]	**0.006**	0.317	**0.045**
LV end-diastolic volume, mL	94 [73;118]	78 [62;100]	**<0.001**	101 [55;128]	88 [49;114]	**0.006**	0.209	**0.029**

Values expressed by median [minimum; maximum].

Identification of Proteins in Urine Sample

Over 2700 proteins were identified in urine samples through LC-MS/MS, after excluding reverse sequences, potential contaminants and proteins only identified by site. To explore the predictive power of the urinary proteins, we only considered proteins with two or more peptides identified and proteins with less than 20% of missing values, resulting in a selection of 324 proteins (Supplemental table 2).

Urinary proteins showing an association with Cardiac Reverse Remodeling

The LVM was defined as the outcome in a multivariable generalized linear mixed-effects model (GLMM) to identify urinary proteins associated with postpartum cardiac RR. In the pre-specified GLMM containing only clinical variables, the presence of at least one CVR factor (β: 1.4, 95% CI [3.30; 24.0], p=0.011) was associated with low LVM regression after delivery (Table 4). Upon addition of each one of 324 proteins on TOP to the pre-specified model, seventeen urinary proteins were associated with LVM (Figure 1A and 1B). According to STRING network configuration, many of these proteins interact amongst them, participating in common pathways. This includes the regulation of insulin-like growth factor (IGF) transport and uptake by IGF binding proteins (involving Complement C3 [C3], Fibronectin [FN1], Serotransferrin [TF] and Trans-Golgi network integral membrane protein 2 [TGOLN2]); platelet activation, signaling and aggregation (including Inter-alpha-trypsin inhibitor heavy chain H4 [ITIH4], Serotransferrin [TF], Fibronectin [FN1] and Cell division control protein 42 homolog [CDC42]) and immune system (namely, Complement C3 [C3], Fibronectin [FN1], Cell division control protein 42 homolog [CDC42], ADP-ribosylation factor 1 [ARF1], Alpha-galactosidase A [GLA], Ubiquitin-like modifier-activating enzyme 1 [UBA 1], Pro-cathepsin H [CTSH] and Glutathione S-transferase P [GSTP1]) (Figure 2).

We then evaluate the added value of the proteins to the clinical model to predict LVM regression. For this, we only considered relevant urinary proteins with an absolute magnitude (expressed by the beta coefficient) higher than 6 (Inter-alpha-trypsin inhibitor heavy chain H4 [ITIH4], Glutathione S-transferase P [GSTP1], Fibronectin [FN1], ADP-ribosylation factor 1 [ARF1], Alpha-galactosidase A [GLA], Sialate O-acetylesterase [SIAE], Sortilin [SORT1], Desmoglein-2 [DSG2]). Nevertheless, the model performance improved significantly ($p<0.001$, Table 5). Of note, the combination of the 8 proteins, or the single addition of the proteins GSTP1, SORT1, or DSG2 increased model performance regarding LVM regression (lowest AIC and higher R^2).

Table 4: Pre-specified clinical variables multivariable GLMM for LVM

Parameter	Clinical Model	
	β, 95% CI	p-value
Follow-up time	-20.00 [-26.00;-15.00]	**<0.001**
Age	-0.79 [-2.10;0.50]	0.200
Cardiovascular risk group	14.00 [3.30;24.00]	**0.011**
Nulliparous	-5.70 [-17.00;5.10]	0.300
Weight gain during pregnancy	0.28 [-0.75;1.30]	0.600

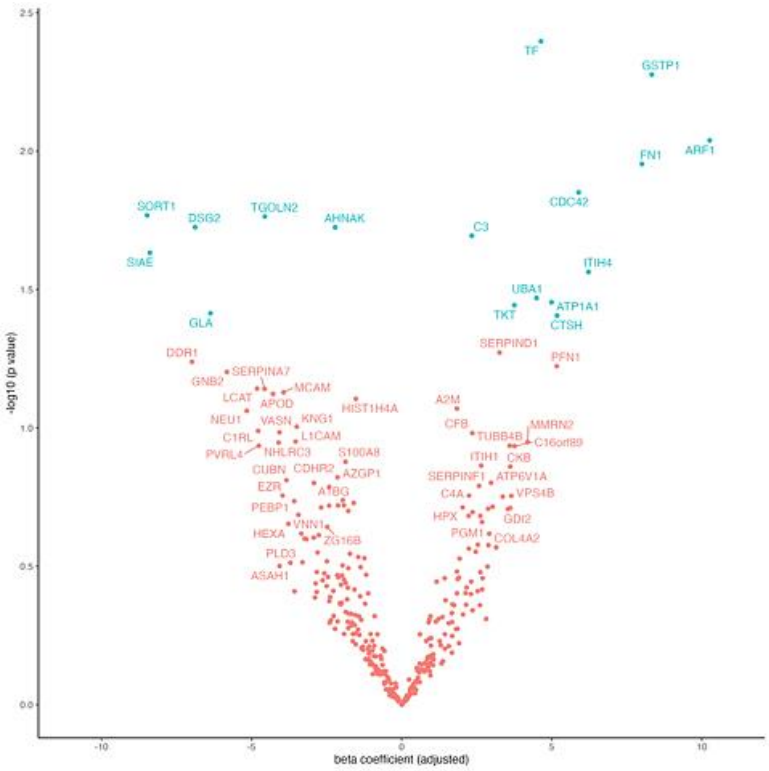

Figure 1A: Volcano Plot from multivariable generalized linear mixed-effects models (adjusted to cardiovascular risk factors, age at recruitment moment, parity, weight gain until 3rd trimester, and evaluation moments) showing the distribution of the proteins according to their effect (beta coefficient) and significance level (-$\log_{10}$(p-value)) regarding LVM regression. Proteins are identified with the respective gene name. Red labels are non-significant associations whereas blue labels signify significant associations.

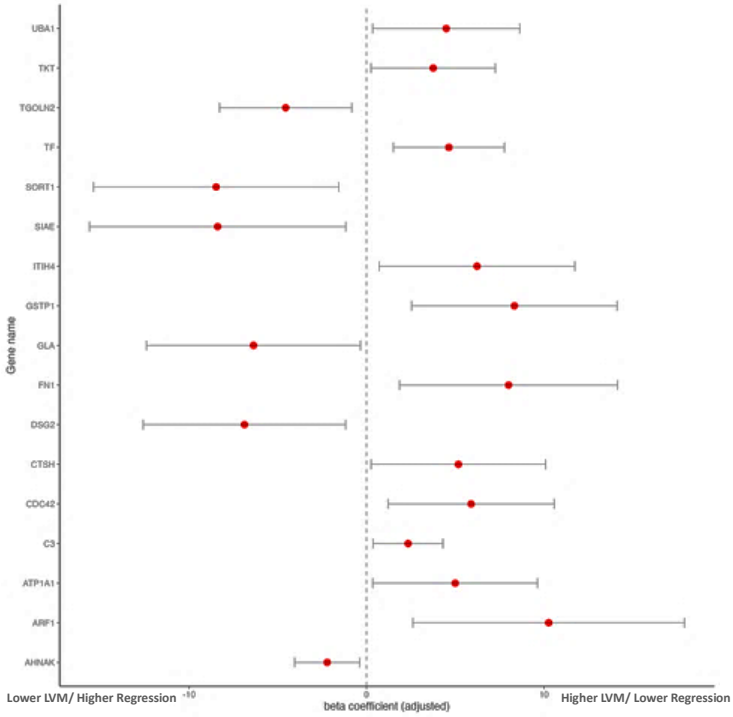

Figure 1B: Urine proteins showing a significant association with LVM, according to the GLMM.

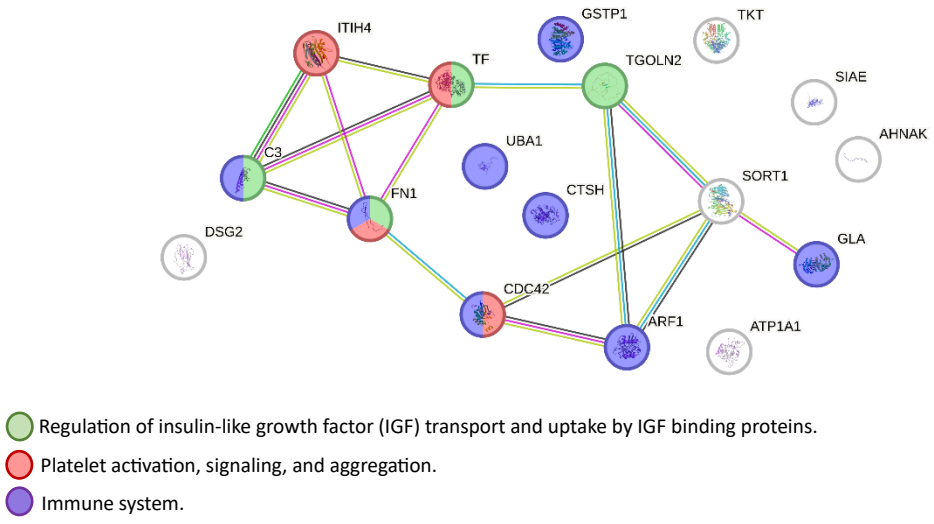

Figure 2: STRING Protein-Protein interaction network of the 17 most relevant proteins associated with postpartum LVM, according to a GLMM. Each node represents a protein, identified with the respective gene name. The most relevant biological processes found through gene ontology enrichment analysis are depicted through node colors. Each type of protein interaction is represented by a different edge color (cyan: known interaction from curated databases, pink: experimentally determined; yellow: text mining; black: co-expression; green: predicted interaction from gene neighborhood).

Table 5: Performance of multivariable generalized linear mixed-effects model.

	AIC	R^2 (cond)	R^2 (marg.)	RMSE
Pre-specified clinical variables multivariable GLMM	1032.8	0.63	0.24	12.087
Pre-specified clinical variables multivariable GLMM + GSTP1	861.9	0.70	0.25	10.015
Pre-specified clinical variables multivariable GLMM + ARF1	864.6	0.68	0.25	10.622
Pre-specified clinical variables multivariable GLMM + FN1	1024.3	0.67	0.28	11.368
Pre-specified clinical variables multivariable GLMM + SORT1	870.1	**0.72**	0.26	9.829
Pre-specified clinical variables multivariable GLMM + SIAE	889.8	0.61	0.29	12.535
Pre-specified clinical variables multivariable GLMM + GLA	959.2	0.62	0.28	12.226
Pre-specified clinical variables multivariable GLMM + ITIH4	1027.8	0.61	0.27	12.309
Pre-specified clinical variables multivariable GLMM + DSG2	837.2	**0.70**	0.25	10.482
Pre-specified clinical variables multivariable GLMM + panel of last 8 urinary proteins	621.0	**0.70**	0.28	9.638

Legend: AIC, Akaike Information Criterion; ARF1, ADP-ribosylation factor 1; cond, conditional; DSG2, desmoglein-2; FN1, fibronectin; GLA, alpha-galactosidase A; GSTP1, glutathione S-transferase P1; ITIH4, inter-α-trypsin inhibitor heavy chain 4; marg., marginal; R^2, coefficient of determination; RMSE, root mean square error; SORT1, sortilin; SIAE, sialate O-acetylesterase.

Insulin-like growth factor-1 showing an association with Cardiac Reverse Remodeling

Insulin-like growth factor-1 (IGF-1) is involved in the pathway of the regulation of insulin-like growth factor (IGF) transport and uptake by IGF-binding proteins. In our cohort, the IGF-1, quantified through ELISA Assay Kit in urine samples, was associated with low LVM regression after delivery (Table 6).

Table 6: Multivariable generalized linear mixed model for LVM including IGF-1 quantification.

Parameter	β, 95% CI	p-value
Follow-up time	-13.00 [-22.00;-4.60]	**0.003**
Age	-0.82 [-2.10;0.50]	0.200
Cardiovascular risk group	10.00 [-0.47;21.00]	0.060
Nulliparous	-6.90 [-18.00, 4.10]	0.200
Weight gain during pregnancy	0.09 [-0.93;1.10]	0.900
Log-transformed insulin-like growth factor-1	6.10 [0.18;12.00]	0.044

Discussion

This work is the first exploratory prospective cohort study that included healthy pregnant women and with CVR factors to identify urinary proteins associated with cardiac RR, as assessed through LVM variation, from the 3rd trimester to 6 months after delivery.

Our cohort showed a significant regression of LVM and volume, and LAV, associated with an improvement of diastolic function (E/e') from the 3rd trimester to 6 months after delivery, confirming previous reports (8, 40). Although pregnant women with and without CVR factors have similar LVM in the 3rd trimester, pregnant women with CVR factors showed lower LVM regression, that is, incomplete cardiac RR at 6 months postpartum compared to healthier ones. The same holds true for diastolic function in the CVR factors group. Indeed, the presence of at least one CVR factor (arterial hypertension, obesity, gestational diabetes or type-II diabetes) was associated with worse LVM regression. Additionally, women with these cardiovascular comorbidities had higher postpartum cardiac volumes and LV filling pressure than those in the healthy group, as confirmed by previous studies focusing on hypertensive diseases of pregnancy (43, 72, 80, 91, 92). In addition, both study groups showed an increase of arterial stiffness between evaluation moments, being significantly elevated in women with CVR factors, which is in line with previous reports (63, 79-81, 102, 103, 105).

To identify proteins associated with higher cardiac RR, we profiled the urine proteome before and after delivery in pregnant women with and without CVR factors. We then assessed whether these proteins could independently predict postpartum LVM. Alpha-galactosidase A (GLA), sialate O-acetylesterase (SIAE), trans-golgi network integral membrane protein 2 (TGOLN2), sortilin (SORT1), desmoglein-2 (DSG2) and neuroblast differentiation-associated protein (AHNAK) were found to be potential indicators of a higher LVM regression ($\beta<0$). In contrast, serotransferrin (TF), inter-α-trypsin inhibitor heavy chain 4 (ITIH4), complement C3 (C3), transketolase (TKT), fibronectin (FN1), ubiquitin-like modifier-activating enzyme 1 (UBA1), cell division control protein 42 homolog (CDC42), glutathione s-transferase P1 (GSTP1), sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1), ADP-ribosylation factor 1 (ARF1) and pro-cathepsin H (CTSH) were associated with a lower LVM regression after delivery ($\beta>0$).

Many putative proteins associated with LVM were found to be functionally integrated with biological pathways, such as the *regulation of insulin-like growth factor (IGF) transport and uptake by IGF-binding proteins*; *platelet activation, signaling and aggregation* and *immune system activity*. These signaling pathways evidence a complex systemic interplay between organs during pregnancy, including women's response to partum-induced cardiac RR.

For instance, the liver mainly produces insulin-like growth factor-I (IGF-1) under the control of growth hormone and insulin endocrine stimulation (153-155). IGF-1 can bind to IGF-1 receptors and activate the Akt pathway (155), thereby promoting growth. IGF-1/PI3K/Akt pathway is involved in LV hypertrophy (156), including pregnancy-induced cardiac hypertrophy (157). Thus, IGF bioavailability regulation, through binding to IGF-binding proteins, may impact IGF-induced pro-hypertrophic activity in cardiac muscle. In addition, IGF-1 can stimulate nitric oxide synthase in endothelial and vascular smooth muscle cells through Akt-catalysed phosphorylation, leading to vasodilatory, anti-inflammatory, anti-apoptotic and angiogenic effects (154, 158). Interestingly, three out of four proteins associated with IGF transport and uptake (TF, C3 and FN1) were associated with worse LVM regression, which suggests that IGF signaling blockage can be a protective mechanism against cardiac hypertrophy in the pregnancy setting. Corroborating it, the IGF-1 levels quantified in urine samples of our cohort were associated with low LVM regression after delivery. Among the proteins mapped to this pathway, we reported for the first time that FN1 is associated with low LVM regression. Indeed, FN1 has been correlated with cardiac remodeling induced by volume overload and arterial hypertension (159). Fibronectin accelerates pathological hypertrophy, characterized by increased cardiomyocyte area, enhanced myofibrillogenesis and sarcomeric assembly, leading to the expression of fetal genes, such as ANP, BNP, SkA, and MHC (159, 160). Thereby, FN1 is a potential target for modification of hypertrophic phenotype (160). Intimately correlated with an increase in FN1 is the ubiquitin-proteasome pathway, responsible for the degradation of most intracellular proteins in the heart (161, 162). For instance, UBA1, a protein involved in the initial step of ubiquitylation (161) whose role in cardiac remodeling and RR regulation remains unclear, was associated with lower LVM regression in our cohort. In fact, these results did not come as a surprise as we have previously shown that ubiquitin correlated positively with interventricular septum and posterior wall thickness (surrogates of cardiac hypertrophy) in patients with aortic stenosis undergoing valve replacement (163). Moreover, the inhibition of UBA1 by PYR-41 was linked to a significant decrease in blood pressure, cardiac hypertrophy, fibrosis, oxidative stress and inflammation and improved angiotensin II-induced cardiac contractile dysfunction (161).

According to STRING's functional analysis, some proteins associated with postpartum LVM, namely FN1, TF, ITIH4, and CDC42, are involved in *platelet activation, signaling and aggregation*. Platelets are actively involved in regulating vascular tone and inflammation and participate in the immune system, showing an important role in hemostasis, thrombosis and in the pathophysiology of uteroplacental diseases (164, 165). Increased platelet aggregation

accompanied by a reduction of circulating platelets has been reported throughout pregnancy (165-167). The decrease in platelet count may result from physiologic hemodilution or from platelet aggregation. In this case, it may lead to uteroplacental diseases such as recurrent miscarriage and pre-eclampsia (165). In a preeclamptic condition, decreased prostacyclin combined with an increased thromboxane A2 production leads to uteroplacental and systemic vasoconstriction, increased platelet aggregation and a consequent reduction of uteroplacental blood flow. The low number of preeclampsia cases present in our cohort, may be explained since approximately half of the participants with CVR factors were taking acetylsalicylic acid, a platelet aggregation inhibitor, according to the risk for preeclampsia estimated by *The Fetal Medicine Foundation* algorithm. Indeed, the American College of Obstetricians and Gynecologists (ACOG) recommended low-dose aspirin prophylaxis for women with a high risk of pre-eclampsia, prescribed before the 16^{th} week of pregnancy (168). Syncycial transferrin receptors have been reported to be increased in gestational diabetes and arterial hypertension as a possible compensatory mechanism to prevent iron depletion due to placental dysfunction (169). Serotransferrin (TF) is involved in iron transfer from the placenta to the fetus through the transferrin receptor in the syncytiotrophoblast membrane. Therefore, it rises towards the end of pregnancy to meet the elevated iron requirements for fetus development (170). Our findings suggest that CVR factors mediate the positive association between TF and LVM (i.e., higher gestational TF is associated with worse cardiac mass regression), as observed in our study and supported by studies showing TF overexpression in women with CVR factors (169). Regarding *platelet activation, signaling and aggregation*, ITIH4 belongs to the ITIH family and stabilizes the extracellular matrix (171). Therefore, high levels of ITIH proteins may have an inhibitory effect on the degradation of extracellular matrix, thereby maintaining anti-angiogenic (172) and hypertrophy effects. This might explain its inverse association with LVM regression. Besides, higher urine ITIH4 levels have been observed in gestational diabetes and correlate with vascular inflammation and immune dysfunction as the disease progresses (173). CDC42 holds a critical role in cardiomyocyte proliferation, sarcomere organization and cell-cell adhesion (174). Furthermore, CDC42 is a member of the Rho GTPase family and may regulate LV hypertrophy, directly controlling non-contractile actin and microtubules cytoskeleton formation through JNK anti-hypertrophic pathway (175, 176). However, CDC42 shows controversial results, having been described as either pro- or anti-cardiac hypertrophic signaling actions in response to physiological and pathological conditions, depending on the experimental setting (177, 178). In our cohort, which included diabetic and hypertensive women, CDC42 demonstrated a positive correlation with LVM. This observation is consistent

with the hormone-induced proliferative state of pregnancy. Of note, if and how the regulation of platelet-mediated hemostasis through FN1/TF/ITITH4/CDC42 impacts cardiovascular remodeling during pregnancy remains elusive and deserves further scrutiny.

According to STRING, the *immune system* is also an important player among the proteins deemed to be more strongly associated with cardiac RR. All phases of pregnancy, from implantation to fetal growth and parturition, require a fine-tuning of immunological processes (179-181), consistent with pregnancy's cardiovascular adaptations. We found the complement system protein C3 to be positively correlated with LVM. Several studies support this observation, i.e., that C3 inhibition did not affect the cardiac maladaptive remodeling in response to pressure overload (182) as well as restrains hypertrophy and improves cardiac function and survival in coronary heart disease (183). Among the 8 proteins mapped to the immune system, GSTP1, an anti-inflammatory protein, was upregulated in the hypertrophied LV to balance elevated levels of reactive oxygen species in heart failure patients (184, 185). Furthermore, GSTP1 was a sensitive predictor of LV function in heart failure, with higher specificity than NT-proBNP, and an independent predictor of adverse cardiac remodeling (184, 185).

The pre-specified clinical model to assess the probability of postpartum RR found that 8 proteins (ITIH4, GSTP1, FN1, ARF1, GLA, SIAE, SORT1, DSG2) could improve the model's performance individually (GSTP1, DSG2 and SORT-1) or combined. Therefore, we developed the first model, including clinical variables and urinary proteins, to assess cardiac RR, through LVM quantification in pregnancy. Regarding the proteins relevant to improve the robustness of the GLM model, we found an inverse association between GLA, DSG2, SIAE and SORT1 expression and LVM. Curiously, despite most of these not being described previously in the context of cardiac remodeling or RR (186-189), they do predict postpartum cardiac RR.

In the end, we found that 8 proteins (ITIH4, GSTP1, FN1, ARF1, GLA, SIAE, SORT1, DSG2) could singly or in multiplex improve the performance of a pre-specified clinical model to assess the probability of cardiac RR in the postpartum setting. Therefore, we developed the first model, including clinical variables and urinary proteins, to assess cardiac RR, through LVM quantification, in pregnancy context.

Curiously, despite most of these not having been described previously in the literature in the context of cardiac remodeling or RR, they improve the robustness of the GLM model to predict postpartum cardiac RR. This highlights the relevance of the present study and the need to validate and unravel the involved molecular mechanisms and pathways.

Regarding study limitations, COVID-19 pandemic strongly impacted the progression of this study by limiting participants' recruitment and cardiovascular evaluation. This resulted in follow-up losses and, consequently, a reduced sample size, compromising the proteomics analysis according to the study group.

In conclusion, we found that cardiac RR was characterized by significant LV chamber regression and diastolic function improvement. However, pregnant women with CVR factors revealed compromised LVM regression and diastolic function improvement at postpartum. The proteins GSTP1, ARF1, FN1, SORT1, SIAE, GLA, ITIH4 and DGS2 were independently associated with LVM regression, and all improved the performance of a model for predicting cardiac RR. Indeed, the proteomic profile of urine reflected the cardiac reverse remodeling process, endorsing urine protein analysis as an alternative or complementary approach to assess the risk of postpartum incomplete cardiac RR.

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Availability of data and materials

The mass spectrometry proteomics data have been deposited to the PRIDE Archive (http://www.ebi.ac.uk/pride/archive/) via the PRIDE partner repository(190) with the data set identifier PXD042655.

Author contribution

Conceived the ideas or experimental design of the study	Inês Falcão-Pires
Performed cardiovascular evaluations/data collection	Ana F. Ferreira; Maria J. Azevedo
Performed LC MS/MS	Thibaut Douché; Mariette Matondo
Data analysis and interpretation	Ana F. Ferreira; Juliana Morais; Fábio Trindade; Francisca A. Saraiva; Sílvia O. Diaz; António S. Barros
Primary author	Ana F. Ferreira
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Provided support to crucial research component: echocardiographic assessment	Carla Sousa
Provided support to crucial research component: urinary proteomic profile	Fábio Trindade; Thibaut Douché; Mariette Matondo; Rui Vitorino; António S. Barros
Provided support to crucial research component: statistical analysis	Francisca A. Saraiva; Sílvia O. Diaz; António S. Barros
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Supplemental Table 1: Echocardiographic equations applied in the present study.

Echocardiographic Variables	**Equations**	**Unit**
LVM	*0.8×(1.04×[(LVEDd+PWd+IVSd)^3–LVEDd^3]+0.6)*	g
RWT	*2×PWd/LVEDd*	n/a
Ejection fraction	*LVIDd^3–LVIDs^3/LVIDd^3×100*	%
LV cardiac output	*LV stroke volume x Heart rate*	L/min
Mean arterial pressure	*(SBP + 2 x DBP)/3*	*mmHg*
Systemic vascular resistance	*80 x mean arterial pressure / LV CO*	dyn.s.cm^{-5}
E/e'	*E mitral inflow velocity / average of Early Diastolic Septal and Lateral mitral annular velocity*	n/a

Legend: CO – cardiac output; DBP – diastolic blood pressure; IVSd - interventricular septum thickness in diastole; LV – left ventricular; LVEDd - left ventricular end-diastolic diameter; LVIDd - left ventricular internal end-diastolic dimension; LVISd - left ventricular internal end-systolic dimension; LVM – left ventricular mass; PWd - left ventricular posterior wall thickness in diastole; RWT – relative wall thickness; SBP – systolic blood pressure.

Supplemental Table 2: Urinary proteins identified through LC-MS/MS and included in pre-specified clinical variables multivariable GLMM for LVM.

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
P07911	UMOD	118	-1.7	-4.9, 1.5	0.3
P0DOY2	IGLC2	102	-1.6	-5.1, 2.0	0.4
P01619	IGKV3-20	116	0.79	-3.3, 4.9	0.7
P41222	PTGDS	117	-0.70	-3.9, 2.5	0.7
P0DJD8	PGA3	105	-2.0	-4.9, 0.94	0.2
P01857	IGHG1	117	2.1	-2.8, 7.1	0.4
P01009	SERPINA1	118	2.6	-1.5, 6.7	0.2
P01876	IGHA1	117	-1.5	-6.7, 3.6	0.6
P05154	SERPINA5	118	0.82	-2.2, 3.8	0.6
P62805	HIST1H4A	99	-1.5	-3.2, 0.18	0.079
P01042	KNG1	118	-3.5	-7.7, 0.67	0.10
P01011	SERPINA3	118	0.32	-3.4, 4.0	0.9
P05155	SERPING1	118	-1.9	-6.9, 3.1	0.5
P10909	CLU	118	-1.3	-5.2, 2.5	0.5
P08571	CD14	118	0.38	-3.5, 4.2	0.8
B9A064	IGLL5	117	-0.81	-3.5, 1.9	0.6
P04083	ANXA1	118	-1.6	-4.0, 0.79	0.2
Q08380	LGALS3BP	118	-0.24	-4.7, 4.2	>0.9
Q01469	FABP5	117	-0.58	-3.0, 1.9	0.6
P04746	AMY2A	117	-1.7	-6.3, 2.9	0.5
Q9UBC9	SPRR3	114	-1.2	-3.6, 1.1	0.3
P01833	PIGR	117	-1.3	-6.7, 4.1	0.6
Q6EMK4	VASN	116	-4.1	-9.0, 0.85	0.10
P02787	TF	118	4.6	1.5, 7.8	**0.004**
P29508	SERPINB3	118	0.90	-1.8, 3.6	0.5
Q14624	ITIH4	118	6.2	0.71, 12	**0.027**
P02760	AMBP	117	0.04	-4.5, 4.5	>0.9
P01133	EGF	118	-2.5	-7.6, 2.7	0.3
P07355	ANXA2	118	-1.4	-4.2, 1.3	0.3
P60709	ACTB	118	2.9	-2.8, 8.5	0.3

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
P54802	NAGLU	118	-1.5	-7.4, 4.3	0.6
P80188	LCN2	111	-0.64	-3.2, 2.0	0.6
P10253	GAA	118	-1.3	-6.4, 3.8	0.6
P01019	AGT	116	0.91	-2.3, 4.2	0.6
P24855	DNASE1	116	1.1	-3.7, 5.8	0.7
Q9UNN8	PROCR	110	-1.1	-5.3, 3.2	0.6
Q13510	ASAH1	118	-4.1	-12, 3.9	0.3
P07339	CTSD	118	-1.1	-6.4, 4.2	0.7
P11117	ACP2	117	-0.40	-6.4, 5.6	0.9
P10451	SPP1	115	-0.60	-4.4, 3.2	0.8
P02647	APOA1	115	0.61	-1.4, 2.7	0.6
P05109	S100A8	110	-1.9	-4.3, 0.58	0.13
P05090	APOD	110	-4.3	-9.0, 0.45	0.075
P01871	IGHM	116	-0.64	-4.1, 2.9	0.7
Q9NQ84	GPRC5C	117	0.48	-4.0, 5.0	0.8
O00560	SDCBP	118	0.59	-3.0, 4.2	0.7
Q96DA0	ZG16B	113	-2.5	-6.5, 1.6	0.2
P15144	ANPEP	118	-0.12	-6.7, 6.4	>0.9
P16278	GLB1	118	-2.5	-8.0, 3.0	0.4
P08185	SERPINA6	116	1.7	-2.7, 6.2	0.4
P00738	HP	107	-0.20	-2.2, 1.8	0.8
P19835	CEL	118	-0.24	-4.9, 4.4	>0.9
Q12805	EFEMP1	117	0.41	-4.5, 5.3	0.9
P30086	PEBP1	115	-3.6	-8.9, 1.7	0.2
P07195	LDHB	118	-0.80	-6.4, 4.7	0.8
Q8NFZ8	CADM4	111	-2.0	-7.0, 3.0	0.4
P15586	GNS	118	-2.5	-7.3, 2.3	0.3
Q9NZZ3	CHMP5	109	-1.8	-6.6, 3.0	0.5
P05543	SERPINA7	117	-4.6	-9.6, 0.42	0.072
P02790	HPX	111	2.0	-1.0, 5.1	0.2
P02671	FGA	108	0.96	-1.7, 3.7	0.5
P00450	CP	118	-1.0	-4.5, 2.4	0.6

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
P22891	PROZ	116	0.47	-3.6, 4.6	0.8
P05062	ALDOB	115	-0.10	-4.5, 4.3	>0.9
P16444	DPEP1	118	-0.21	-5.3, 4.9	>0.9
P53990	IST1	117	0.86	-4.0, 5.7	0.7
P25311	AZGP1	114	-2.1	-5.1, 0.79	0.2
P30740	SERPINB1	115	-0.90	-3.4, 1.6	0.5
P12109	COL6A1	117	-0.26	-5.9, 5.4	>0.9
Q7LBR1	CHMP1B	108	-1.8	-5.8, 2.2	0.4
Q9Y646	CPQ	118	-1.0	-6.3, 4.2	0.7
P06733	ENO1	118	1.8	-2.4, 6.1	0.4
P15309	ACPP	115	0.98	-2.8, 4.8	0.6
P02649	APOE	117	-0.57	-5.8, 4.6	0.8
O43633	CHMP2A	111	0.69	-3.5, 4.9	0.7
P12830	CDH1	114	-1.8	-5.3, 1.8	0.3
O60494	CUBN	118	-3.8	-9.2, 1.5	0.2
P04217	A1BG	105	-2.4	-5.8, 1.0	0.2
Q93088	BHMT	115	-0.98	-4.5, 2.6	0.6
P01024	C3	118	2.3	0.37, 4.3	**0.020**
P19440	GGT1	116	-0.19	-5.8, 5.4	>0.9
P08294	SOD3	96	-0.34	-5.3, 4.6	0.9
P63104	YWHAZ	116	0.89	-4.2, 6.0	0.7
P31949	S100A11	98	-1.9	-5.9, 2.2	0.4
Q14019	COTL1	94	-0.53	-3.9, 2.8	0.8
P09210	GSTA2	95	-1.4	-5.4, 2.6	0.5
P04004	VTN	114	0.92	-1.7, 3.6	0.5
Q969P0	IGSF8	117	2.2	-2.7, 7.0	0.4
P33908	MAN1A1	115	1.9	-1.7, 5.6	0.3
P04075	ALDOA	118	-2.0	-6.5, 2.4	0.4
P39059	COL15A1	113	-2.0	-6.1, 2.1	0.3
Q16769	QPCT	100	0.30	-5.2, 5.8	>0.9
Q9NZP8	C1RL	113	-4.8	-11, 0.98	0.10
Q16270	IGFBP7	97	1.2	-2.3, 4.8	0.5

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
P14618	PKM	117	-1.5	-6.1, 3.2	0.5
Q01459	CTBS	112	-2.7	-6.7, 1.4	0.2
P00918	CA2	116	-0.27	-5.6, 5.1	>0.9
P98164	LRP2	118	-1.1	-6.6, 4.4	0.7
P50995	ANXA11	115	1.5	-2.1, 5.0	0.4
P0C0L5	C4B	118	2.4	-1.3, 6.0	0.2
Q9UBX5	FBLN5	117	1.9	-4.0, 7.7	0.5
P01008	SERPINC1	116	0.04	-3.5, 3.6	>0.9
P05156	CFI	113	0.27	-4.4, 4.9	>0.9
O95336	PGLS	116	-0.14	-5.1, 4.8	>0.9
P62879	GNB2	111	-5.8	-12, 0.32	0.063
P15941	MUC1	111	-2.6	-7.9, 2.7	0.3
P02750	LRG1	107	-1.2	-4.3, 1.8	0.4
P26038	MSN	118	0.84	-4.3, 5.9	0.7
O75882	ATRN	118	-1.7	-6.8, 3.5	0.5
P27105	STOM	116	0.18	-6.5, 6.8	>0.9
P00338	LDHA	113	-0.30	-5.5, 4.9	>0.9
P27487	DPP4	118	0.00	-6.0, 6.0	>0.9
P22792	CPN2	112	-0.86	-6.1, 4.4	0.7
P08758	ANXA5	115	0.86	-3.3, 5.0	0.7
P98160	HSPG2	118	1.1	-4.1, 6.2	0.7
P07737	PFN1	97	5.2	-0.22, 11	0.060
Q9UHL4	DPP7	113	-0.75	-5.5, 4.0	0.8
P12429	ANXA3	109	-1.2	-3.6, 1.3	0.3
Q07075	ENPEP	117	-0.29	-4.1, 3.5	0.9
A8K2U0	A2ML1	116	-0.18	-2.3, 1.9	0.9
P29622	SERPINA4	118	0.18	-4.4, 4.8	>0.9
Q92820	GGH	108	-1.6	-7.1, 3.8	0.6
Q6UX06	OLFM4	116	-0.94	-6.1, 4.2	0.7
P00558	PGK1	116	2.2	-1.8, 6.3	0.3
P09467	FBP1	113	0.04	-4.1, 4.2	>0.9
P13473	LAMP2	105	0.10	-3.8, 4.0	>0.9

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
Q8WUM4	PDCD6IP	118	-0.49	-4.8, 3.8	0.8
P60174	TPI1	115	2.6	-2.7, 8.0	0.3
Q06830	PRDX1	106	-2.4	-8.4, 3.5	0.4
P78324	SIRPA	115	-2.0	-5.8, 1.9	0.3
P43251	BTD	115	2.6	-4.0, 9.2	0.4
Q5VW32	BROX	112	-1.3	-6.7, 4.1	0.6
P23284	PPIB	106	0.12	-6.1, 6.3	>0.9
Q9NP79	VTA1	107	2.3	-3.1, 7.7	0.4
P08236	GUSB	109	-2.0	-7.2, 3.1	0.4
P08473	MME	118	1.6	-4.6, 7.7	0.6
P01023	A2M	112	1.8	-0.26, 3.9	0.085
Q9H3G5	CPVL	113	1.7	-2.9, 6.2	0.5
P52758	HRSP12	100	-1.1	-5.9, 3.7	0.7
P15289	ARSA	116	1.3	-3.4, 5.9	0.6
P07686	HEXB	118	-2.3	-8.6, 4.0	0.5
Q92692	PVRL2	110	-2.1	-8.4, 4.1	0.5
P04066	FUCA1	114	-2.4	-9.5, 4.7	0.5
Q9UKU9	ANGPTL2	111	0.95	-3.4, 5.3	0.7
P09525	ANXA4	117	-0.84	-6.2, 4.6	0.8
P68104	EEF1A1	102	1.0	-4.1, 6.2	0.7
P35858	IGFALS	114	0.77	-3.3, 4.8	0.7
P11597	CETP	105	-0.36	-3.8, 3.1	0.8
P36955	SERPINF1	118	2.6	-1.1, 6.2	0.2
P06865	HEXA	116	-3.8	-9.9, 2.3	0.2
P23528	CFL1	99	-0.96	-7.6, 5.7	0.8
P62834	RAP1A	104	-0.41	-5.7, 4.9	0.9
Q9H8L6	MMRN2	112	4.2	-1.0, 9.4	0.11
P53634	CTSC	113	-1.2	-7.1, 4.7	0.7
P04899	GNAI2	105	-2.6	-8.3, 3.0	0.4
Q9BRK3	MXRA8	109	-0.48	-4.1, 3.1	0.8
P40925	MDH1	103	0.93	-5.0, 6.9	0.8
O00391	QSOX1	118	2.5	-1.9, 7.0	0.3

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
P13796	LCP1	102	0.25	-1.8, 2.3	0.8
Q8IV08	PLD3	103	-3.7	-11, 3.5	0.3
P09211	GSTP1	100	8.3	2.5, 14	**0.005**
P32119	PRDX2	102	-2.9	-9.1, 3.4	0.4
P30530	AXL	105	1.6	-3.1, 6.3	0.5
P15311	EZR	117	-4.0	-9.7, 1.8	0.2
P58499	FAM3B	97	-1.0	-6.8, 4.7	0.7
P14384	CPM	104	2.5	-3.3, 8.3	0.4
P11142	HSPA8	118	3.1	-2.5, 8.8	0.3
P00352	ALDH1A1	117	-2.1	-6.6, 2.3	0.3
P02751	FN1	118	8.0	1.9, 14	**0.011**
P51654	GPC3	113	0.87	-3.2, 4.9	0.7
P00966	ASS1	106	0.60	-2.6, 3.8	0.7
P26992	CNTFR	100	-3.2	-8.6, 2.3	0.3
Q6UX73	C16orf89	115	3.8	-0.95, 8.5	0.12
P09668	CTSH	107	5.2	0.26, 10	**0.039**
P17174	GOT1	116	-2.8	-8.6, 2.9	0.3
Q12860	CNTN1	117	0.60	-3.3, 4.5	0.8
O75351	VPS4B	104	3.7	-1.7, 9.0	0.2
P42785	PRCP	103	-2.9	-9.8, 4.1	0.4
Q8WZ75	ROBO4	114	0.58	-3.0, 4.1	0.7
Q9H0W9	C11orf54	113	-2.8	-7.4, 1.9	0.2
P63000	RAC1	97	1.4	-5.6, 8.3	0.7
Q12794	HYAL1	113	0.76	-4.1, 5.7	0.8
P37837	TALDO1	99	-1.4	-4.1, 1.3	0.3
P00751	CFB	101	2.4	-0.50, 5.2	0.10
P06727	APOA4	105	1.2	-1.3, 3.7	0.4
Q9BYE9	CDHR2	116	-2.9	-7.0, 1.2	0.2
Q5JS37	NHLRC3	94	-4.1	-9.2, 1.0	0.11
P04406	GAPDH	108	1.7	-4.1, 7.5	0.6
O14773	TPP1	116	0.95	-4.0, 5.9	0.7
P84077	ARF1	100	10	2.6, 18	**0.009**

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
Q03154	ACY1	99	0.98	-2.5, 4.4	0.6
P60953	CDC42	110	5.9	1.2, 11	**0.014**
Q13621	SLC12A1	115	-0.69	-5.2, 3.9	0.8
O43505	B4GAT1	107	-0.35	-5.2, 4.5	0.9
Q99519	NEU1	99	-5.2	-11, 0.76	0.087
O43490	PROM1	115	-1.4	-6.2, 3.4	0.6
Q9HB40	SCPEP1	102	-1.7	-6.6, 3.3	0.5
Q9H444	CHMP4B	100	1.0	-4.7, 6.8	0.7
O43451	MGAM	116	0.67	-3.0, 4.3	0.7
O00462	MANBA	117	-0.43	-3.9, 3.1	0.8
Q8NBJ4	GOLM1	106	-1.9	-4.8, 0.98	0.2
P19801	AOC1	107	0.53	-2.8, 3.8	0.8
P0DMV9	HSPA1B	118	2.7	-3.0, 8.4	0.3
O43895	XPNPEP2	103	-0.10	-3.7, 3.5	>0.9
Q9HBB8	CDHR5	113	-1.6	-6.3, 3.2	0.5
P00491	PNP	112	-2.1	-7.2, 3.1	0.4
Q13228	SELENBP1	115	-0.29	-5.6, 5.0	>0.9
O75874	IDH1	113	-1.8	-6.2, 2.6	0.4
P16152	CBR1	112	1.3	-3.8, 6.4	0.6
Q96KP4	CNDP2	112	0.16	-3.4, 3.7	>0.9
P08195	SLC3A2	115	-0.52	-7.4, 6.3	0.9
Q16651	PRSS8	95	-2.2	-9.3, 4.8	0.5
P51688	SGSH	101	0.01	-5.3, 5.3	>0.9
Q15907	RAB11B	106	-0.29	-7.6, 7.0	>0.9
P55287	CDH11	97	-1.8	-4.5, 0.95	0.2
Q14914	PTGR1	113	-0.57	-6.2, 5.1	0.8
P14550	AKR1A1	109	1.9	-2.1, 5.9	0.3
P51149	RAB7A	101	2.8	-5.2, 11	0.5
Q9H6X2	ANTXR1	102	-1.2	-5.6, 3.2	0.6
P43121	MCAM	111	-3.9	-8.3, 0.39	0.074
P52209	PGD	109	1.4	-2.5, 5.2	0.5
Q8NCC3	PLA2G15	106	-0.21	-5.7, 5.3	>0.9

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
P40121	CAPG	112	1.8	-3.9, 7.6	0.5
Q9HCN6	GP6	96	-1.8	-7.1, 3.5	0.5
P12277	CKB	103	3.6	-1.2, 8.4	0.14
P34059	GALNS	105	-0.87	-5.8, 4.0	0.7
Q96PD5	PGLYRP2	107	-1.4	-4.7, 1.9	0.4
P68371	TUBB4B	112	3.6	-0.90, 8.1	0.12
P29992	GNA11	102	2.1	-3.3, 7.6	0.4
P11021	HSPA5	115	1.7	-3.3, 6.6	0.5
P14543	NID1	95	-0.93	-5.2, 3.4	0.7
P06744	GPI	115	3.0	-1.6, 7.6	0.2
P51148	RAB5C	100	1.7	-5.6, 9.0	0.6
P34896	SHMT1	104	0.13	-4.0, 4.3	>0.9
Q3LXA3	DAK	109	0.28	-3.4, 4.0	0.9
Q9UGT4	SUSD2	103	2.4	-3.9, 8.6	0.5
O00241	SIRPB1	101	-0.87	-6.0, 4.3	0.7
P54760	EPHB4	111	-3.3	-9.0, 2.3	0.2
P08697	SERPINF2	112	-1.9	-6.0, 2.2	0.4
P61106	RAB14	111	-0.40	-8.8, 8.0	>0.9
Q8NHP8	PLBD2	108	1.3	-5.7, 8.4	0.7
P55017	SLC12A3	112	2.9	-1.5, 7.3	0.2
P55290	CDH13	113	0.43	-5.3, 6.1	0.9
P19827	ITIH1	112	2.6	-0.86, 6.2	0.14
P09603	CSF1	107	-3.3	-9.7, 3.1	0.3
Q92520	FAM3C	104	1.4	-4.5, 7.3	0.6
P50395	GDI2	109	3.5	-1.9, 8.9	0.2
Q08345	DDR1	109	-7.0	-14, 0.23	0.058
Q14393	GAS6	117	-0.42	-6.0, 5.1	0.9
O00533	CHL1	110	-0.92	-4.5, 2.7	0.6
P48594	SERPINB4	104	-1.1	-3.7, 1.5	0.4
Q9Y2E5	MAN2B2	113	0.05	-3.7, 3.8	>0.9
Q08174	PCDH1	95	-0.29	-4.1, 3.5	0.9
P04180	LCAT	109	-4.8	-10, 0.44	0.072

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
P07900	HSP90AA1	111	2.2	-1.3, 5.7	0.2
P06280	GLA	110	-6.4	-12, -0.34	**0.039**
P05546	SERPIND1	95	3.3	-0.05, 6.6	0.053
Q99816	TSG101	101	3.4	-1.5, 8.3	0.2
Q8IUL8	CILP2	117	-1.9	-8.3, 4.5	0.6
O75487	GPC4	110	2.3	-2.7, 7.3	0.4
P18085	ARF4	98	2.7	-3.4, 8.8	0.4
P08582	MFI2	111	-0.70	-5.6, 4.2	0.8
Q9UKU6	TRHDE	112	2.9	-2.2, 8.0	0.3
P20711	DDC	102	1.8	-1.9, 5.6	0.3
Q12913	PTPRJ	114	-2.1	-6.5, 2.3	0.3
Q9UIB8	CD84	109	-0.75	-5.7, 4.2	0.8
P35052	GPC1	109	0.96	-6.0, 7.9	0.8
Q8N271	PROM2	109	-2.4	-8.1, 3.3	0.4
Q9H6S3	EPS8L2	101	-0.86	-4.8, 3.1	0.7
Q96NY8	PVRL4	96	-4.8	-11, 1.2	0.12
Q6UVK1	CSPG4	113	0.22	-4.4, 4.8	>0.9
P02748	C9	95	1.7	-2.6, 6.0	0.4
P20073	ANXA7	110	3.6	-1.9, 9.1	0.2
P31946	YWHAB	96	-2.8	-9.4, 3.7	0.4
P50502	ST13	104	-1.6	-7.6, 4.4	0.6
O95497	VNN1	98	-3.4	-8.8, 1.9	0.2
O00299	CLIC1	99	0.44	-7.2, 8.1	>0.9
Q9HAT2	SIAE	102	-8.4	-16, -1.2	**0.023**
P08183	ABCB1	114	0.09	-4.4, 4.6	>0.9
Q12929	EPS8	106	-0.56	-4.5, 3.3	0.8
Q9H223	EHD4	106	0.93	-3.4, 5.3	0.7
P50895	BCAM	110	-1.8	-7.3, 3.8	0.5
P07384	CAPN1	98	-2.1	-5.3, 1.1	0.2
P05023	ATP1A1	115	5.0	0.35, 9.6	**0.035**
O43493	TGOLN2	107	-4.6	-8.3, -0.83	**0.017**
Q00796	SORD	94	2.0	-3.6, 7.7	0.5

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
P15313	ATP6V1B1	95	0.02	-4.0, 4.0	>0.9
O43707	ACTN4	109	1.4	-1.6, 4.5	0.3
P38606	ATP6V1A	101	3.0	-1.2, 7.1	0.2
Q9Y6W3	CAPN7	108	-0.15	-3.8, 3.5	>0.9
Q5JWF2	GNAS	100	-3.6	-12, 4.6	0.4
P12821	ACE	109	-1.1	-5.7, 3.5	0.6
Q86YQ8	CPNE8	102	1.9	-5.3, 9.1	0.6
P08572	COL4A2	109	2.9	-2.0, 7.8	0.2
P29401	TKT	98	3.8	0.25, 7.3	**0.036**
O00159	MYO1C	106	-0.97	-5.0, 3.1	0.6
P61019	RAB2A	98	0.01	-7.9, 8.0	>0.9
Q16706	MAN2A1	109	2.5	-2.0, 7.0	0.3
P35241	RDX	103	-0.82	-5.8, 4.1	0.7
Q9BYF1	ACE2	108	0.48	-5.9, 6.8	0.9
Q92673	SORL1	115	-2.9	-7.9, 2.1	0.2
Q13332	PTPRS	115	1.9	-2.1, 5.8	0.4
P55786	NPEPPS	99	0.17	-5.0, 5.3	>0.9
P54753	EPHB3	109	-2.4	-9.2, 4.5	0.5
Q7Z7M0	MEGF8	118	0.24	-5.4, 5.9	>0.9
P36871	PGM1	98	2.7	-1.6, 7.0	0.2
Q92896	GLG1	101	1.4	-3.4, 6.3	0.6
P13797	PLS3	104	-0.23	-5.9, 5.5	>0.9
P0C0L4	C4A	110	2.2	-1.0, 5.5	0.2
P12111	COL6A3	116	-0.68	-4.0, 2.6	0.7
O75339	CILP	106	-2.3	-7.5, 2.9	0.4
Q9UQB8	BAIAP2	98	-1.5	-5.8, 2.7	0.5
P18206	VCL	105	-2.4	-6.1, 1.2	0.2
Q5SZK8	FREM2	108	1.1	-2.2, 4.5	0.5
O00468	AGRN	106	0.55	-3.7, 4.8	0.8
Q99523	SORT1	101	-8.5	-15, -1.6	**0.017**
P23470	PTPRG	98	-3.2	-8.8, 2.3	0.3
P22314	UBA1	98	4.5	0.35, 8.6	**0.034**

Protein ID	Gene Name	N	Beta	95% CI[1]	p-value
Q14126	DSG2	96	-6.9	-13, -1.2	**0.019**
P32004	L1CAM	105	-3.5	-7.9, 0.84	0.11
Q6V0I7	FAT4	107	0.51	-2.5, 3.5	0.7
P22105	TNXB	104	0.60	-4.9, 6.1	0.8
Q09666	AHNAK	96	-2.2	-4.1, -0.38	**0.019**
P11717	IGF2R	99	-2.8	-8.0, 2.3	0.3

[1]CI = Confidence Interval

Subchapter iii. Stretch-Induced Compliance mechanism in pregnancy-induced cardiac hypertrophy and the impact of cardiovascular risk factors.

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Title: Stretch-Induced Compliance mechanism in pregnancy-induced cardiac hypertrophy and the impact of cardiovascular risk factors

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Abstract

Aim: Considering that pressure-overload-induced hypertrophy compromises cardiac stretch-induced compliance (SIC) following acute volume overload (AVO), we hypothesized that SIC might be exacerbated in physiological hypertrophy induced by pregnancy's chronic volume overload. We aimed to evaluate the SIC-response in pregnant women with or without cardiovascular risk factors.

Methods: Thirty-seven (1st trimester, 1stT) and 31 (3rd trimester, 3rdT) women independently recruited undergone prospectively echocardiography's before (T0), immediately after (T1), and 15 min after AVO induced by passive leg elevation (T2). Blood samples were collected before and after the AVO.

Results: A significant increase in the inferior vena cava diameter and stroke volume from T0 to T1 in both trimesters confirmed an effective AVO. A significant increase in the left ventricle (LV) end-diastolic volume (LVEDV) and E/e' was observed immediately after AVO in the 1stT and 3rdT groups. SIC-response (15 min after AVO) was characterized by a significant decrease of E/e' in both trimesters, counterbalanced with additional expansion of LVEDV only in the 1stT. During the entire AVO period, LV stiffness decreased significantly in both trimesters. NT-proBNP concentration increased slightly after AVO only in the 1stT. The presence of cardiovascular risk factors significantly impacted the SIC-response, contrasting with the non-significant effect of parity and age.

Conclusion: A distinct functional response to SIC was observed between 1stT and 3rdT, being influenced by cardiovascular risk factors. Despite the LV of 3rdT pregnant women showing a structural limitation to dilate and accommodate increased volume upon AVO, its physiological hypertrophy did not compromise the SIC-mechanism, suggesting it to be exacerbated.

Abstract word count: 256

Keywords

Pregnancy

Stretch induced compliance

Acute volume overload

Myocardial hypertrophy

Cardiovascular risk factors

Haemodynamic overload

Introduction

In the cardiovascular system, homeostasis is maintained due to continuous cardiac adaptation to hemodynamic conditions. An increase in venous return (preload) triggers an acute myocardial stretch, characterized by an immediate rise in contractility by the Frank-Starling mechanism, followed by a progressive increase known as the slow force response (111, 191). Concomitantly to these adaptations, we have described a novel adaptive mechanism following acute myocardial stretch, named stretch-induced compliance (SIC), whereby myocardial compliance increases owing to cyclic guanosine 3', 5'-monophosphate-dependent protein kinase G (cGMP-PKG) pathway activation and titin phosphorylation (111). Interestingly, we described a reduced diastolic response to stretch in hypertrophied hearts, suggesting that the SIC mechanism is compromised in pathological hypertrophy induced by chronic pressure overload (111). However, the response to SIC remains unclear in physiological hypertrophy induced by pregnancy that imposes a volume overload.

Pregnancy is characterized by hemodynamic overload that induces physiological hypertrophy (3, 6, 9, 10, 12-15, 17, 20) combined with an improvement in aortic compliance and a decrease in systemic vascular resistance (3, 6, 13, 99). These adaptations aim to accommodate the increasing preload and to maintain homeostasis in the cardiovascular system. Nitric oxide and natriuretic peptides are involved in left ventricular (LV) remodeling during gestation through cyclic guanosine monophosphate (cGMP) (192). Indeed, natriuretic peptides rise in early pregnancy, contributing to stroke volume increments and systemic vasodilation (193). Several factors are known to affect hemodynamic adaptations during pregnancy. Such is the case of cardiovascular risk factors that are associated with cardiac remodeling characterized by increased LV dimensions and mass combined with a higher incidence of diastolic and subclinical systolic dysfunction (53, 64, 68, 72, 73, 144, 194). Additionally, maternal age substantially impacts diastolic function, being reported as an independent predictor of diastolic function impairment during gestation (195). Lastly, parity also influences anatomic heart adaptation to pregnancy's hemodynamic overload, since higher cardiac dimensions have been documented in primiparous compared to nulliparous women (195, 196).

Considering the progressive hemodynamic adaptation that occurs during pregnancy in response to increased preload, our hypothesis is that SIC mechanism may be exacerbated in cases of pregnancy-induced physiological hypertrophy. Within this framework, our objective is to comprehensively characterize SIC in pregnancy and examine how cardiovascular risk factors, parity, and age might influence this process.

Methods and Design

Study design and setting

This study was conducted at the Obstetrics Department of *the Centro Hospitalar Universitário São João* (CHUSJ) and was approved by the Ethics Committee of CHUSJ (ID 201/18). All participants were willing to provide written informed consent. The confidentiality and data anonymity complied with the Declaration of Helsinki of 1964, revised in Fortaleza, in 2013. The complete protocol for the present study has been published (117).

Participants

Participants were recruited during their hospital appoitments from February 2019 to October 2022 (in the 1st [1stT] or 3rd trimester [3rdT] of pregnancy) or voluntarily through online forms available at https://perimyrobb.wordpress.com/. Two independent study groups were created according to the time of cardiovascular assessment: 1stT or 3rdT. Study groups are independent meaning that there is no overlap of participants at both evaluation times. This allowed us to analyze the average mean effect SIC in these two stages of cardiac remodeling induced by hemodynamic overload inherent to pregnancy.

Inclusion criteria were: adult pregnant women (≥18 years old) with or without cardiovascular risk factors, namely chronic and/or gestational hypertension, gestational diabetes mellitus (DM), and/or obesity. Arterial hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg measured in the office or in-hospital before 20 weeks of gestation (118). Gestational hypertension was defined as arterial hypertension diagnosed after 20 weeks of gestation that resolved within 42 days postpartum. Gestational DM was considered if fasting glucose was [92; 126] mg/dL at 1st trimester or fasting glucose ≥ 92 mg/dL 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 pregnancy weeks. Obesity was defined as a body mass index before pregnancy ≥ 30 kg/m^2.

Women with twin pregnancies, pre-existing cardiomyopathy, renal disease, chronic obstructive pulmonary disease, active systemic infection, genetic syndromes, or type-1 or type-2 DM were excluded.

Measurements

In the first cardiovascular evaluation in the 1st trimester [1stT, 11-14 weeks, at the beginning of cardiac remodeling] or 3rd trimester [3rdT, 30-35 weeks, peak of cardiac remodeling, when cardiovascular adaptations should be most noticeable], the included participants underwent

the following evaluations: 1) clinical characterization, 2) transthoracic echocardiogram before (T0), immediately after (T1) and 15 minutes after (T2) acute volume overload (AVO) induced by passive leg elevation, 3) pulse wave velocity (PWV) before AVO, 4) blood sample collection before and after 15 minutes of AVO at the Department of Surgery and Physiology of the Faculty of Medicine of the University of Porto. Clinical characterization included information on maternal cardiovascular health, maternal health-related habits, maternal smoking habits (include currently and former smokers, as well as those participants that stopped smoking only during pregnancy or at the beginning of pregnancy), parity, medical history and demographics, obstetric and perinatal outcomes. These data were collected from the questionnaires and electronic medical records.

Vascular stiffness evaluation

Brachial blood pressure was measured after 10 minutes of resting.

Arterial stiffness was estimated by carotid-femoral pulse wave velocity (PWV), calculated from carotid-femoral distance/transit time, and peripheral augmentation index, defined as the ratio of late systolic pressure to early systolic pressure (Complior®; Alam Medical, France). Repeated measurements were carried out and averaged until the agreement was ≤0.5 m/s.

Echocardiographic assessment

Conventional transthoracic echocardiography evaluation with a 3 MHz phased-array probe (ACUSON SC2000 PRIME^{TM}) was performed by a single operator, and measurements were obtained from standard views according to the European Society of Cardiology recommendations for chamber quantification and diastolic function evaluation (109, 110). At least three cardiac cycles were acquired for data analysis. Two certified cardiologists independently analyzed, interpreted, and harmonized the results. Relative wall thickness was set as 2×LV posterior wall thickness in diastole/LV end-diastolic diameter and LV mass quantified as $0.8 \times (1.04 \times [(\text{LV end-diastolic diameter} + \text{LV posterior wall thickness in diastole} + \text{Interventricular septum thickness in diastole})^3 - (\text{LV end-diastolic diameter})^3] + 0.6)$g. Simpson's biplane method was used to estimate end-diastolic and end-systolic volumes, stroke volume, and ejection fraction (calculated through $([\text{LV internal end-diastolic dimension}]^3 - [\text{LV internal end-systolic dimension}])^3/[\text{LV internal end-diastolic dimension}]^3 \times 100\%$). LV cardiac output (CO) was calculated as the product of the LV stroke volume and heart rate (L/min). Mean blood pressure was calculated using the following formula: systolic blood pressure + (2 × diastolic blood pressure)/3. In addition, E/e', a surrogate of LV filling pressures, was assessed

by the ratio between E mitral inflow velocity to peak velocity of early (e') myocardial motion at the mitral annulus (average of septal and lateral e') by tissue Doppler imaging (TDI).

To evaluate the response to AVO, echocardiography exam was repeated in the left lateral decubitus position at baseline (T0), immediately after passive leg elevation to 45° (T1), and after 15 minutes in this position (T2). AVO was performed raising both legs to 45° by wedge pillow positioned at the lower portion of the bed.

Quantification of NT-proBNP

Blood samples were centrifuged for 15 minutes, 5000 rpm at 4°C. Plasma was aliquoted and stored at −80°C until analysis. We quantified NT-proBNP (DY3604-05, R&D Systems) in pairwise plasma samples collected before and 15 minutes after passive leg elevation using an ELISA Assay Kit, according to the manufacturer's instructions.

Statistical analysis

Clinical parameters, echocardiographic parameters, pulse wave velocity, and biochemical plasma measurements were gathered from a database and analyzed using R statistical software (version 4.2.1; namely, lme4(197), marginaleffects(198), gtsummary(199), ggstatplot(200), and ggplot2(201) packages)**.**

For descriptive statistics, continuous variables were expressed as means and standard deviations. Data normality was gauged by histogram and Q-Q plot analyses (data not shown). Categorical variables were expressed as absolute values and relative frequencies. Chi-squared or Fisher exact tests were applied to assess associations among the categorical variables.

All participants performed passive leg elevation maneuvers, resulting in three paired echocardiographic analyses (T0, T1, and T2) in each time point group (1^{st}T *vs* 3^{rd}T).

Independent-samples t-tests were applied to compare the physical assessment, pulse wave velocity, and clinical and echocardiographic parameters between the time points/study groups (1^{st}T *vs* 3^{rd}T). One-way ANOVA with random intercept was applied to assess NT-proBNP variation between 1^{st} and 3^{rd} trimesters.

Within each trimester, we applied mixed-effects models to study the average mean differences between evaluation moments (T0, T1 and T2), taking into account a set of pre-specified predictors (presence of at least one cardiovascular risk factor [gestational diabetes, arterial hypertension or obesity], primiparous [yes or no], age; trimester of pregnancy [1^{st}T or 3^{rd}T]) while accounting for within-group variability. In addition, to understand the impact of these predictors on the outcome, we computed the marginal effects, which provide estimates of

how the mean response changes when a predictor varies while averaging over the other predictors. The marginal effects help clarify the practical implications of each model result and facilitate comparisons of contrasts between different levels of categorical predictors or the effect of continuous predictors on the outcome variable. The outcome variable was NT-proBNP and each echocardiographic parameter evaluated.

Results

Baseline demographic, clinical and echocardiographic characterization

The demographic and clinical characteristics of both study groups are shown in Table 1. No significant differences were found between groups regarding age, body mass index before pregnancy, parity or gestational age at delivery. Likewise, the prevalence of systemic arterial hypertension, gestational diabetes, and obesity was similar between groups, but in 1stT the percentage of smokers was higher and the systolic and mean blood pressure were lower (Table 1).

Table 1: Demographic, clinical and physical characterization.

	1stT (n=37)	**3rdT** (n=31)	**p-value**
Timing of the tests (pregnancy weeks)	14 ± 1	32 ± 2	n/a
Age, years	33 ± 4	33 ± 4	0.880
Pre-pregnancy body mass index, kg/m^2	24.3 ± 5.3	26.0 ± 4.7	0.171
Cardiovascular risk factors			
- Arterial hypertension, n (%)	1 (2.7)	4 (12.9)	0.170
- Obesity n (%)	6 (16.2)	6 (19.4)	0.760
- Gestational diabetes, n (%)	3 (8.1)	4 (12.9)	0.694
Smokers, n (%)	17 (45.9)	6 (19.4)	**0.038**
Pharmacological therapy during pregnancy			
- Aspirin	4 (10.8)	5 (16.7)	0.500
- Metformin	0 (0)	3 (9.7)	0.588
Primiparity, n (%)	9 (24.3)	11 (35.5)	0.424
Pregnancy weeks at Delivery	39 ± 2	39 ± 1	0.895
Type of delivery			
- Vaginal delivery, n (%)	17 (50.0)	17 (68.0)	0.193
- Caesarean delivery, n (%)	17 (50.0)	8 (32.0)	
Preeclampsia diagnosis, n (%)	1 (2.7)	1 (3.2)	>0.999
Physical Examination			
- Maternal body weight, kg	67 ± 14	77 ± 13	**<0.001**
- Systolic blood pressure, mmHg	106 ± 9	117 ± 12	**<0.001**
- Diastolic blood pressure, mmHg	68 ± 12	72 ± 11	0.127
- Mean blood pressure, mmHg	80 ± 10	87 ± 10	**0.009**
- Pulse wave velocity, cm/s	6.5 ± 0.7	6.3 ± 1.0	0.241

Values expressed by mean±standard deviation. Categorical variables were expressed as absolute values and relative frequencies. Absence of missing data. n/a: not applicable

Regarding baseline echocardiographic values (T0), pregnant women in the 3rdT group showed higher heart rate, stroke volume, cardiac output, relative wall thickness, left ventricular (LV) mass, left atrial (LA) volume, and LV end-diastolic and end-systolic volumes. Moreover, pregnant women in the 3rdT group presented lower ejection fraction, E/A ratio, LV elastance (ratio between E/e' and LV end-diastolic volume), and inferior vena cava diameter than those in the 1stT group, while E/e' (an echocardiographic surrogate of myocardial filling pressures) was similar between these two time points (Table 2).

Table 2: Echocardiographic assessment before leg elevation (T0).

	1stT	**3rdT**	**p-value**
Heart rate, beats/min	74 ± 10	81 ± 11	**0.009**
LVEF, %	62 ± 4	59 ± 4	**0.007**
E/A	1.67 ± 0.39	1.41 ± 0.37	**0.006**
E/e'	6.04 ± 1.17	6.22 ± 1.30	0.559
Stroke volume, mL	49 ± 7	55 ± 9	**0.005**
Cardiac output, L/min	3.6 ± 0.8	4.4 ± 0.8	**<0.001**
Maximum LA volume, mL	44 ± 9	51 ± 12	**0.007**
LV end-diastolic volume, mL	79 ± 11	93 ± 14	**<0.001**
LV end-systolic volume, mL	30 ± 5	38 ± 7	**<0.001**
LV Elastance, ([E/e']/LV end-diastolic volume)	0.08 ± 0.01	0.07 ± 0.01	**0.006**
Inferior vena cava, mm	12 ± 2	9 ± 3	**<0.001**
Relative Wall Thickness	0.31 ± 0.05	0.34 ± 0.05	**0.014**
LV mass, g	98 ± 16	122 ± 32	**<0.001**

Values expressed by mean±standard deviation.

Characterizing and comparing SIC mechanism between the 1st and 3rd trimesters

A passive leg elevation-induced AVO was confirmed in both trimesters by a significant increase in the inferior vena cava diameter from baseline (T0) to immediately after passive leg elevation (T1), which further increases 15 minutes after AVO (T2) in the 3rdT group (p=0.004, Figure 1A). The same trend was also observed in the stroke volume, indicating that the Frank-Starling mechanism was preserved (Figure 1B).

Regarding chamber volume changes in response to this AVO, LA (Figure 1C) and LV end-diastolic (Figure 1D) volumes enlarged immediately after leg elevation (T1) in both 1stT and 3rdT. These volumes remained elevated (3rdT) or further increased (1stT) over the following 15 minutes (T2), suggesting a distinct LV response between these two pregnancy moments. Nevertheless, the LV filling pressures were similar between the 1stT and 3rdT groups (difference:

0.22, 95% CI [-0.30; 0.74], p=0.413, Table 3). In the 1stT and 3rdT, E/e' increased immediately after passive leg elevation (T1), followed by a significant decrease 15 minutes after AVO (T2, Figure 1E), paralleled by a reduction in LV elastance (an echocardiographic surrogate of LV stiffness) 15 minutes after AVO (Figure 1F).

As summarized in Table 3, comparing each parameter's global variation upon AVO between 1stT and 3rdT, there are significant inferior vena cava diameter (difference: -4.14 mm, 95% CI [-5.40; -2.88], p<0.001), stroke volume (difference: 6.79 mL, 95% CI [3.43; 10.16], p<0.001), LA volume (difference: 5.30 mL, 95% CI [0.69; 9.90], p=0.024) and LV end-diastolic volume (difference: 13.24 mL, 95% CI [7.74; 18.75], p<0.001).

This distinct response to SIC between the 1stT and 3rdT is further reinforced and graphically represented in Figure 2, depicting LV stiffness changes, that is, the relationship between E/e' and LV end-diastolic volume upon AVO. Although both trimesters showed a similar response to AVO (T0 to T1), the higher LV end-diastolic volumes at baseline (T0) in the 3rdT may impose a ventricular limitation to progressively enlarge further 15 minutes after AVO (T2), contrasting to what is observed in the 1stT, suggesting a higher LV stiffness in the 3rdT. Indeed, these results are corroborated by the higher filling pressures (E/e') immediately after (T1) passive leg elevation observed in 3rdT, despite its significant reduction 15 minutes after AVO (T2), whose values normalized to values similar to those of 1stT T2, being inferior to 3rdT T0 (Figure 7, Supplemental Table 1). This fact suggested an exacerbated SIC-response in physiological hypertrophy, corroborated by significantly increased slope T2/T1 in 3rdT compared to 1stT (-0.78 vs -0.15, p=0.025).

Variation of NT-proBNP

NT-proBNP levels at baseline and in response to SIC were similar in both trimesters (p=0.110, Supplemental Table 7a). Nevertheless, there was a marginal increment in NT-proBNP levels from T0 to T2 in the 1stT (102±10pg/mL to 106±15pg/mL, p=0.160), which contrasts with the descending trend from T0 to T2 in the 3rd T (109±15pg/mL to 107±13pg/mL, p=0.510). Interestingly, these changes mimic the echocardiographic findings and support the disparities observed in the response to SIC between the two trimesters.

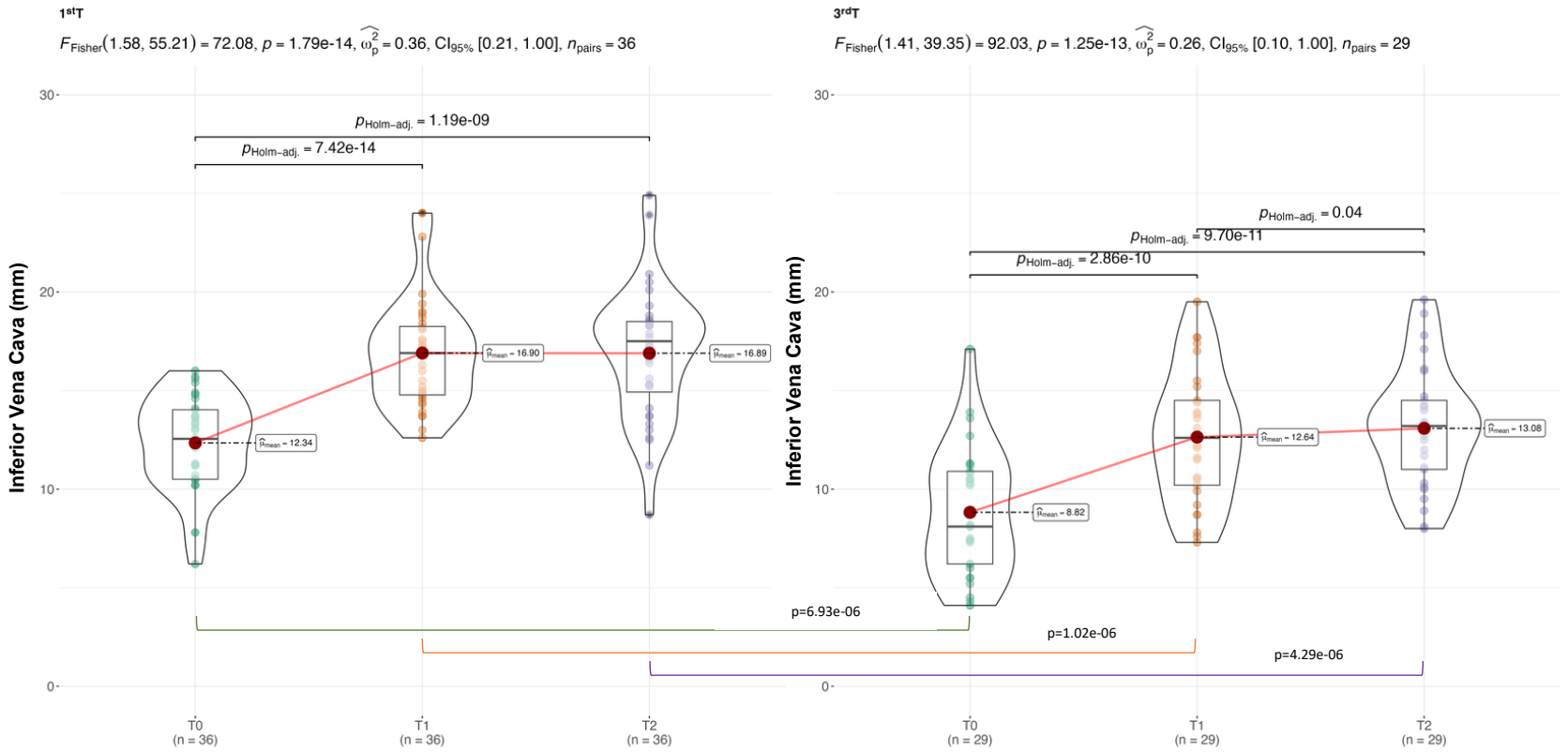


Figure 1A: Progression of inferior vena cava diameter before (T0), immediately after (T1), and 15 minutes after passive leg elevation (T2) in participants evaluated in the 1st trimester (1stT) and another assessed in the 3rd trimester (3rdT). The green, orange and purple points in T0, T1 and T2, respectively, represent each participant with quantification of the inferior vena cava diameter in each trimester of pregnancy, 1stT and 3rdT. The global trend is shown by a red line, connecting average population means in the three evaluation moments in each trimester of pregnancy (1stT - mean in T0: 12.34mm *vs* mean in T1: 16.90mm *vs* mean in T2: 16.89mm, p<0.001; 3rdT - mean in T0: 8.82mm *vs* mean in T1: 12.64mm *vs* mean in T2: 13.06mm, p<0.001). Data median and interquartile range are, also, depicted through box plots and data distribution with violin plots.

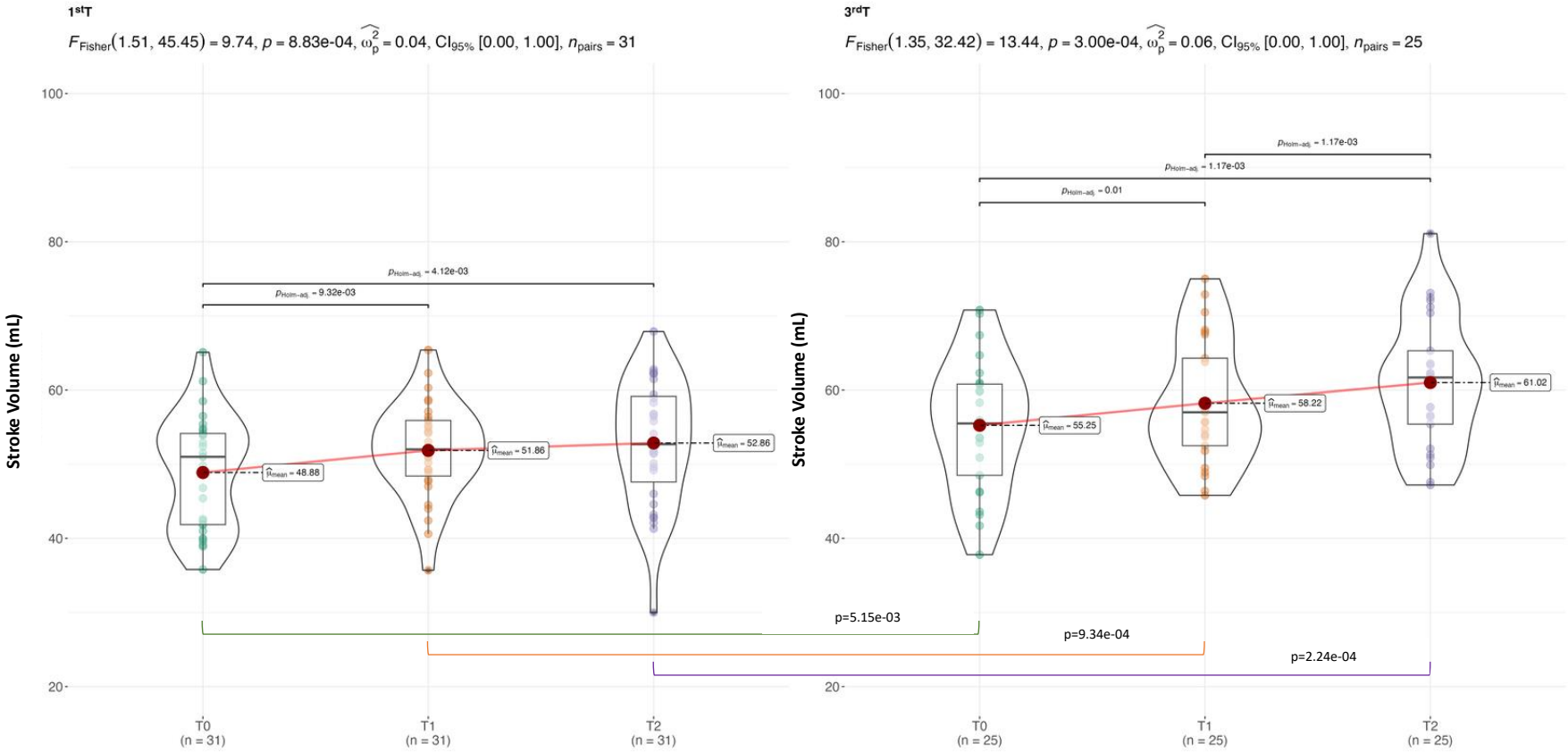


Figure 1B: Progression of stroke volume before (T0), immediately after (T1), and 15 minutes after passive leg elevation (T2) in participants evaluated in 1st trimester (1stT) and another assessed in 3rd trimester (3rdT). The green, orange and purple points in T0, T1 and T2, respectively, represent each participant with stroke volume quantification in each trimester of pregnancy, 1stT and 3rdT. The global trend is shown by a red line, connecting average population means in the three evaluation moments in each trimester of pregnancy (1stT - mean in T0: 48.88mL *vs* mean in T1: 51.86mL *vs* mean in T2: 52.86mL, p<0.001; 3rdT - mean in T0: 55.25mL *vs* mean in T1: 58.22mL *vs* mean in T2: 61.02mL, p<0.001). Data median and interquartile range are, also, depicted through box plots and data distribution with violin plots.

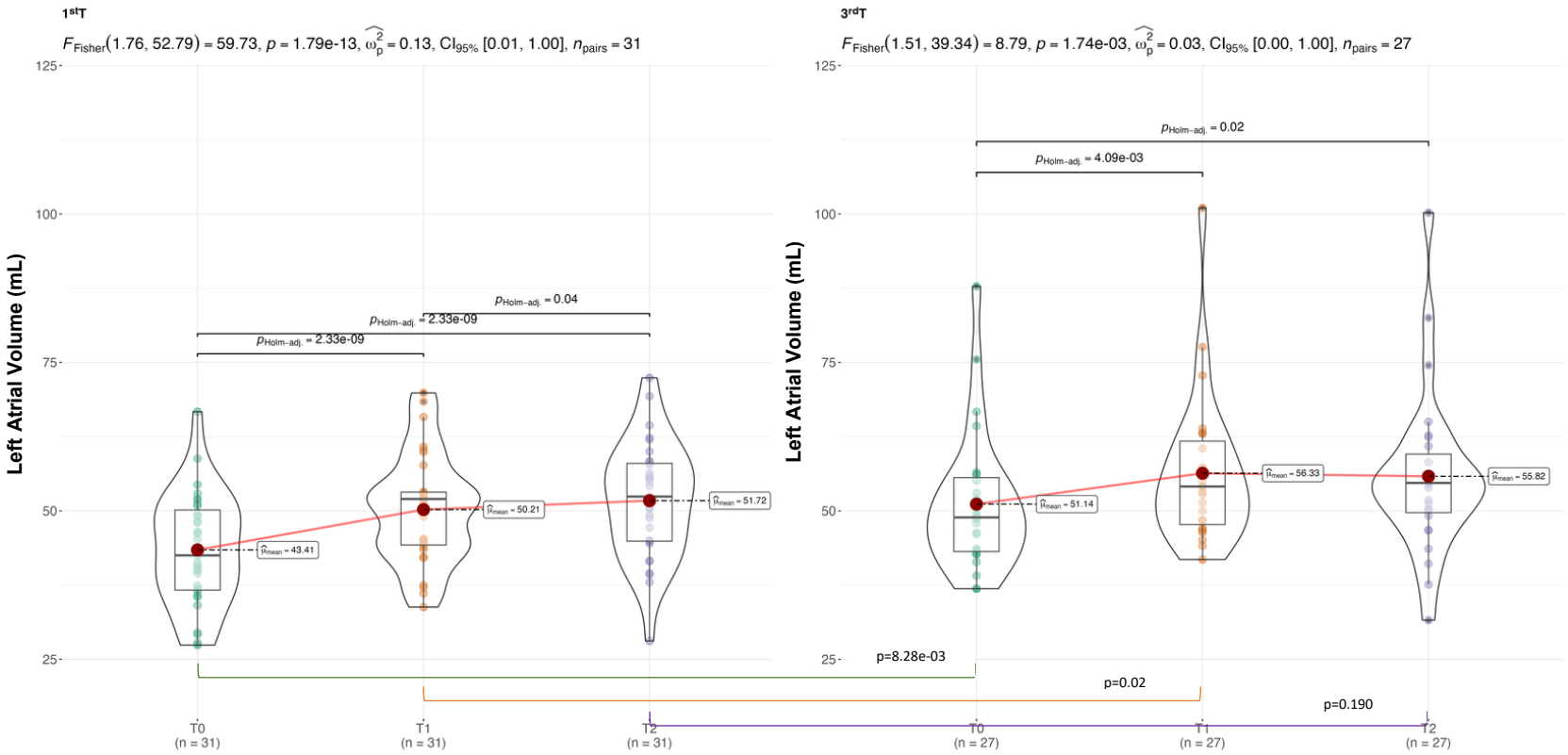


Figure 1C: Progression of left atrial volume before (T0), immediately after (T1), and 15 minutes after passive leg elevation (T2) in participants evaluated in 1st trimester (1stT) and another assessed in 3rd trimester (3rdT). The green, orange and purple points in T0, T1 and T2, respectively, represent each participant with left atrial volume quantification in each trimester of pregnancy, 1stT and 3rdT. The global trend is shown by a red line, connecting average population means in the three evaluation moments in each trimester of pregnancy (1stT - mean in T0: 43.41mL *vs* mean in T1: 50.21mL *vs* mean in T2: 51.72mL, p<0.001; 3rdT - mean in T0: 51.14mL *vs* mean in T1: 56.33mL *vs* mean in T2: 55.82mL, p=0.002). Data median and interquartile range are, also, depicted through box plots and data distribution with violin plots.

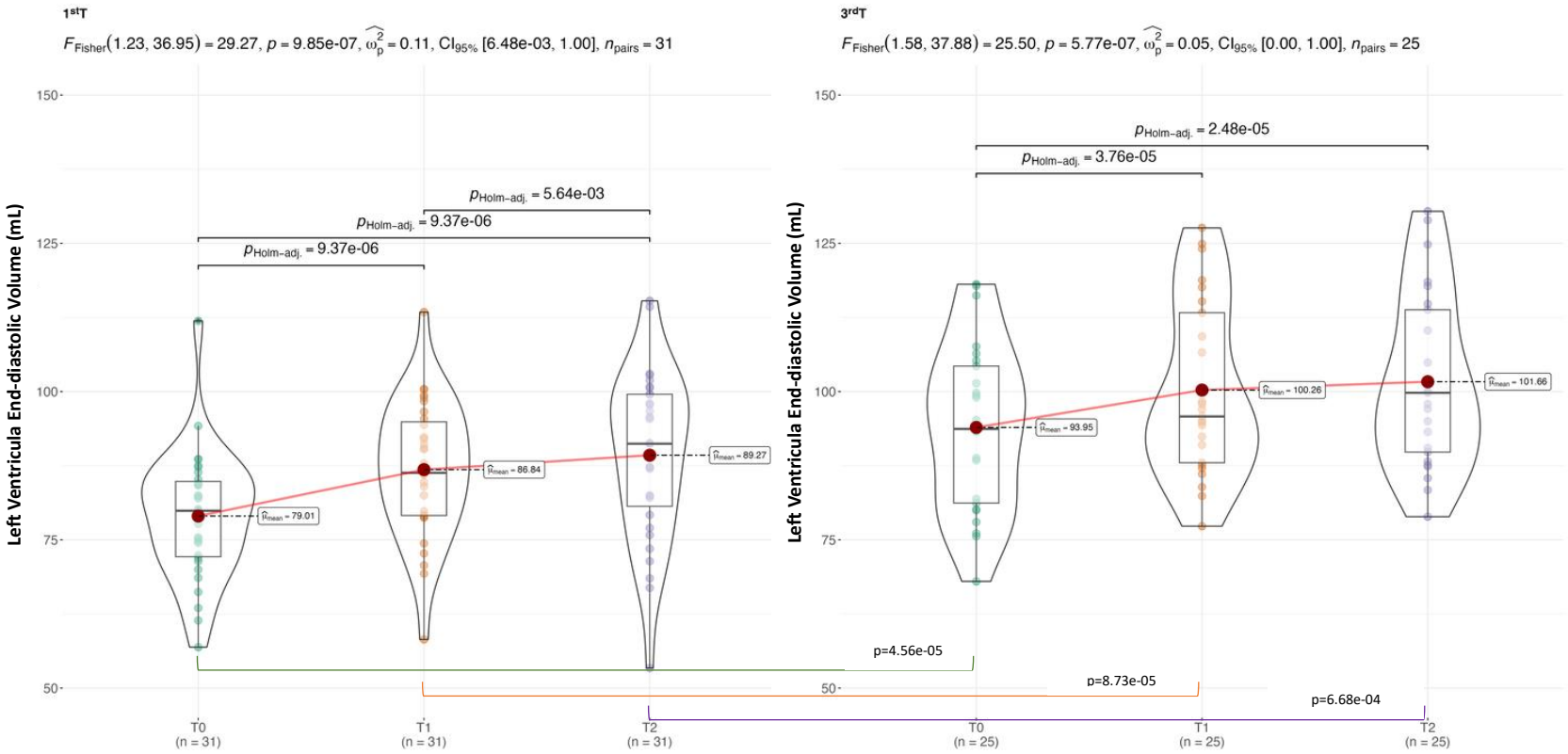


Figure 1D: Progression of left ventricular end-diastolic volume before (T0), immediately after (T1), and 15 minutes after passive leg elevation (T2) in participants evaluated in 1st trimester (1stT) and another assessed in 3rd trimester (3rdT). The green, orange and purple points in T0, T1 and T2, respectively, represent each participant with left ventricular volume quantification in each trimester of pregnancy, 1stT and 3rdT. The global trend is shown by a red line, connecting average population means in the three evaluation moments in each trimester of pregnancy (1stT - mean in T0: 79.01mL *vs* mean in T1: 86.84mL *vs* mean in T2: 89.27mL, p<0.001; 3rdT - mean in T0: 93.95mL *vs* mean in T1: 100.26mL *vs* mean in T2: 101.66mL, p<0.001). Data median and interquartile range are depicted through box plots and data distribution with violin plots.

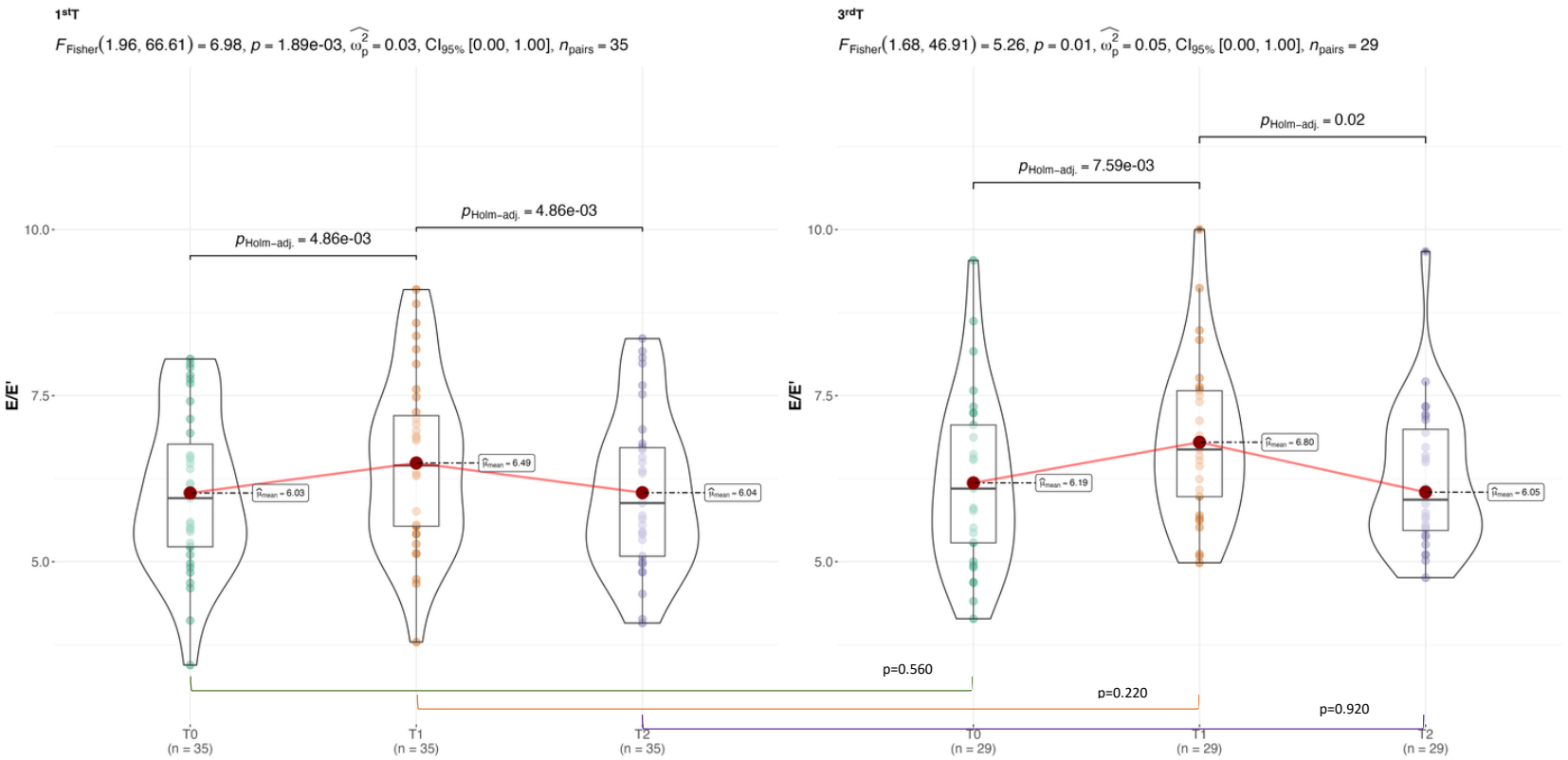


Figure 1E: Progression of E/e' before (T0), immediately after (T1), and 15 minutes after passive leg elevation (T2) in participants evaluated in 1st trimester (1stT) and another assessed in 3rd trimester (3rdT). The green, orange and purple points in T0, T1 and T2, respectively, represent each participant with E/e' quantification in each trimester of pregnancy, 1stT and 3rdT. The global trend is shown by a red line, connecting average population means in the three evaluation moments in each trimester of pregnancy (1stT - mean in T0: 6.03 *vs* mean in T1: 6.49 *vs* mean in T2: 6.04, p=0.002; 3rdT - mean in T0: 6.19 *vs* mean in T1: 6.80 *vs* mean in T2: 6.05, p=0.001). Data median and interquartile range are, also, depicted through box plots and data distribution with violin plots.

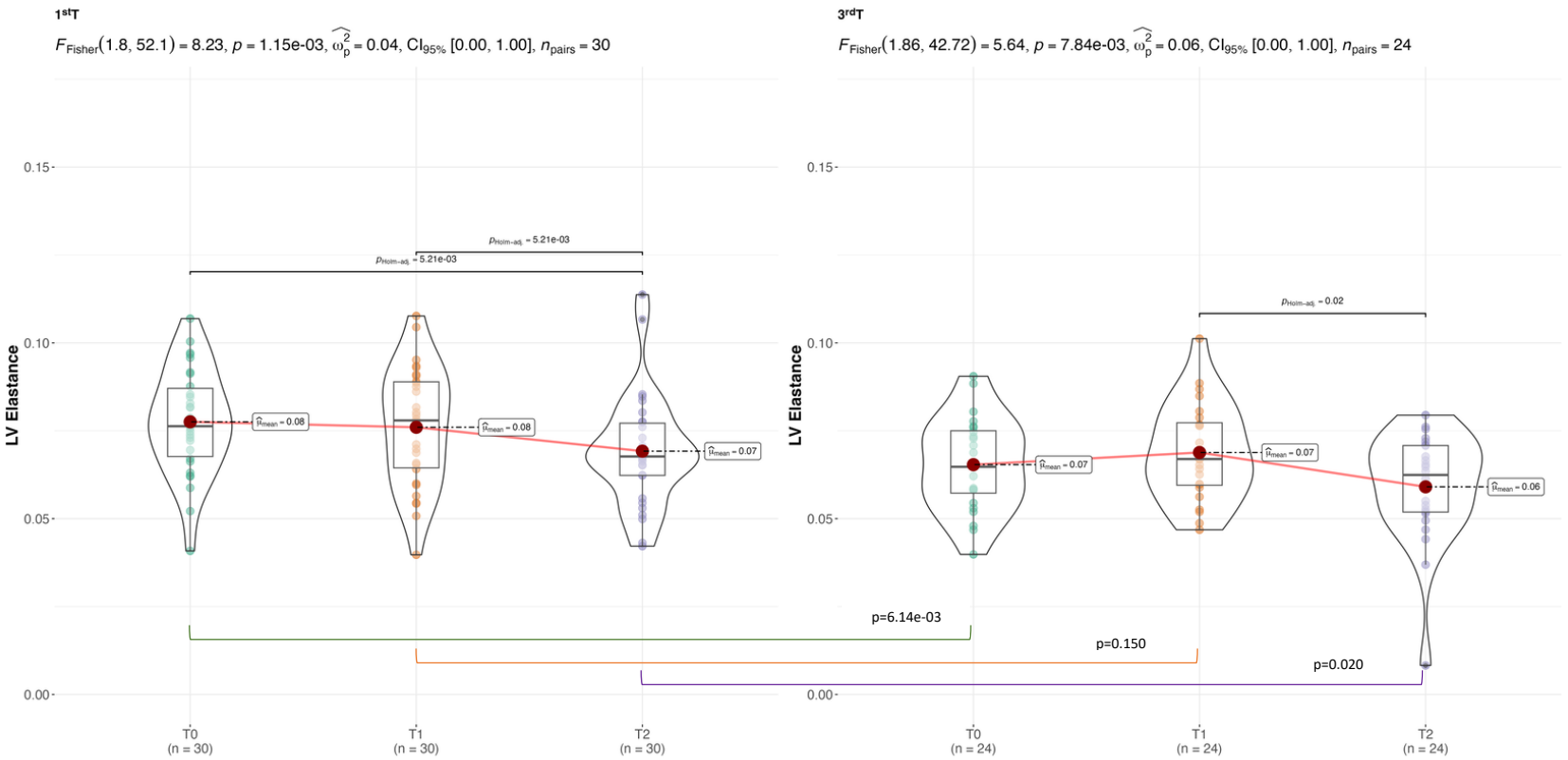
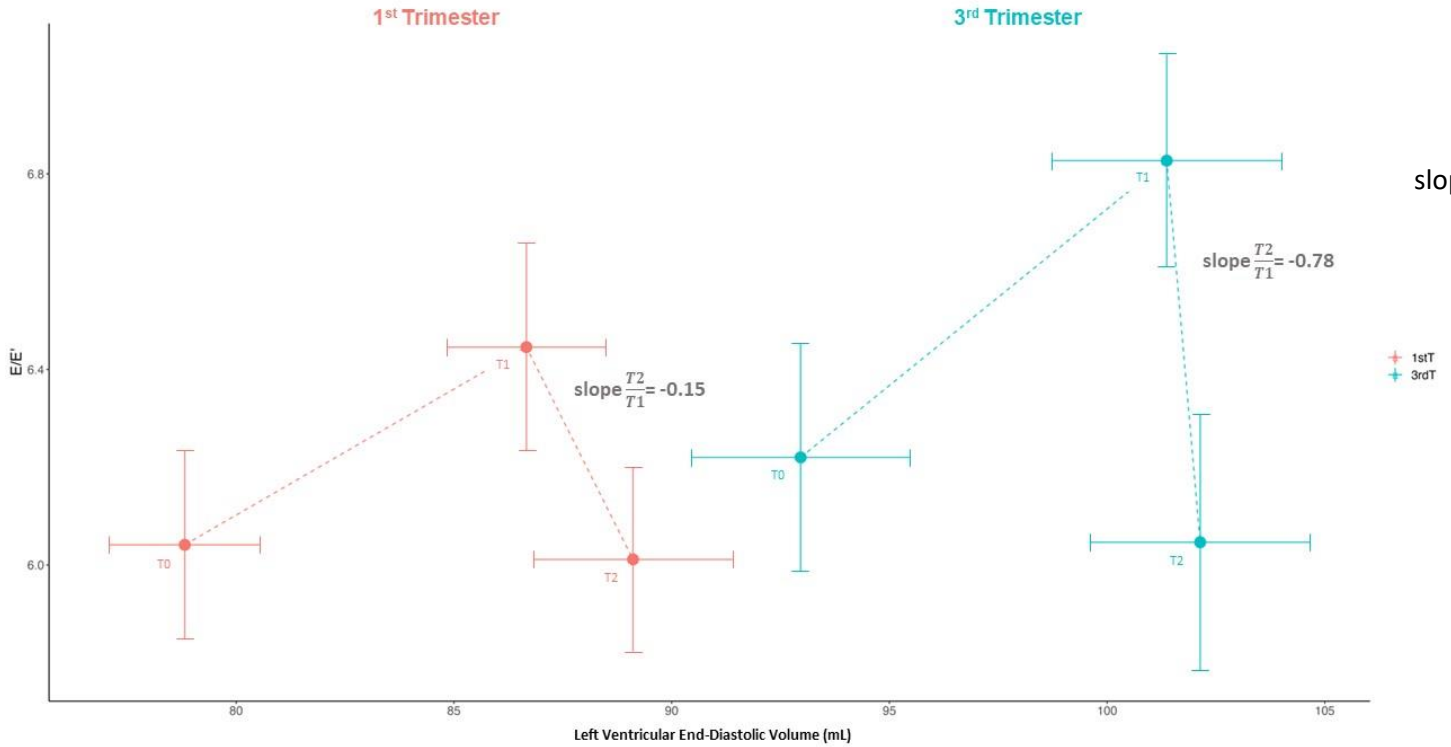


Figure 1F: Progression of left ventricular (LV) elastance before (T0), immediately after (T1), and 15 minutes after passive leg elevation (T2) in participants evaluated in 1st trimester (1stT) and another assessed in 3rd trimester (3rdT). The green, orange and purple points in T0, T1 and T2, respectively, represent each participant with left ventricular elastance calculation in each trimester of pregnancy, 1stT and 3rdT. The global trend is shown by a red line, connecting average population means in the three evaluation moments in each trimester of pregnancy (1stT - mean in T0: 0.08 *vs* mean in T1: 0.08 *vs* mean in T2: 0.07, p=0.001; 3rdT - mean in T0: 0.07 *vs* mean in T1: 0.07 *vs* mean in T2: 0.06, p=0.008). Data median and interquartile range are, also, depicted through box plots and data distribution with violin plots.



slope $\frac{T2}{T3}$ 1st T *vs* slope $\frac{T2}{T3}$ 3rd T

p-value: **0.025**

Figure 2: Filling pressures, measured by E/e', rose immediately after sudden leg elevation (T1) and dropped to baseline values 15 minutes later (T2), in pregnant women at 1st T. Despite superior E/e' values, a similar trend was observed in 3rd T. In contrast, left ventricular end-diastolic volume showed a persistent increase through passive leg elevation maneuver in 1st T. In 3rd T, pregnant women displayed higher left ventricular end-diastolic volume at baseline (T0), which may limit its response to persistent enlargement induced by acute volume overload 15 minutes later. A distinct slope magnitude was observed between T2 and T1 in 1st T and 3rd T (1st T: -0.15 *vs* 3rd T: -0.78, p=0.025). Each point represents the mean±standard deviation of E/e' and left ventricular end-diastolic volume in T0, T1 and T2 in 1st T (orange) and 3rd T (blue). The verticle lines linked the standard error values of E/e' measures in T0, T1, and T2 in 1st T (orange) and 3rd T (blue). The horizontal lines linked the standard error values of left ventricular end-diastolic volume measures in T0, T1, and T2 in 1st T (orange) and 3rd T (blue).

Impact of Cardiovascular Risk Factors, Parity, and Age

In contrast to parity and age, the presence of cardiovascular risk factors had a significant impact on the SIC-response (Table 3). Pregnant women with cardiovascular risk factors showed higher values of inferior vena cava diameter (difference: 1.62mm, 95% CI [0.09; 3.15], p=0.039), stroke volume (difference: 6.97mL, 95% CI [2.90; 11.03], p=0.001), LA volume (difference: 9.82mL, 95% CI [4.21; 15.43], p=0.001), LV volume (difference: 13.13 mL, 95% CI [6.47; 19.79], p<0.001), and E/e' (difference: 0.71, 95% CI [0.06; 1.35], p=0.032). Accordingly, cardiovascular risk factors were important modulators of the SIC-response, as shown in the multivariable generalized linear mixed models (Table 3). However, these cardiovascular risk factors (-0.07, 95% CI [-6.70, 6.50], p>0.999, Supplemental Table 7b), parity (-3.50, 95% CI [-9.90, 2.90], p=0.200, Supplemental Table 7b), and age (-0.67, 95% CI [-1.40, 0.09], p=0.082, Supplemental Table 7b) had no impact on NT-proBNP levels.

Table 3: Compilation of significant results from contrasts computations and from pre-specified predictors assessed through multivariable linear mixed models (Supplemental Tables 1-6 a and b) performed in each echocardiographic parameter to characterize SIC-response. The contrast computations calculate the difference between two levels of categorical or continuous predictors on the evaluated echocardiographic parameter. The column of the GLM model provides the impact of each pre-specified predictor (trimester of pregnancy/ cardiovascular risk factors/ evaluation moment) in each echocardiographic parameter (using the marginal effects).

Parameter	Trimester of Pregnancy [3rdT *vs* 1stT]		Cardiovascular Risk Factors [Yes *vs* No]		Evaluation moment [T1 *vs* T0]		Evaluation moment [T2 *vs* T0]	
	Contrasts	**GLM model**	**Contrasts**	**GLM model**	**Contrasts**	**GLM model**	**Contrasts**	**GLM model**
Inferior vena cava diameter	-4.14[1] [-5.40, 2.88][2] p<0.001[3]	-4.10[1] [-5.40, -2.90][2] p<0.001[3]	1.62[1] [0.09, 3.15][2] p=0.039[3]	-4.10[1] [-5.40, -2.90][2] p<0.001[3]	4.24[1] [3.68, 4.79][2] p<0.001[3]	4.20[1] [3.70, 4.80][2] p<0.001[3]	4.42[1] [3.87, 4.97][2] p<0.001[3]	4.40[1] [3.90, 5.00][2] p<0.001[3]
Stroke Volume	6.79[1] [3.43, 10.16][2] p<0.001[3]	6.80[1] [3.40, 10.00][2] p<0.001[3]	6.970[1] [2.90, 11.03][2] p=0.001[3]	7.00[1] [2.80, 11.00][2] p=0.001[3]	3.08[1] [1.71, 4.46][2] p<0.001[3]	3.10[1] [1.70, 4.50][2] p<0.001[3]	4.87[1] [3.52, 6.22][2] p<0.001[3]	4.90[1] [3.50, 6.20][2] p<0.001[3]
LA Volume	5.30[1] [0.69, 9.90][2] p=0.024[3]	5.30[1] [0.60, 10.00][2] p=0.028[3]	9.82[1] [4.21, 15.43][2] p=0.001[3]	9.80[1] [4.10, 16.00][2] p=0.001[3]	5.78[1] [4.28, 7.29][2] p<0.001[3]	5.80[1] [4.30, 7.30][2] p<0.001[3]	6.45[1] [4.95, 7.96][2] p<0.001[3]	6.50[1] [4.90, 8.00][2] p<0.001[3]
LV End-diastolic Volume	13.24[1] [7.74, 18.75][2] p<0.001[3]	13.00[1] [7.60, 19.00][2] p<0.001[3]	13.13[1] [6.47, 19.79][2] p<0.001[3]	13.00[1] [6.30, 20.00][2] p<0.001[3]	7.13[1] [5.34, 8.92][2] p<0.001[3]	7.10[1] [5.30, 8.90][2] p<0.001[3]	8.98[1] [7.23, 10.75][2] p<0.001[3]	9.00[1] [7.20, 11.0][2] p<0.001[3]
E/e'			0.71[1] [0.06, 1.35][2] p=0.032[3]	0.71[1] [0.05, 1.40[2] p=0.036[3]	0.51[1] [0.25, 0.77][2] p<0.001[3]	0.51[1] [0.25, 0.77][2] p<0.001[3]		

Legend: GLM model: multivariable generalized linear mixed model; LA: left atrial; LV: left ventricular.(1) coefficient,(2) 95% CI, (3) p-value.

Discussion

The present study is the first to characterize echocardiographically the SIC mechanism in the context of physiological hypertrophy induced by pregnancy. We performed a passive leg elevation maneuver to induce AVO in different groups of pregnant women undergoing distinct basal hemodynamic preload and cardiovascular remodelling (1stT *vs* 3rdT). We found that the SIC-response was different between 1stT and 3rdT and was significantly influenced by the presence of cardiovascular risk factors.

As expected, pregnant women evaluated in the 3rdT revealed higher baseline values in most echocardiographic parameters than those evaluated in the 1stT. The exceptions were LV elastance, ejection fraction, E/A ratio, and inferior vena cava diameter, which were lower in the 3rdT group. The lower LV elastance in the 3rdT group may result from an elevated LV end-diastolic volume induced by an augmented volume overload at the end of gestation, which is necessary to meet the demands of the growing fetus. This cardiac dilation, associated with higher LV mass, leads to increased LV filling pressures and, consequently, deterioration of diastolic function, as evidenced by decreased E/A (9, 33). The diameter of the inferior vena cava in 3rdT pregnant women was unexpectedly lower than that in 1stT, which may be explained by compression of the distal part of this vein by the enlarged uterus, as already described by Ryo *et al.* (202).

Immediately after the passive leg elevation maneuver, effective preload augmentation was confirmed at both trimesters through a significant enlargement of the inferior vena cava. Concomitantly, the E/e' and LV end-diastolic volume increased significantly in both stages of pregnancy. By virtue of SIC, E/e' decreased significantly 15 minutes after AVO in both time points, but LV end-diastolic volume continued to expand only in the 1stT. As we have previously shown (203), this LV's inability to fill further in the 3rdT might result from increased levels of procollagen type I carboxy-terminal propeptide (PICP), a fibrosis marker that may compromise myocardial compliance and limit LV expansion (130). In this context, collagen synthesis may arise as a compensatory response to pregnancy hemodynamic overload to maintain the structural integrity of the cardiac extracellular matrix (30). In addition, it contributes to an increase in myocardial passive tension during diastolic filling (31), which was confirmed in the present study by increased E/e' values in 3rdT participants in moment T1.

Despite this LV structural limitation to further enlarge 15 minutes after AVO (T2) in 3rdT, our results suggested that SIC-response is exacerbated in these pregnant women when compared to those in 1stT , as confirmed by the significantly different slopes (T2/T1, 1stT: -0.15 vs 3rdT: - 0.78).

In addition, the further reduction of E/e´ 15 minutes after AVO (T2) compared to baseline (T0) in 3rdT, but not in 1stT, corroborate our hypothesis.

This distinct adaptation in the 3rdT group, suggesting an exacerbated SIC-response, was paralleled by a significant increment of stroke volume and cardiac output. Indeed, according to the Frank-Starling law, the transient volume overload caused by passive leg elevation in our cohort sample induced a significant increase in stroke volume and cardiac output, even in 3rdT pregnant women. Our results contrast with the study by Vårtun *et al.*, who showed no significant increase in stroke volume upon leg elevation in healthy women after 32 weeks of pregnancy, indicating reduced cardiac contractility with advancing gestation (107). The different evaluation time points in their cohort may explain these divergent results.

Interestingly, both 1stT and 3rdT pregnant women showed reduced LV stiffness after sustained AVO, being significantly lower in the 3rdT hypertrophic hearts, indicating an improvement of LV compliance and SIC-response. These cardiac adaptations to AVO were accompanied by opposite trends for NT-proBNP to increase in 1stT, in contrast to a slight decrease in 3rdT. Indeed, in 1stT, AVO increases ventricular myocardial stretching, leading to a slight NT-proBNP increase, which is consistent with LV further enlarging 15 minutes upon passive leg elevation (16, 204). In contrast, in the 3rdT, NT-proBNP levels are stable, which likely results from the fact that the LV may be close to its maximal capacity to accommodate blood and, thereby, unable to dilate further, as confirmed by a non-significant LV end-diastolic volume enlargement 15 minutes after AVO. These modest NT-proBNP results have to be complemented by future studies focusing on pregnancy-derived neuroendocrine players, such as estrogen, progesterone, relaxin, and oxytocin, whose diuretic, vasodilator, and direct myocardial effects may surpass those induced by natriuretic peptides (11, 205-207). For instance, relaxin presented a crucial anti-fibrotic and anti-apoptotic effect throughout pregnancy's cardiac remodelling, which may reduce LV stiffness and concomitantly promote vasodilatation (206). Some of the beneficial actions of oxytocin in cardiac remodeling seem to be mediated by the production of cGMP stimulated by the local release of atrial natriuretic peptide and the synthesis of nitric oxide (207). Moreover, progesterone and estrogens have shown LV hypertrophic and anti-hypertrophic effects, respectively, and their balance is essential for pregnancy maintenance and cardiovascular homeostasis (11).

The baseline NT-proBNP levels in our cohort were similar between both trimesters, which contrasts with previous studies that reported lower values at term (204, 208, 209). However, our cohort deliberately included pregnant women with cardiovascular risk factors (19% in 1stT *vs* 26% in 3rdT), thereby possibly increasing NT-proBNP levels as the hemodynamic load progressed

throughout pregnancy. Indeed, increased NT-proBNP values were documented according to the severity of hypertensive diseases of pregnancy (preeclampsia *vs* chronic arterial hypertension) in 3rd trimester (210). Moreover, the independence of groups in our cohort precluded the longitudinal assesmento of NT-proBNP levels throughout gestation.

We also observed that cardiovascular risk factors affect the SIC mechanism in the context of pregnancy. In our cohort, pregnant women with cardiovascular risk factors showed increased cardiac chamber volumes and impaired diastolic function (augmented E/e'). Accordingly, previous studies have documented a higher prevalence of concentric hypertrophy or concentric remodeling associated with cardiac chambers' enlargement (17, 59, 63, 67, 72-75) and deterioration of diastolic function (64, 68, 72, 81, 144). These changes, primarily described in hypertensive disorders of pregnancy (10, 29, 30, 34, 35), are also associated with stroke volume reduction (79); the latter not confirmed by us. In addition, reduced stroke volume in the 3rdT has been documented in obesity (53, 54), the most prevalent cardiovascular risk factor in our cohort sample. In line with our previous results (203), this discrepancy might be due to the lower severity of the risk factors in our cohort, which resulted from regular medical appointments combined with nutritional advice and strict weight, glucose, and blood pressure control provided in our hospital to pregnant women with cardiovascular risk factors.

In this work, maternal age and parity did not impact SIC-response. Previous studies have documented significant age-related diastolic decline during gestation (195). In our cohort, maternal age was similar between groups, being the diastolic impairment observed in the 3rdT mostly ascribed to the pregnancy's significant volume overload rather than aging. Higher parity has been associated with increased cardiac volumes, LVM and diastolic dysfunction (42, 44). The relatively low parity (≤ 2 previous live births) in our cohort may explain the homogeneity in SIC.

The present study has limitations, such as the small sample size and the absence of within-subject analysis of SIC-response in the 1stT and 3rdT. These were consequences of the COVID-19 pandemic that precluded follow-up cardiovascular evaluation in 3rdT and compromised the appointment duration, thereby limiting the maneuver of passive leg elevation.

Conclusion

A distinct functional response to SIC was observed between 1stT and 3rdT of pregnancy, which was influenced by cardiovascular risk factors. Despite the LV of 3rdT pregnant women showing a structural limitation to dilate and accommodate increased volume upon AVO, its physiological hypertrophy did not compromise the SIC-mechanism, suggesting its exacerbation.

Conflict of interest

The authors have no conflicts of interest to declare.

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Availability of data and materials

Data will be available upon request.

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Supplemental Table 1: Echocardiographic assessment before (T0), immediately after (T1), and 15 minutes after passive leg elevation (T2).

	1st T						3rd T			
Evaluation Moment	n	T0	n	T1	n	T2	n	T0	n	T1	n	T2
Heart rate, beats/min	37	74 ± 10	37	73 ± 10	37	74 ± 9	31	81 ± 11	31	80 ± 9	31	80 ± 10
LVEF, %	33	62 ± 4	33	60 ± 3	32	59 ± 3	29	59 ± 4	27	58 ± 3	28	60 ± 4
Mitral inflow parameters												
E, cm/s	36	93 ± 15	37	103 ± 18	37	95 ± 14	30	83 ± 13	30	96 ± 13	30	87 ± 19
A, cm/s	36	57 ± 10	37	59 ± 12	37	60 ± 10	30	62 ± 13	30	65 ± 17	30	63 ± 15
E/A	36	1.67 ± 0.39	37	1.80 ± 0.43	37	1.61 ± 0.34	30	1.41 ± 0.37	30	1.57 ± 0.45	30	1.49 ± 0.38
TDI mitral annulus												
Septal												
e' sept, cm/s	37	12.7 ± 1.8	37	13.3 ± 1.9	37	13.2 ± 2.1	37	11.2 ± 1.7	37	11.8 ± 1.5	37	12.0 ± 1.7
Lateral												
e' lat, cm/s	37	16.0 ± 2.4	36	19.2 ± 3.1	37	18.8 ± 3.0	30	16.0 ± 2.4	30	16.7 ± 2.6	29	16.7 ± 1.9
E/e' average (E/e')	36	6.04 ± 1.17	36	6.45 ± 1.29	37	6.01 ± 1.14	30	6.22 ± 1.30	30	6.83 ± 1.21	29	6.0 ± 1.5
Stroke volume, mL	33	49 ± 7	33	52 ± 6	32	53 ± 8	29	55 ± 9	27	59 ± 9	28	61 ± 9
Cardiac output, L/min	33	3.6 ± 0.8	33	3.8 ± 0.7	32	3.9 ± 0.7	29	4.4 ± 0.8	27	4.7 ± 0.7	28	4.9 ± 0.9
Maximum LA volume, mL	34	44 ± 9	35	50 ± 9	32	52 ± 10	31	51 ± 12	28	57 ± 12	28	56 ± 13
LV end-diastolic volume, mL	33	79 ± 11	33	87 ± 11	32	89 ± 14	29	93 ± 14	27	101 ± 15	28	102 ± 14
LV end-systolic volume, mL	33	30 ± 5	33	35 ± 6	32	36 ± 6	29	38 ± 7	27	42 ± 7	28	41 ± 7
LV Elastance	33	0.08 ± 0.01	33	0.08 ± 0.02	32	0.07 ± 0.02	28	0.07 ± 0.01	27	0.07 ± 0.01	28	0.06 ± 0.01
Inferior vena cava, mm	37	12 ± 2	36	17 ± 3	37	17 ± 3	30	9 ± 3	29	13 ± 3	31	13 ± 3
Relative Wall Thickness	37	0.31 ± 0.05		-		-	31	0.34 ± 0.05		-		-
LV mass, g	37	98 ± 16		-		-	31	122 ± 32		-		-

Values expressed by mean±standard deviation. n: number of participants.

Supplemental Table 2a: Estimated difference (contrasts) of clinical variables and trimesters of pregnancy related to variation of inferior vena cava diameter during acute volume overload induced by passive leg elevation maneuver.

Variables	**Components**	**Difference**	**p-value**	**95% CI**
Age at Recruitment Moment	dY/dX	-0.06	0.491	[-0.24, 0.12]
Cardiovascular Risk Factors	Yes - No	1.62	**0.039**	[0.09, 3.15]
Evaluation Moment	T1 - T0	4.24	**<0.001**	[3.68, 4.79]
Evaluation Moment	T2 - T0	4.42	**<0.001**	[3.87, 4.97]
Multiparous	Yes - No	1.10	0.146	[-0.38, 2.58]
Trimester of Pregnancy	$3^{rd}T$ - $1^{st}T$	-4.14	**<0.001**	[-5.40, -2.88]

Supplemental Table 2b: Multivariable generalized linear mixed model for inferior vena cava diameter.

Variables	**Beta 95% CI[1]**	**p-value**
Trimester of Pregnancy	-4.10 [-5.40, -2.90]	**<0.001**
Acute Volume Overload Moments		
T1	4.20 [3.70, 4.80]	**<0.001**
T2	4.40 [3.90, 5.00]	**<0.001**
Age at Recruitment Moment	-0.06 [-0.24, 0.12]	0.500
Multiparous	1.10 [-0.41, 2.60]	0.200
Cardiovascular Risk Factors	1.60 [0.05, 3.20]	**0.043**
Conditional R^2	0.847	
Marginal R^2	0.504	
[1]CI = Confidence Interval		

Supplemental Table 3a: Estimated difference (contrasts) of clinical variables and trimesters of pregnancy related to stroke volume variation during acute volume overload induced by passive leg elevation maneuver.

Variables	**Components**	**Difference**	**p-value**	**95% CI**
Age at Recruitment Moment	dY/dX	-0.01	0.960	[-0.49, 0.46]
Cardiovascular Risk Factors	Yes - No	6.97	**0.001**	[2.90, 11.03]
Evaluation Moment	T1 - T0	3.08	**<0.001**	[1.71, 4.46]
Evaluation Moment	T2 - T0	4.87	**<0.001**	[3.52, 6.22]
Multiparous	Yes - No	0.02	0.990	[-3.91, 3.96]
Trimester of Pregnancy	3^{rd}T - 1^{st}T	6.79	**<0.001**	[3.43, 10.16]

Supplemental Table 3b: Multivariable generalized linear mixed model for stroke volume.

Variables	**Beta 95% CI[1]**	**p-value**
Trimester of Pregnancy	6.80 [3.40, 10.00]	**<0.001**
Acute Volume Overload Moments		
T1	3.10 [1.70, 4.50]	**<0.001**
T2	4.90 [3.50, 6.20]	**<0.001**
Age at Recruitment Moment	-0.01 [-0.50, 0.47]	>0.999
Multiparous	0.02 [-4.00, 4.00]	0.300
Cardiovascular Risk Factors	7.0 [2.80, 11.00]	**0.001**
Conditional R^2	0.826	
Marginal R^2	0.312	
[1]CI = Confidence Interval		

Supplemental Table 4a: Estimated difference (contrasts) of clinical variables and trimesters of pregnancy related to left atrial volume variation during acute volume overload induced by passive leg elevation maneuver.

Variables	**Components**	**Difference**	**p-value**	**95% CI**
Age at Recruitment Moment	dY/dX	0.04	0.895	[-0.61, 0.69]
Cardiovascular Risk Factors	Yes - No	9.82	**0.001**	[4.21, 15.43]
Evaluation Moment	T1 - T0	5.78	**<0.001**	[4.28, 7.29]
Evaluation Moment	T2 - T0	6.45	**<0.001**	[4.95, 7.96]
Multiparous	Yes - No	-1.74	0.528	[-7.14, 3.67]
Trimester of Pregnancy	3^{rd}T - 1^{st}T	5.30	**0.024**	[0.69, 9.90]

Supplemental Table 4b: Multivariable generalized linear mixed model for left atrial volume.

Variables	**Beta 95% CI[1]**	**p-value**
Trimester of Pregnancy	5.30 [0.60, 10.00]	**0.028**
Acute Volume Overload Moments		
T1	5.80 [4.30, 7.30]	**<0.001**
T2	6.50 [4.90, 8.00]	**<0.001**
Age at Recruitment Moment	0.04 [-0.62, 0.71]	0.900
Multiparous	-1.70 [-7.20, 3.80]	0.500
Cardiovascular Risk Factors	9.8 [4.10, 16.00]	**0.001**
Conditional R^2	0.866	
Marginal R^2	0.240	
[1]CI = Confidence Interval		

Supplemental Table 5a: Estimated difference (contrasts) of clinical variables and trimesters of pregnancy related to left ventricular end-diastolic volume variation during acute volume overload induced by passive leg elevation maneuver.

Variables	**Components**	**Difference**	**p-value**	**95% CI**
Age at Recruitment Moment	dY/dX	0.01	0.987	[-0.77, 0.78]
Cardiovascular Risk Factors	Yes - No	13.13	**<0.001**	[6.47, 19.79
Evaluation Moment	T1 - T0	7.13	**<0.001**	[5.34, 8.92]
Evaluation Moment	T2 - T0	8.99	**<0.001**	[7.23, 10.75]
Multiparous	Yes - No	0.73	0.824	[-5.71, 7.18]
Trimester of Pregnancy	3^{rd}T - 1^{st}T	13.24	**<0.001**	[7.74, 18.75]

Supplemental Table 5b: Multivariable generalized linear mixed model for left ventricular end-diastolic volume.

Variables	**Beta 95% CI[1]**	**p-value**
Trimester of Pregnancy	13.00 [7.60, 19.00]	**<0.001**
Acute Volume Overload Moments		
T1	7.10 [5.30, 8.90]	**<0.001**
T2	9.00 [7.20, 11.00]	**<0.001**
Age at Recruitment Moment	0.01 [-0.78, 0.80]	>0.999
Multiparous	0.73 [-5.80, 7.30]	0.800
Cardiovascular Risk Factors	13 [6.30, 20.00]	**<0.001**
Conditional R^2	0.899	
Marginal R^2	0.402	
[1]CI = Confidence Interval		

Supplemental Table 6a: Estimated difference of clinical variables and trimesters of pregnancy related to E/e' variation during acute volume overload induced by passive leg elevation maneuver.

Variables	**Components**	**Difference**	**p-value**	**95% CI**
Age at Recruitment Moment	dY/dX	0.001	0.970	[-0.07, 0.08]
Cardiovascular Risk Factors	Yes - No	0.71	**0.032**	[0.06, 1.35]
Evaluation Moment	T1 - T0	0.51	**<0.001**	[0.25, 0.77]
Evaluation Moment	T2 - T0	-0.07	0.621	[-0.32, 0.19]
Multiparous	Yes - No	-0.37	0.246	[-0.99, 0.25]
Trimester of Pregnancy	3^{rd}T - 1^{st}T	0.22	0.413	[-0.30, 0.74]

Supplemental Table 6b: Multivariable generalized linear mixed model for E/e'.

Variables	**Beta 95% CI[1]**	**p-value**
Trimester of Pregnancy	0.22 [-0.31, 0.75]	0.4
Acute Volume Overload Moments		
T1	0.51 [0.25, 0.77]	**<0.001**
T2	-0.07 [-0.33, 0.20]	0.600
Age at Recruitment Moment	0.00 [-0.07, 0.08]	>0.999
Multiparous	-0.37 [-1.00, 0.27]	0.300
Cardiovascular Risk Factors	0.71 [0.05, 1.40]	**0.036**
Conditional R^2	0.668	
Marginal R^2	0.101	
[1]CI = Confidence Interval		

Supplemental Table 7a: Estimated difference of clinical variables and trimesters of pregnancy related to NT-proBNP variation during acute volume overload induced by passive leg elevation maneuver.

Variables	Components	Difference	p-value	95% CI
Age at Recruitment Moment	dY/dX	-0.67	0.077	[-1.42, 0.07]
Cardiovascular Risk Factors	Yes - No	-0.07	0.982	[-6.54, 6.39]
Evaluation Moment	T2 - T0	1.06	0.547	[-2.39, 4.50]
Multiparous	Yes - No	-3.49	0.273	[-9.73, 2.75]
Trimester of Pregnancy	$3^{rd}T - 1^{st}T$	4.34	0.110	[-0.98, 9.66]

Supplemental Table 7b: Multivariable generalized linear mixed model for NT-proBNP.

Variables	Beta 95% CI[1]	p-value
Trimester of Pregnancy	4.30 [-1.10, 9.80]	0.110
Acute Volume Overload Moments		
T2	1.10 [-2.40, 4.60]	0.500
Age at Recruitment Moment	-0.67 [-1.40, 0.09]	0.082
Multiparous	-3.50 [-9.90, 2.90]	0.200
Cardiovascular Risk Factors	-0.07 [-6.70, 6.50]	>0.999
Conditional R^2	0.447	
Marginal R^2	0.084	
[1]CI = Confidence Interval		

Subchapter iv. Cardiovascular remodelling and reverse remodelling during pregnancy and postpartum: looking at the right side of the heart.

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Title: Cardiovascular remodelling and reverse remodelling during pregnancy and postpartum: looking at the right side of the heart

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Resumo

Introdução: Durante a gravidez, o sistema cardiovascular (CV) está exposto a uma sobrecarga hemodinâmica necessária ao desenvolvimento do feto. Vários estudos têm-se focado na remodelagem ventricular esquerda induzida pela gravidez, sendo escassa a evidência científica referente às câmaras cardíacas direitas.

Objetivo: Caracterizar a remodelagem CV direita e remodelagem reversa (RR) induzida pela gravidez e pós-parto, respetivamente, bem como explorar o impacto dos fatores de risco cardiovasculares (FRCV) nestes processos.

Métodos: Este estudo de coorte prospetivo recrutou mulheres grávidas voluntárias (saudáveis e com FRCV – obesas e/ou hipertensas e/ou com diabetes gestacional e/ou com hábitos tabágicos) em dois centros terciários entre 2019 e 2022. Foram avaliadas por ecocardiografia transtorácica bidimensional no 1º trimestre de gravidez – 1T, no 3º trimestre – 3T, um mês – PP1, seis meses – PP2, e um ano pós-parto – PP3. Em cada visita foram recolhidos dados clínicos maternos, assim como desfechos perinatais. Para a análise da remodelagem CV direita e RR foram usados modelos lineares generalizados de efeitos mistos, assim como para avaliar o impacto dos FRCV neste processo.

Resultados: Neste estudo foram incluídas 130 grávidas, 79% primíparas, com uma mediana de idades de 33 anos. Dois grupos de estudo foram definidos de acordo com a ausência (n=51, grupo saudável) ou a presença de pelo menos um FRCV (n=79, grupo FRCV). Apesar dos resultados ecocardiográficos do nosso estudo se encontrarem dentro dos limites da normalidade, observamos diferenças importantes entre grupos ao longo da gravidez e pós-parto, tais como: 1) Um aumento semelhante e significativo das dimensões da aurícula direita (AD) e do ventrículo direito (VD) durante a gestação com recuperação no momento PP2, à excepção da AD no grupo FRCV; 2) A função sistólica permaneceu preservada durante a gravidez nos dois grupos, observando-se durante a normalização hemodinâmica no pós-parto uma diminuição da excursão sistólica do plano valvular tricúspide (TAPSE) e pela velocidade do S' tricúspide. 3) Diminuição do *strain* da AD em ambos os grupos durante a gravidez, mais evidente no grupo com FRCV, que teve uma recuperação mais lenta e prolongada ao longo do pós-parto. 4) Manutenção do *strain* global longitudinal do VD durante todos os momentos de avaliação, tendo estado significativamente reduzido no 3T do grupo com FRCV, recuperando ao momento PP2. 5) Deterioração da função diastólica do 1T para o 3T nos dois grupos, com recuperação no momento PP1, observada através da diminuição significativa da razão E/A tricúspide, sendo este valor significativamente mais elevado no grupo saudável. 6). Os parâmetros pulmonares permaneceram constantes durante toda a gravidez e pós-parto, demonstrando de forma consistente valores mais elevados de pressão sistólica da artéria pulmonar (PSAP) no grupo com FRCV.

Conclusão: Este estudo descreve alterações funcionais e estruturais subtis do coração direito, dentro dos limites da normalidade/fisiológicos, com recuperação aos seis meses pós-parto. Os FRCV parecem afetar a magnitude das alterações observadas relativamente à função diastólica do VD, PSAP e

deformação miocárdica (strain), sem impacto significativo na função sistólica do VD. Deste modo, concluimos que a gravidez representa uma janela de oportunidade para detetar alterações precoces no VD, podendo anticipar disfunção cardíaca futura.

Abstract

Introduction: During pregnancy, the cardiovascular (CV) system is exposed to hemodynamic overload to meet the increased demands of the growing foetus. Many studies have focused on pregnancy-induced left ventricular remodelling, overlooking the changes in right cardiac chambers.

Aim: To characterize the right CV remodelling and reverse remodelling (RR) induced by pregnancy and postpartum, respectively, and the impact of CV risk (CVR) factors in these processes.

Methods: This prospective cohort was recruited at two tertiary centres from 2019 to 2022 and included volunteer pregnant women with or without CVR factors - obese and/or hypertensive and/or with gestational diabetes and/or smoking habits. Women were evaluated by bidimensional transthoracic echocardiography at 1st trimester of pregnancy (1T), 3rd trimester (3T), one-month (PP1), six-months (PP2), and one-year postpartum (PP3). At each visit, maternal clinical data as well as obstetric and perinatal outcomes were collected. Generalized linear mixed-effects models were used for the analysis of right CV remodelling and RR, and to evaluate the impact of CVR factors in these processes.

Results: This study included 130 pregnant women, 79% primiparous, with a median age of 33 years old. Participants were subdivided into those without (n=51, healthy group) or with at least one CVR factor (n=79 CVR group). Despite all echocardiographic results are within the normality range, we describe the progression of right heart structural and functional adaptations throughout pregnancy and postpartum, such as: 1) Similar enlargement of the right atrium (RA) and RV (right ventricle) dimensions throughout pregnancy, recovering at PP2 except for RA in the CVR group; 2) Preserved RV systolic function throughout pregnancy in both groups, while the postpartum hemodynamic normalization triggers a reduction of tricuspid annular plane systolic excursion (TAPSE) and tricuspid S' wave velocity; 3) Reduced RA strain in both groups during pregnancy, more patent in CVR group, which had a slower postpartum recovery; 4) Preserved RV global longitudinal strain (GLS) throughout all time points, being significantly reduced in 3T of CVR group and recovering at PP2; 5) Deterioration of diastolic function from 1T to 3T in both groups with an early recovery at PP1, assessed by the tricuspid E/A ratio, being these values significantly higher in the healthy group, and 6) Steady pulmonary artery systolic pressure (PASP) values throughout pregnancy and postpartum, showing consistently higher values in the CVR group.

Conclusion: This study described subtle right cardiac functional and structural changes within the normal/physiological range, which recovered as soon as 6 months after delivery. CVR factors seemed to affect the magnitude of RV diastolic function changes, PASP and myocardial deformation (strain) without any impact on the RV systolic function. We conclude that pregnancy represents a window for detecting early RV changes, possibly foreseeing future cardiac dysfunction.

Keywords

Pregnancy

Postpartum

Right Heart

Right Cardiovascular Remodelling

Reverse Remodelling

Cardiovascular risk factors

Introduction

During pregnancy, the cardiovascular (CV) system is exposed to a series of adaptations to meet the needs of the foetus and the normal progression of gestation (1,2). Many studies have focused on pregnancy-induced left ventricular remodelling, overlooking the changes in the right cardiac chambers (3,4) (5–7).

Hemodynamic overload underlying pregnancy is the primary stimulus to induce cardiac remodelling. This overload results from volume increment inherent to the augmentation of plasma volume (nearly 40%) and erythropoiesis (8). This preload elevation is counterbalanced by the decrease of peripheral vascular resistance, leading to the decline of arterial pressure (1,9,10).

Transthoracic echocardiography (TTE) is the recommended imaging method to monitor cardiac remodelling (11), being of great utility to characterise physiological remodelling during pregnancy. This physiological remodelling comprises chamber enlargement and left ventricular hypertrophy, which return to pre-pregnancy values after delivery (12,13). During gestation and the postpartum period, the ejection fraction remains constant, while global longitudinal strain, a more sensitive method for evaluating systolic function, presents divergent results (14,15). While Cong *et al.* described a transient reduction in all strain parameters in 3rd trimester, recovering postpartum, Sengupta *et al.* reported increased radial strain to compensate for the lower longitudinal strain during gestation. These differences could be explained by the use of a more sensitive and specific method by Cong *et al.*, such as 3D strain. Regarding diastolic function, an increase in left ventricular filling pressure was observed until 3rd trimester, recovering at postpartum (16).

Regarding the right CV system, namely the right cardiac chambers and pulmonary circulation, data describing cardiac adaptations during pregnancy and the postpartum period are scarce. The right ventricle (RV) displays distinct anatomical and physiological characteristics from the LV. The RV is a system – pump – of low pressure, divided into three regions: inflow tract chamber, trabecular apical myocardium, and outflow tract chamber (17,18). Its free wall width ranges from one to five millimetres, includes two muscle layers with different orientations: circumferential and longitudinal (17–19). Along with these features and his trapezoid form, the RV has a simpler mechanical function, sharing the interventricular septum (IVS) with the LV, pericardial space, and epicardial circumferential myocytes. Thus, modifications in volume or pressure overload on the LV can directly affect the RV (18).

Limited information is available on right cardiac remodelling during pregnancy. Transient changes have been described by postpartum normalization (16). These changes include an

increase in right ventricular volume and a significant decrease in systolic function, measured by tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC), and longitudinal strain of the RV (16,20). Deterioration of the right diastolic function was also documented during gestation, mainly in the 3rd trimester.

Studies focusing on pulmonary circulation have documented an increase in pulmonary flow in 2nd and 3rd trimesters, associated with reduced pulmonary vascular resistance, while pulmonary artery pressure remains unchanged (21). These findings indicate the remarkable capacity of pulmonary circulation to adapt to pregnancy.

Pregnancy is a state of "physiological stress" and some studies have demonstrated that pregnant women with CVR factors or who develop pregnancy complications, such as preeclampsia and gestational diabetes mellitus (DM), have a higher risk of developing CV diseases in the long-term (22–27). Thus, cardiac remodelling that develops throughout pregnancy represents a key stage for screening subclinical cardiovascular disease, thereby predicting and preventing future cardiovascular events and HF in women with traditional risk factors, such as arterial hypertension and diabetes (28,29).

The present study aimed to characterize the right CV changes induced by pregnancy and the postpartum period, and to explore the impact of CVR factors.

Methods and Design

Study design and setting

This was a prospective cohort study conducted at the Obstetrics Departments of Centro Hospitalar Universitário São João (CHUSJ) and Unidade Local de Saúde de Matosinhos - Hospital Pedro Hispano (ULSM-HPH), approved by the Ethics Committees of CHUSJ and ULSM-HPH (ID 201/18 and ID 154/20/RS, respectively). Participants were willing and able to provide written informed consent. The confidentiality and data anonymity complied with the Declaration of Helsinki of 1964, revised in Fortaleza, in 2013. The full protocol of the present study has been previously published (30).

Participants

Participants were recruited from February 2019 to July 2022, at their first medical appointment (in the 1st or 3rd trimester of pregnancy) or voluntarily through online forms available at https://perimyrobb.wordpress.com/, and then were followed up until 1 year after delivery.

Patient's inclusion criteria: adult pregnant women (≥18 years old), with or without cardiovascular risk factors namely chronic and/or gestational hypertension, gestational DM, and/or obesity and/or with smoking habits. Arterial hypertension was defined as systolic blood pressure [SBP] ≥140mmHg and/or diastolic blood pressure [DBP] ≥90mmHg measured in the office or in-hospital before 20 weeks of gestation (26). Gestational hypertension was defined as arterial hypertension diagnosed after 20 weeks of gestation and resolving within 42 days postpartum. Gestational DM was considered if fasting glucose was [92, 126] mg/dL at 1st trimester or ≥180mg/dL or ≥153mg/dL 1 or 2 hours after an oral glucose tolerance test [75g oral glucose load] performed at 24-28 pregnancy weeks. Obesity was defined as body mass index was≥30kg/m^2 before pregnancy.

Patient's exclusion criteria: Women with twin pregnancies, pre-existing cardiomyopathy, renal disease, chronic obstructive airway disease, active systemic infection, genetic syndromes, or type-1 or type-2 DM.

Measurements

The included participants underwent the following evaluations: 1) clinical characterization, 2) brachial blood pressure measurement, 3) bidimensional TTE, at the Department of Surgery

and Physiology of the Faculty of Medicine of the University of Porto, during the following time points: 1) 1st trimester of pregnancy – 1T [11-14 weeks], the beginning of cardiovascular remodelling); 2) 3rd trimester – 3T [30-35 weeks], at the peak of cardiovascular remodelling, when cardiovascular adaptations should be most noticeable; 3) postpartum (PP1, 6-8 weeks; PP2, 6-7 months; and PP3, 1 year after delivery) – during RR to assess cardiovascular recovery. Clinical characterization included maternal cardiovascular health, maternal health-related habits, maternal smoking habits, parity, medical history and demographics, as well as obstetric and perinatal outcomes. These data were collected through questionnaires and electronic medical records.

Echocardiographic assessment

A single operator performed a 2D conventional evaluation with a 3 MHz phased-array probe (ACUSON SC2000 PRIME™). Measurements were obtained from standard views, according to the European Society of Cardiology (ESC) recommendations for chamber quantification and diastolic function evaluation (32). At least three cardiac cycle images were acquired for data analysis. Although only one operator performed the echocardiograms, a second operator performed the myocardial deformation analysis. Two certified cardiologists independently analyzed, interpreted, and harmonized the results.

Right Heart Assessment

Right ventricle systolic function was evaluated using fractional area change (FAC), defined as $((end\ diastolic\ area\ - end\ systolic\ area)/(end\ diastolic\ area)) \times 100$, tricuspid annular plane systolic excursion (TAPSE), right ventricle index of myocardial performance (RIMP), defined as $(\text{Isovolumic Relaxation Time} + \text{Isovolumic Contraction Time})/\text{Ejection Time}$, and pulsed tissue Doppler S', according to guidelines of American Society of Echocardiography (ASE), endorsed by European Association of Echocardiography, from 2010 (19). Evaluation of the diastolic function of RV and chamber quantification followed the same guidelines. The TAPSE/PASP ratio was also calculated, as it is a validated noninvasive measure of RV-arterial coupling (33).

Myocardial deformation

Myocardial deformation was assessed in accordance with the ESC recommendations for two-dimensional echocardiography speckle tracking analysis of the right ventricle and right atrium, a consensus document of the European Association of Cardiovascular Imaging and ASE from 2018 (34).

Strain analysis of the RV and RA were calculated using an apical four-chamber view with Syngo Velocity Vector Imaging software, version 3.5 (Siemens Healthcare, Erlangen, Germany). The endocardium was traced manually using a point-and-click approach, whereas the system automatically traced the epicardium and generated six segments for the RV and three segments for the RA. The tracing was readjusted manually to increase the tracking accuracy, and strain curves were generated. In this study, RV strain values corresponded to the global longitudinal strain (GLS) of the free wall and septum (all six segments), and the RA strain values represented the average of the conduit and contraction phases of the RA cycle.

Pulmonary vascular resistance and pulmonary arterial pressure

Pulmonary circulation was evaluated based on calculations of pulmonary vascular resistance (PVR) and pulmonary arterial systolic pressure (PASP) using TTE values. The evaluation of PASP can be estimated by measuring the tricuspid regurgitant (TR) jet velocity, a method that is already part of conventional TTE. PVR was calculated using the following formula: $10 \times TR\ velocity\ \max / \text{Velocity Time Integral}\ of\ \text{Right Ventricle Outflow Tract}$, reported first from Abbas *et al.* (35).

Statistical analysis

Clinical and echocardiographic parameters were gathered from a database and analyzed using IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp. The level of significance was defined as a *p*-value less than 0.05.

Regarding descriptive statistics, continuous variables were expressed as median, 1st and 3rd quartiles (Q1, Q3). Data normality was assessed by inspection of the histogram and Q-Q plots (not shown). Categorical variables were expressed by absolute values and relative frequencies.

Generalized linear mixed-effects models (GLMM) were used for the analysis of right cardiovascular remodelling and reverse remodelling during pregnancy and postpartum, respectively, as well as the impact of the CVR factors in these processes using R statistical software (version 4.2.1; namely, lme4, gtsummary, and ggplot2 packages). Regarding statistical modeling, the evaluation moment at 3rd trimester (peak of cardiovascular remodelling) was used as a reference.

Results

Baseline demographic and clinical characterisation

The demographic and clinical information of the study population is presented in Table 1.

This study included 130 pregnant women, 60.8% primiparous, with a median age of 33 years old and median pre-pregnancy body mass index (BMI) of 23.8 kg/m^2. Fifty-one (39.2%) participants were classified as healthy, while seventy-nine (60.8%) had at least one cardiovascular risk (CVR) factor. The CVR factor group included 44 women with smoking habits (55.7%), 21 with arterial hypertension (26.6%), 19 with obesity (24.1%), and 14 with gestational DM (17.7%). Furthermore, 36.7% of participants had more than one CVR factor. Women with CVR factors were older (35 [32; 38] years *vs* 32 [29; 35] years, p<0.001) than healthy participants. However, similar proportions of primiparous and c-section deliveries were found in both study groups (Table 1). Women with CVR factors had a significantly higher BMI before pregnancy than healthy ones (24.7 kg/m^2 *vs* 22.8 kg/m^2, p=0.001). From 1st to 3rd trimester, both study groups registered a similar weight increase, approximately 10kg (p=0.001, Table 2).

Regarding hemodynamics, mean arterial pressure (MAP) remained unchanged from 1st to 3rd trimester, while heart rate increased significantly in both groups (Table 2). After delivery, MAP showed a significant increase while heart rate markedly decreased at one-month postpartum in both study groups (Table 2). Pregnant women with CVR factors had superior MAP throughout pregnancy and postpartum period (p<0.001, Table 2). In addition, SV exhibited a more evident reduction at six-months after delivery in the healthy group (p=0.064) and at one-month postpartum in the CVR group (p<0.001, Table 3).

Table 1. Demographic and clinic characteristics of the study groups.

	Healthy Group	CVR Group	***p*-value**
Variables	51 (39.2%)	79 (60.8%)	
Age, years Median (IQR)	32 (29; 35)	35 (32; 38)	<0.001
Comorbidities			
Arterial hypertension, n (%)	0	21 (26.6)	
Obesity, n (%)	0	19 (24.1)	
Gestational diabetes, n (%)	0	14 (17.7)	
Smoking habits, n (%)	0	44 (55.7)	
Body mass index, kg/m²			
Median (IQR)	22.8 (20.7; 26.6)	24.7 (21.7; 30.6)	0.001
Caesarean delivery, n (%)	21 (41.2)	30 (38)	0.717
Primiparous, n (%)	32 (62.7)	47 (59.5)	0.854

CVR – cardiovascular risk factor; IQR – interquartile range; n – number of participants.

Characterization of right cardiovascular remodelling during pregnancy

The echocardiographic characterization of right cardiovascular remodelling and RR during pregnancy and postpartum is displayed in Tables 2-7.

Regarding RA dimensions during pregnancy, both study groups showed a significant increase of areas (healthy group, p=0.001 and CVR group, p=0.034, Table 3, Figure 1A) and volumes from the 1st to 3rd trimester, except for RA volume in the CVR group at the postpartum period.

The RV dimensions (Figure 1B) of the CVR group increased significantly from 1st to 3rd trimester (area, p=0.011 and volume, p=0.025, Table 3), despite a notorious tendency to be patent in the healthy group.

Systolic function, assessed by FAC, TAPSE, tricuspid S', maintained preserved throughout pregnancy without any differences between groups (Table 4). CVR factors presented higher RIMP values, but both groups remained unchanged throughout the evaluation moments (p=0.047, Table 4).

Regarding myocardial deformation, both groups showed a significant decline of RA strain from 1st to 3rd trimester (Table 5 and Figure 1C), being more pronounced in the CVR group. The RV global longitudinal strain (GLS) followed the same tendency, being statistically significant only

in the CVR group (p=0.004, Table 5 and Figure 1D). Changes in RA and RV myocardial deformation troughout pregnancy and postpartum were similar between the two groups (Table 5).

Diastolic function measured by E/A tricuspid ratio decreased significantly in the CVR group from 1st to 3rd trimester (p=0.003, Table 6 and Figure 1E), becoming significantly lower than the healthy group (p=0.003, Table 6), which is ascribed to a significant increase of A wave velocity during pregnancy (healthy group, p=0.05 and CVR group, p=0.008, Table 6). Regarding E/e', both groups showed no significant differences throughout pregnancy (Table 6).

Characterization of right cardiovascular reverse remodelling after delivery

In healthy pregnant women, the RA area and volume reduction began as soon as one month postpartum, becoming significant six months after delivery (area, p<0.001 and volume, p=0.002, Table 3). Interestingly, in the CVR group, the regression of RA dimension was slower and continued beyond one-year postpartum (Table 3). Regarding RV dimensions, both groups decreased similarly from the 3rd trimester to six-months postpartum (Table 3).

Regarding RV systolic function, the FAC was constant throughout all the time points evaluated (Table 4). At the same time, the TAPSE and tricuspid S' wave (Figure 1F) decreased significantly one-month postpartum and continued the same trend thereafter (Table 4). At the same time point, a significant recovery of RA strain was observed in the healthy group (p=0.027, Table 5), while no significant differences were found in the CVR group (p=0.170, Table 5). Recovery of RA strain in the CVR group only became significant at six-months postpartum (*p*=0.001, Table 5). The RV strain of the healthy group remained stable during the postpartum period, while the CVR group recovered six-months postpartum (p=0.013, Table 5).

Diastolic function, assessed by increased E/A, improved significantly as soon as one-month after delivery (Table 6). Nevertheless, the CVR group displayed increased values of A wave velocity throughout the five evaluation moments, leading to a distinct response between groups (p=0.023, Table 6). Indeed, CVR group showed lower values of E/A ratio over time (p=0.002, Table 6).

Lastly, the progression of pulmonary parameters during pregnancy and postpartum was stable throughout the evaluation moments and similar between groups, except for PVR that increased six-months postpartum in the CVR group (p=0.027, Table 7). Interestingly, CVR factors were associated with higher PASP values (p=0.031, Table 7).

Table 2. Maternal weight, blood pressure and heart rate through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

	Healthy Group			CVR Group			Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
Maternal body weight, kg							
1T	47	64.00 [59.00; 71.00]	<0.001	77	70.90 [61.00; 84.50]	<0.001	
3T	48	71.00 [66.05; 78.75]	-	77	80.00 [69.50; 89.00]	-	
PP1	47	65.00 [59.00; 73.00]	<0.001	66	72.50 [63.00; 84.25]	<0.001	<0.001
PP2	51	64.00 [56.00; 70.00]	<0.001	79	70.00 [60.00; 84.00]	<0.001	
PP3	30	64.50 [54.00; 69.25]	<0.001	47	65.00 [58.00; 80.00]	<0.001	
Weight gain in pregnancy, kg							
1T	47	2.00 [1.00; 3.00]	<0.001	77	2.00 [0.50; 4.00]	<0.001	
3T	48	10.00 [7.63; 11.95]	-	76	10.00 [7.00; 13.60]	-	
PP1	44	-6.25 [-7.58; -4.13]	<0.001	65	-7.00 [-8.75; -4.65]	<0.001	<0.001
PP2	48	-8.00 [-11.00; -6.00]	<0.001	77	-8.00 [-11.00; -4.30]	<0.001	
PP3	27	-9.00 [-12.00; -6.50]	<0.001	45	-7.00 [-12.40; -4.90]	<0.001	
Systolic blood pressure, mmHg							
1T	36	110.0 [100.0; 110.0]	0.060	40	114.0 [103.0; 124.0]	0.598	
3T	45	110.0 [100.0; 118.0]	-	77	110.0 [108.5; 120.0]	-	
PP1	47	110.0 [100.0; 120.0]	0.598	63	120.0 [110.0; 120.0]	0.274	<0.001
PP2	51	111.0 [102.0; 119.0]	0.568	79	120.0 [110.0; 130.0]	0.004	
PP3	31	113.0 [105.0; 121.0]	0.075	48	119.0 [110.0; 126.8]	0.018	
Diastolic blood pressure, mmHg							
1T	36	70.0 [60.0; 80.0]	0.937	40	70.0 [70.0; 80.0]	0.579	
3T	45	70.0 [60.0; 70.5]	-	77	70.0 [67.5; 80.0]	-	

Table 2. Maternal weight, blood pressure and heart rate through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

	Healthy Group			CVR Group		Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
PP1	47	76.0 [70.0; 80.0]	<0.001	63	80.0 [70.0; 84.0]	<0.001	<0.001
PP2	51	75.0 [70.0; 80.0]	<0.001	79	80.0 [74.0; 88.0]	<0.001	
PP3	31	77.0 [70.0; 84.0]	<0.001	48	80.0 [74.5; 89.8]	<0.001	
Mean arterial pressure, mmHg							
1T	36	80.0 [74.2; 89.5]	<0.384	40	86.7 [82.2; 93.3]	0.790	
3T	45	80.0 [75.0; 89.7]	-	77	86.0 [80.0; 94.5]	-	
PP1	47	86.7 [80.0; 93.3]	<0.001	63	90.0 [83.3; 98.7]	0.002	<0.001
PP2	51	87.7 [83.0; 92.3]	<0.001	79	93.3 [87.0; 102.7]	<0.001	
PP3	31	88.0 [83.3; 97.3]	<0.001	48	90.8 [86.7; 101.3]	<0.001	
Heart rate, beats/min							
1T	37	73 [67; 81]	<0.001	38	74 [68; 82]	<0.001	
3T	45	78 [72; 83]	-	76	80 [71; 86]	-	
PP1	47	60 [55; 64]	<0.001	63	62 [57; 67]	<0.001	0.164
PP2	51	64 [58; 70]	<0.001	79	65 [61; 69]	<0.001	
PP3	31	65 [61; 67]	<0.001	48	67 [60; 75]	<0.001	

1T – first trimester; 3T – third trimester, settled as the reference category; PP1 – 1 month postpartum; PP2 – 6 months postpartum; PP3 – one year postpartum; IQR – interquartile range; N – number of participants; CVR – cardiovascular risk group.

Table 3. Stroke volume and dimensions of RA and RV from TTE, through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

	Healthy Group			CVR Group			Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
Stroke volume							
1T	36	57.05 [50.79; 67.01]	0.568	35	59.64 [52.72; 66.79]	0.945	
3T	38	57.46 [51.12; 66.49]	-	70	60.06 [52.76; 65.99]	-	
PP1	43	55.41 [51.75; 66.71]	0.752	59	54.75 [47.11; 62.98]	<0.001	0.405
PP2	49	54.90 [49.36; 62.34]	0.064	75	58.68 [48.62; 64.65]	0.019	
PP3	28	55.98 [48.77; 62.48]	0.003	45	54.94 [49.82; 61.84]	0.003	
Right atrium area, cm^2							
1T	35	12.53 [11.43; 13.40]	<0.001	36	12.67 [11.50; 13.96]	0.034	
3T	35	13.75 [12.37; 15.46]	-	66	13.35 [12.26; 15.11]	-	
PP1	42	13.33 [11.93; 14.88]	0.220	57	14.22 [11.94; 15.98]	0.220	0.171
PP2	48	12.46 [11.43; 14.19]	<0.001	75	13.32 [12.15; 14.96]	0.517	
PP3	31	13.15 [12.21; 14.12]	0.031	44	13.00 [12.05; 14.58]	0.238	
Right atrium volume, mL							
1T	36	29.15 [25.38; 33.05]	0.004	70	30.60 [25.40; 32.90]	0.135	
3T	39	33.30 [27.30; 39.10]	-	39	32.05 [26.63; 37.88]	-	
PP1	43	32.60 [25.80; 39.20]	0.452	59	34.90 [27.30; 41.70]	0.118	0.290
PP2	48	28.65 [24.98; 34.80]	0.002	75	32.50 [27.30; 38.60]	0.777	
PP3	31	32.40 [27.60; 36.20]	0.206	44	30.00 [27.58; 35.88]	0.604	
Right ventricle area, cm^2							
1T	28	15.87 [14.61; 17.71]	0.160	26	15.92 [13.95; 17.46]	<0.011	
3T	27	17.12 [16.46; 18.29]	-	55	17.67 [15.50; 19.88]	-	

Table 3. Stroke volume and dimensions of RA and RV from TTE, through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

	Healthy Group			CVR Group			Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
PP1	35	16.87 [14.69; 18.01]	0.676	42	16.79 [14.65; 18.40]	0.080	0.475
PP2	38	14.93 [12.57; 18.03]	0.031	57	15.80 [13.37; 17.97]	<0.001	
PP3	21	15.40 [13.58; 17.77]	0.066	33	15.07 [12.28; 16.64]	<0.001	
Right ventricle volume, mL							
1T	28	29.80 [24.83; 35.28]	0.072	26	30.05 [26.10; 36.50]	0.025	
3T	27	34.30 [29.40; 41.80]	-	55	33.68 [29.10; 44.90]	-	
PP1	35	34.40 [27.90; 38.90]	0.611	42	34.25 [28.95; 41.65]	0.412	0.183
PP2	38	28.85 [21.35; 35.83]	0.059	57	31.00 [23.70; 38.15]	0.001	
PP3	21	29.20 [25.00; 38.05]	0.153	33	28.90 [20.75; 33.45]	<0.001	

1T – first trimester; 3T – third trimester, settled as the reference category; PP1 – 1 month postpartum; PP2 – 6 months postpartum; PP3 – one year postpartum; IQR – interquartile range; N – number of participants; CVR – cardiovascular risk group.

Table 4. Right ventricular function through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

		Healthy Group			CVR Group		Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
FAC, %							
1T	28	48.35 [45.78; 52.56]	0.164	26	50.26 [44.28; 55.78]	0.124	
3T	27	51.71 [47.18; 56.44]	-	54	46.52 [41.89; 50.98]	-	
PP1	35	48.53 [44.31; 54.02]	0.861	42	48.22 [44.50; 52.78]	0.197	0.298
PP2	38	49.12 [45.20; 54.54]	0.486	55	49.92 [44.59; 51.90]	0.167	
PP3	21	47.78 [42.79; 50.57]	0.245	33	44.33 [41.11; 51.80]	0.842	
TAPSE, mm							
1T	37	25 [21; 27]	0.475	39	25 [22; 27]	0.669	
3T	45	26 [22; 29]	-	77	24 [22; 27]	-	
PP1	44	24 [21; 26]	<0.001	62	22 [20; 25]	<0.001	0.700
PP2	50	24 [21; 26]	<0.001	77	23 [22; 26]	0.021	
PP3	31	23 [19; 25]	<0.001	48	23 [20; 25]	0.003	
Tricuspid S' wave, cm/s							
1T	36	14.3 [12.9; 15.3]	0.860	39	14.2 [12.8; 15.3]	0.735	
3T	45	14.5 [12.9; 15.9]	-	76	14.1 [13.0; 15.3]	-	
PP1	44	12.7 [11.7; 13.7]	<0.001	62	12.1 [11.2; 13.0]	<0.001	0.561
PP2	51	12.7 [11.8; 14.4]	<0.001	79	12.6 [11.7; 14.0]	<0.001	
PP3	31	13.0 [12.1; 14.4]	0.002	48	12.9 [12.0; 14.3]	<0.001	
RIMP							
1T	31	0.46 [0.39; 0.52]	0.225	31	0.46 [0.40; 0.52]	0.664	
3T	36	0.44 [0.38; 0.48]	-	60	0.45 [0.41; 0.53]	-	

Table 4. Right ventricular function through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

	Healthy Group			CVR Group			Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
PP1	43	0.40 [0.37; 0.49]	0.195	60	0.44 [0.39; 0.53]	0.541	0.047
PP2	47	0.44 [0.39; 0.49]	0.727	78	0.45 [0.40; 0.50]	0.299	
PP3	31	0.45 [0.41; 0.49]	0.949	47	0.45 [0.41; 0.52]	0.989	

1T – first trimester; 3T – third trimester, settled as the reference category; PP1 – 1 month postpartum; PP2 – 6 months postpartum; PP3 – one year postpartum; IQR – interquartile range; N – number of participants; CVR – cardiovascular risk group; FAC – fractional area change; TAPSE – tricuspid annular plane systolic excursion; RIMP – right index of myocardial performance.

Table 5. Myocardial deformation of RA and RV through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

	Healthy Group			CVR Group			Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
Right atrium strain, %							
1T	36	36.85 [33.55; 42.00]	0.036	34	39.15 [35.25; 43.70]	<0.001	
3T	36	34.00 [30.63; 39.98]	-	65	33.50 [29.95; 37.50]	-	
PP1	42	37.75 [33.23; 42.43]	0.027	59	35.30 [32.10; 38.90]	0.170	0.347
PP2	45	38.20 [35.15; 42.65]	0.002	74	36.80 [32.93; 41.55]	<0.001	
PP3	31	37.80 [34.90; 42.70]	0.003	45	37.80 [34.20; 41.05]	0.002	
Right ventricle GLS, %							
1T	36	-25.15 [-27.88; -21.88]	0.429	32	-26.40 [-30.43; -20.93]	0.004	
3T	35	-24.70 [-27.80; -20.80]	-	60	-23.20 [-25.28; -20.48]	-	
PP1	37	-22.80 [-26.50; -20.65]	0.344	55	-23.00 [-26.70; -20.00]	0.590	0.430
PP2	40	-24.90 [-28.10; -22.00]	0.600	70	-24.30 [-27.70; -21.08]	0.013	
PP3	28	-24.50 [-28.98; -20.98]	0.604	41	-24.80 [-28.75; -20.70]	0.030	

1T – first trimester; 3T – third trimester, settled as the reference category; PP1 – 1 month postpartum; PP2 – 6 months postpartum; PP3 – one year postpartum; IQR – interquartile range; N – number of participants; CVR – cardiovascular risk group; GLS – global longitudinal strain.

Table 6. Diastolic function through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

		Healthy Group			CVR Group		Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
A tricuspid wave, cm/s							
1T	28	33.0 [29.5; 37.8]	0.050	29	35.0 [31.0; 40.5]	0.008	
3T	34	35.5 [32.0; 42.0]	-	61	39.0 [32.0; 44.0]	-	
PP1	41	28.0 [25.0; 32.5]	<0.001	56	30.0 [27.0; 34.0]	<0.001	0.023
PP2	47	29.0 [25.0; 34.0]	<0.001	75	29.0 [25.0; 33.0]	<0.001	
PP3	28	26.5 [24.3; 30.0]	<0.001	46	29.5 [25.8; 36.0]	<0.001	
E tricuspid wave, cm/s							
1T	28	54.5 [46.5; 59.8]	0.616	29	54.0 [47.5; 61.0]	0.053	
3T	35	54.0 [44.0; 62.0]	-	62	50.5 [46.0; 56.3]	-	
PP1	41	48.0 [44.5; 55.5]	0.028	56	49.0 [44.3; 55.8]	0.211	0.191
PP2	47	51.0 [46.0; 58.0]	0.291	75	51.0 [44.0; 56.0]	0.335	
PP3	28	53.0 [42.3; 60.0]	0.204	46	48.5 [44.0; 54.3]	0.075	
E/A ratio							
1T	20	1.67 [1.35; 1.82]	0.086	19	1.62 [1.41; 1.78]	0.003	
3T	32	1.47 [1.28; 1.67]	-	58	1.30 [1.15; 1.44]	-	
PP1	41	1.75 [1.45; 1.96]	<0.001	54	1.63 [1.37; 1.81]	<0.001	0.002
PP2	47	1.69 [1.56; 2.00]	<0.001	73	1.70 [1.46; 2.00]	<0.001	
PP3	28	1.91 [1.64; 2.30]	<0.001	46	1.71 [1.45; 1.96]	<0.001	
A' lateral wall, cm/s							
1T	34	12.10 [10.38; 13.98]	0.003	36	12.70 [10.25; 15.88]	0.003	
3T	38	13.85 [11.03; 16.65]	-	65	15.00 [11.90; 17.20]	-	
PP1	43	11.50 [10.00; 13.80]	<0.001	61	11.50 [9.35; 13.45]	<0.001	0.103
PP2	51	10.90 [9.90; 14.00]	<0.001	78	12.25 [10.00; 13.95]	<0.001	

Table 6. Diastolic function through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

	Healthy Group			CVR Group			Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
PP3	30	12.55 [9.68; 14.40]	0.002	47	12.20 [9.90; 14.40]	<0.001	
E' lateral wall, cm/s							
1T	34	15.25 [12.85; 17.90]	0.141	36	14.60 [13.40; 16.93]	0.039	
3T	38	14.15 [11.15; 16.78]	-	66	14.10 [11.48; 16.43]	-	
PP1	43	14.10 [12.20; 16.50]	0.468	61	13.00 [11.30; 15.00]	0.048	0.162
PP2	51	13.80 [12.00; 16.50]	0.931	78	13.45 [11.83; 15.93]	0.765	
PP3	30	14.80 [12.50; 16.75]	0.843	47	13.60 [11.70; 15.90]	0.961	
E/e' ratio							
1T	26	3.45 [3.01; 3.96]	0.127	29	3.59 [3.05; 4.16]	0.298	
3T	35	3.91 [3.08; 4.28]	-	57	3.53 [3.00; 4.62]	-	
PP1	41	3.48 [3.04; 4.46]	0.359	56	3.76 [3.32; 4.10]	0.611	0.570
PP2	47	3.67 [3.18; 4.53]	0.343	74	3.66 [3.08; 4.32]	0.286	
PP3	28	3.53 [2.88; 4.45]	0.268	45	3.83 [2.97; 4.52]	0.219	

1T – first trimester; 3T – third trimester, settled as the reference category; PP1 – 1 month postpartum; PP2 – 6 months postpartum; PP3 – one year postpartum; IQR – interquartile range; N – number of participants; CVR – cardiovascular risk group.

Table 7. Pulmonary circulation parameters through pregnancy (1T, 3T) and postpartum (PP1, PP2, PP3) between groups.

	Healthy Group			CVR Group		Healthy *vs* CVR
Variables	**N**	**Median (IQR)**	***p*-value**	**N**	**Median (IQR)**	***p*-value**	***p*-value**
PASP, mmHg							
1T	24	16.5 [14.0; 20.0]	0.240	28	19.0 [16.0; 22.0]	0.740	
3T	28	17.5 [14.0; 21.0]	-	52	18.5 [16.0; 22.0]	-	0.031
PP1	31	18.0 [15.0; 20.0]	0.781	43	19.0 [16.0; 22.0]	0.848	
PP2	26	18.0 [14.0; 20.0]	0.797	53	18.0 [16.0; 21.5]	0.506	
PP3	22	16.3 [14.0; 19.0]	0.953	31	18.0 [16.0; 20.0]	0.672	
TAPSE/PASP ratio							
1T	24	1.53 [1.11; 1.89]	0.655	28	1.30 [1.06; 1.56]	0.936	
3T	28	1.37 [1.11; 1.89]	-	52	1.34 [1.05; 1.69]	-	
PP1	31	1.26 [1.13; 1.56]	0.150	43	1.17 [0.98; 1.45]	0.102	0.071
PP2	25	1.34 [1.08; 1.60]	0.100	51	1.34 [1.11; 1.46]	0.232	
PP3	22	1.29 [1.09; 1.74]	0.383	31	1.31 [1.16; 1.46]	0.150	
PVR, woods							
1T	7	1.06 [0.82; 1.23]	0.216	14	1.05 [0.95; 1.34]	0.497	
3T	13	1.36 [1.09; 1.47]	-	33	1.27 [1.09; 1.42]	-	
PP1	11	1.22 [1.07; 1.41]	0.668	32	1.29 [1.16; 1.46]	0.249	0.502
PP2	7	1.18 [1.16; 1.50]	0.471	38	1.33 [1.19; 1.49]	0.027	
PP3	9	1.27 [1.02; 1.41]	0.706	20	1.33 [1.16; 1.46]	0.074	

1T – first trimester; 3T – third trimester, settled as the reference category; PP1 – 1 month postpartum; PP2 – 6 months postpartum; PP3 – one year postpartum; IQR – interquartile range; N – number of participants; CVR – cardiovascular risk group; PASP – pulmonary artery systolic pre21ssure; PVR – pulmonary vascular resistance.

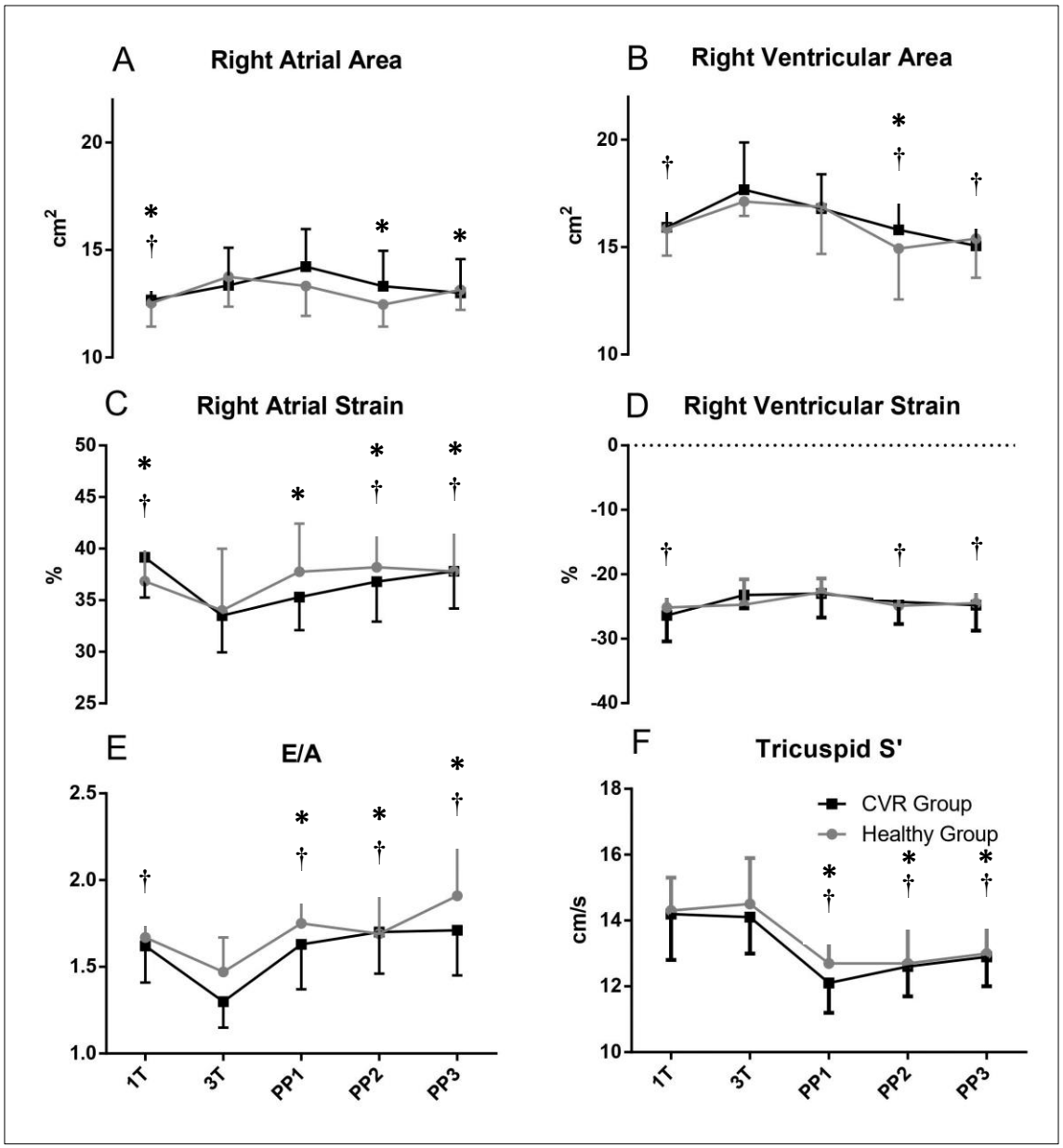

Figure 1. Evolution of RA **(1A)** and RV **(1B)** area, RA strain **(1C)**, GLS RV **(1D)**, E/A tricuspid ratio **(1E)** and tricuspid velocity of S' **(1F)** throughout pregnancy[1T (1st trimester) and 3T (3rd trimester, settled as the reference category)] and postpartum [PP1, PP2 and PP3 (1-,6-, 12 - months postpartum)] between healthy and CVR (cardiovascular risk) group. *p-value: <0.05 in healthy group, †p-value: <0.05 in CVR group.

Discussion

To the best of our knowledge, our study is the first to describe the right heart remodelling during pregnancy and reverse remodelling (RR) at postpartum, including five evaluation moments from 1st trimester of pregnancy to one-year after delivery in healthy women and women with cardiovascular risk (CVR) factors. First, we demonstrated that similarly to the left heart, right cardiac chambers increase their dimensions during pregnancy and normalize mostly up to six months postpartum. The magnitude of these transient changes was small and within the normal range. Second, RV global longitudinal strain (GLS) was preserved throughout all time points and was significantly reduced in the 3rd trimester of the CVR group and recovering six months after delivery. Third, cardiovascular risk factors showed a trend toward delaying RA strain recovery postpartum. Fourth, pregnancy seemed to enhance subclinical deterioration of the right diastolic function in CVR group, as observed by the higher magnitude of change in A and E/A over time. Lastly, the presence of CVR factors affects pulmonary circulation hemodynamics differently.

Concerning right cardiac remodelling during pregnancy, the RA and RV dimensions enlarge in response to the increased venous return (preload), similar to what is documented in the left atrium (LA) (14,15,36) and previously described for the RV during pregnancy (16,20). After delivery, reverse remodelling was evident in RA and RV dimensions at six-months postpartum in healthy group, while a delayed structural RA reverse remodelling was observed when cardiovascular risk factors were present. In line with our results, Diaz *et al.* have reported in healthy pregnant women, a reduction of RA area three months postpartum (20). As for the myocardial deformation, our findings suggest that RA strain decreases during pregnancy, which could be explained by the progressive increase of RA volume, already reported to produce lower peak atrial strain compared to non-dilated atrium (37,38). Another reason may be the deterioration of the RV diastolic function. Indeed, the impact of LV filling pressure during diastole on LA relaxation and strain has been previously shown by several authors (39–41). After delivery, the RA strain increased in both groups of our cohort with a slower recovery in the CVR group, suggesting an inverse relationship with right atrium dimensions, similar to what was observed during pregnancy but in opposite directions. Corroborating our findings, Tasar *et al.* described a reduction of LA strain during healthy pregnancies, recovering up to six-months postpartum (42). Concerning RA strain after delivery in women with CVR factors, as far as we know, this is the first study describing its progression throughout postpartum. Assuming the relationship between atrial myocardial deformation and diastolic dysfunction and the burden of CVR factors, RA strain during postpartum is expected to recover slowly. Regarding RV myocardial deformation, we showed that GLS tends to be unaffected in healthy women throughout

pregnancy and postpartum, which disagrees with Diaz *et al*., who report a significant decrease of RV GLS in healthy women earlier than other echocardiographic parameters, recovering up to three months postpartum (20). These divergent results might be explained by different evaluation moments.

However, when CVR risks are present, RV GLS decreases throughout pregnancy and recovers six months after delivery. Accordingly, Tadic *et al*. and Elaziz *et al*. have reported a progressive reduction of RV GLS throughout pregnancies complicated by gestational hypertension and PE (43,44). Therefore, these findings reinforce the need to monitor RV function by strain analysis when CVR factors are present since this tool has proven to be more sensitive when compared to other RV function parameters.

Regarding systolic function, both groups within our cohort showed a constant FAC, TAPSE and S' tricuspid velocity during pregnancy, contrasting with previous studies in normal gestation that documented decreased FAC and TAPSE (16,20). A possible justification for these discordant results could be the timing of evaluation (2^{nd} vs 3^{rd} trimesters) and the study population clinical characteristics selected by Diaz *et al.,* which included primarily healthy pregnant women, with 16.3% of women presenting smoking habits and one woman with gestational DM. As for the study by Karen *et al*., the explanation could lie in the significant differences between sample sizes (559 healthy pregnant women *vs* 51 in our cohort). After delivery, FAC remained constant, while TAPSE and S' tricuspid velocity decreased significantly, maybe due to postpartum hemodynamic load reduction. From our point of view, FAC stability over time might be explained by the proportional increase/decrease of RV end-diastolic and end-systolic areas. Of note, TAPSE and velocity of tricuspid S' appeared to respond to volume variations occurring during pregnancy and postpartum, evidenced by the significant differences, particularly at one month postpartum, when stroke volume decreases. Indeed, this relationship between tricuspid valve and volume or pressure overload has already been described due to specific features of the anatomy of tricuspid valve, which has the largest annulus of the heart and is connected to RV by more than three papillary muscles, making it more susceptible to structural deformations and load-dependent (45,46).

Regarding the impact of CVR factors on pregnancy and postpartum systolic function, other authors have shown reduced values of FAC and TAPSE in pregnant women with PE (43,47). In addition, while Nunez *et al.* reported an inferior TAPSE in women with gestational DM compared to uncomplicated pregnancies (48), Buddeberg *et al.* found no significant differences in right systolic function, measured by TAPSE and S' tricuspid velocity, between morbidly obese pregnant women compared to normal weight pregnant women (49). In our cohort, CVR factors did not influence systolic function, probably due to the different stages of severity of these CV diseases

and the echocardiographic modality used. For example, Elaziz *et al.*, included pregnant women with PE, whereas we included women with arterial hypertension (not complicated with PE). Calabuig *et al*. used three-dimensional echocardiography to assess RV function, which is a more accurate method to evaluate subtle changes in ventricular volumes and ejection fraction compared to two-dimensional echocardiography (48). Regarding RV global myocardial performance, measured by RIMP, we found increased values in the CVR group, which agrees with Çağlar *et al.,* who reported higher RIMP values in women with PE in the 3rd trimester (47). The reason for these results could be related to increased filling pressures of LV, since increased afterload has already been described to negatively impact the right myocardial performance (50).

Deterioration of diastolic function has already been described in studies focusing on LV adaptations to pregnancy (14,51–53). Since LV's passive filling (E-wave) is less effective, given the decrease of the pressure gradient between the LA and LV, atrial contraction (A-wave) increases to improve ventricular filling and maintain cardiac output. Our results suggest a similar pattern in RV diastolic function, which was amplified when CVR factors were present. Consistent with our findings, Çağlar *et al*. reported an inferior E/A tricuspid ratio and superior A velocity when PE was present, compared to healthy pregnant women (47).

Regarding pulmonary circulation, PASP was significantly higher in the CVR group, possibly resulting from the increased LV filling pressures during pregnancy, which has been previously well-documented for most CVR factors (10,54,55). These results are consistent with those of studies reporting higher PASP values in women with gestational hypertension and PE (44) and in pregnant women above 35 years old, which is closer to our cohort's CVR group median age (56).

Study limitations

This study presents the following limitations: 1) small sample size; 2) the COVID-19 pandemic compromised the cardiovascular follow-up evaluations of some participants owing to circulation restrictions and recommendations of the Directorate General of Health for pregnant women; 3) the higher incidence of non-attending participants within the CVR group, which precluded the subgroup analysis of each cardiovascular risk factor and their comparison; 4) the absence of inter-operator echocardiographic variability evaluation; and 5) exclusion of some participants with poor acoustic windows of the RV apical view.

Conclusions

This study described pregnancy's subtle right functional and structural changes (within the normal range), which recover as soon as six months postpartum. We showed that pregnancy increases right atrial and ventricular dimensions while simultaneously decreasing myocardial

deformation of these chambers. These changes subsequently impact diastolic function, more pronouncedly in the presence of CVR factors. After delivery, the normalization of these parameters was delayed in the CVR factors' group. In contrast, the impact of pregnancy and postpartum period on systolic function was negligible in both groups, except for a small reduction in response to the reduced volemia after delivery. The pulmonary circulatory system showed a great capacity to adapt to changes in pregnancy and postpartum hemodynamic adaptation.

Together, our comprehensive and robust methodology suggests that novel and reliable approaches, such as strain and diastolic function measurements, might highlight subtle subclinical changes of the "right" heart during peripartum remodelling.

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Availability of data and materials

The data are available upon request.

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Subchapter v. The Impact of Echocardiographic Indexation to Evaluate Cardiac Reverse Remodelling throughout Pregnancy and Postpartum

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Title: The impact of echocardiographic indexation to evaluate cardiac reverse remodeling throughout pregnancy and postpartum

Título: Impacto da indexação nos parâmetros ecocardiográficos para avaliação da remodelagem cardíaca e remodelagem reversa durante a gravidez e pós-parto

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Resumo

Introdução e objetivos: As *Guidelines* de Ecocardiografia Transtorácica recomendam a indexação dos volumes e massa do ventrículo esquerdo à área de superficie corporal (ASC). Durante a gravidez, a contínua variação do peso e consequentemente da ASC poderão influenciar a avaliação da remodelagem cardíaca (RC) e remodelagem reversa (RR). Os objetivos foram identificar as metodologias de indexação mais utilizadas na avaliação ecocardiográfica em gestantes através de uma revisão sistemática; bem como comparar quatro métodos de indexação [i) valores absolutos; ii) indexação ASC prévia à gestação; iii)coeficientes alométricos(CA); iv)indexação à ASC calculada em cada momento de avaliação] utilizando um exemplo ilustrativo.

Métodos: A revisão sistemática baseou-se na RC e RR na gestação, recorrendo-se a duas bases de dados. O exemplo ilustrativo baseou-se num estudo de coorte prospetivo que incluiu quatro avaliações ecocardiográficas durante a gestação e pós-parto.

Resultados: Foram incluídos 27 estudos, em que na maioria foi aplicada indexação das variáveis ecocardiográficas à ASC calculada em cada momento da avaliação(n=21). Os testes estatísticos baseados na variabilidade intra-sujeito foram os mais utilizados para análise longitudinal dos dados(n=17). No exemplo ilustrativo, a indexação à ASC prévia à gestação ou uso de CA demonstraram um tamanho de efeito superior à indexação à ASC calculada em cada momento de avaliação, e igual à ausência de indexação, na análise intra-sujeito. Testes estatísticos baseados na variabilidade intra-sujeito demonstraram um tamanho de efeito superior aos baseados na variabilidade inter-sujeito para avaliação longitudinal da massa cardíaca.

Conclusão: O presente estudo revelou a necessidade de harmonização dos métodos estatísticos e indexação das variáveis ecocardiográficas para a avaliação da RC e RR na gravidez.

PALAVRAS-CHAVE

Área de superfície corporal; Ecocardiografia; Massa do ventrículo esquerdo indexada; Remodelagem ventricular; Remodelagem reversa; Gravidez

Abstract

Introduction and objectives: Echocardiography guidelines suggest normalizing left ventricular (LV) volumes and mass (LVM) to body size. During pregnancy, continuous weight variation impacts on body surface area (BSA) calculation, limiting the longitudinal analysis of cardiac remodeling (CR) and reverse remodeling (RR) variables. Our aim was to identify the most common indexing methodologies in the literature on pregnant populations through a systematic review; and, to compare four scaling methods: i) none (absolute values); ii) indexing to the BSA before pregnancy; iii) allometric indexing; and iv) indexing to BSA measured at the same day of cardiac assessment, using an illustrative example.

Methods: We performed a systematic review of CR and RR during pregnancy and post-partum using two databases. We included studies reporting longitudinal echocardiographic analysis of cardiac chamber volumes in humans. We used a prospective cohort study of healthy pregnant women who underwent four echocardiographic evaluations during pregnancy and postpartum, as an illustrative example.

Results: Twenty-seven studies were included, most studies indexed to BSA measured at each evaluation moment(n=21). Within-subjects design was the most reported to analyse longitudinal data(n=17). Indexation to the pre-pregnancy BSA or application of allometric indexes revealed a higher effect than BSA measured at each evaluation and an equal effect to not indexing using within-subjects design. The within-subjects designs also revealed a higher effect size value than the between-subjects design for longitudinal analysis of LVM adaptations during pregnancy and postpartum.

Conclusion(s): This study concludes that indexation methods do not impact the clinical interpretation of longitudinal echocardiographic assessment but highlights the need to harmonize normalization procedures during pregnancy.

Keywords:

Body Surface Area; Echocardiography, Left Ventricular Mass Indexed; Ventricular Remodeling; Reverse Remodeling; Pregnancy

Introduction

The European and American guidelines for transthoracic echocardiography recommend indexing the left ventricular size and mass to body surface area (BSA), to enable a more accurate interpretation and comparison of cardiac structural changes between individuals with different body sizes (211). Pregnant women experience continuous BSA variation during gestation and after delivery. This weight change differs significantly between women and is influenced by the women's pre-pregnancy BSA, nutrition and/or physical activity (212, 213). Weight changes during pregnancy and postpartum may interfere in the longitudinal analysis of cardiac remodeling (CR) and reverse remodeling (RR) processes (within-group variation) and in the comparison between distinct study groups, e.g., healthy *versus* pregnant women with cardiovascular risk factors (between-group variation).

In this context, evaluating the most appropriate normalization method for assessing structural and hemodynamic cardiac changes throughout pregnancy and postpartum becomes highly relevant.

We identified four possible methods to display cardiac remodeling during pregnancy, puerperium and postpartum periods: 1) none (absolute values), without indexing; 2) indexing to the baseline BSA (before pregnancy); 3) allometric indexing; or 4) indexing to BSA measured on the same day as the cardiac assessment.

Out of the aforementioned methods, only method four takes into consideration the weight variation of the pregnant women throughout gestation, while methods 1), 2) and 3) overlook the effect of fetus development and maternal adaptations as crude values, baseline BSA (before pregnancy), or allometric indexes, respectively are used. Accordingly, the first three methods do not account for the extra weight from the fetus (such as placenta and blood volume expansion) and for the interpretation of CR and RR.

There is consensus that using absolute values, without any indexation, to compare groups may lead to bias in the results. Vinayagam et al. highlighted the relevance of correcting for BSA when comparing subjects with a wide range of body shapes and sizes, as is standard practice in pediatric cardiology (54). However, the ideal methodology for indexing and embracing fetus development when studying cardiac hemodynamics and structure in pregnancy is still missing.

Furthermore, using a between-subjects or within-subjects design will yield distinct results since the individual variation is not taken into account in the within-subjects test.

Objectives

The purposes of this study were to: 1) identify the most common indexing methodologies in the literature for the pregnant population through a systematic review and 2) compare four methods using an illustrative example from a longitudinal cohort of pregnant women evaluated at four time points (before and after delivery).

Methods and Design

Systematic review

This systematic review complies with PRISMA statement (214).

Eligibility criteria

The search was limited by publication date (1 August 2016 and 1 August 2021) and study language. We included original articles (clinical trials, cohort studies, registry-based cohort studies, case-control studies) with longitudinal echocardiographic analysis of CR and RR during pregnancy and post-partum (quantification of left atrium volume, LV mass, LV end-diastolic or end-systolic volumes) in humans. We excluded case reports or case series, abstract proceedings and original studies with cardiac assessments other than transthoracic echocardiography.

Search strategy

A systematic search was performed on the MEDLINE and Web of Sciences Core Collection databases. A manual search was done on reference lists of the included articles. The search strategy included six search components: pregnancy, echocardiography, publication date and article language, excluding offspring terms and specific publication types. Each component combined MeSH terms (controlled language) and free-text keywords selected by the study authors. The complete search strategy is defined in Table S1.

Selection process

One author (AFF) screened the titles and abstracts of all citations identified by the searches using an online reviewing software (Rayyan Systems Inc., USA). Potentially eligible studies were assessed for inclusion criteria. Reference lists from included articles were also reviewed.

Data extraction

Data extracted from the selected papers included: author(s) name; year of publication; country; study design; study groups, assessment time points (both during pregnancy or post-partum); recruitment period; total number of participants; number of participants in each study group (if applicable); indexation method; cardiac chambers indexed (LV mass and volumes, and left atrium volume); statistical methods for between-group comparison and for within-group / longitudinal comparison; discussion or comment about indexation methods in the article.

Data analysis

Results are presented using descriptive statistics.

Prospective cohort study

A convenience sample of pregnant women who had their first medical appointment at the Obstetrics Department of *Centro Hospitalar Universitário de São João*, Porto, from March 2019 to April 2021 were invited to participate. Pregnant women aged <18 years, with pre-existing cardiomyopathy, renal disease, chronic obstructive airway disease, active systemic infection, genetic syndromes, type-1 diabetes mellitus, obesity, arterial hypertension or gestational hypertension were excluded.

Women were evaluated at the following time points: 1) first trimester (10-15 weeks, baseline conditions, the initial phase of cardiac remodeling); 2) third trimester (30–35 weeks, peak of cardiac remodeling, when cardiac adaptations are expected to be most prominent); 3) 6–8 weeks after delivery; and 4) 6–7 months after delivery (cardiovascular RR stages to assess cardiac recovery). The evaluation included clinical characterization through questionnaires, clinical examination and cardiac assessment by transthoracic echocardiography.

Ethics and consent

All study participants provided written informed consent. The Ethics Committee of Centro Hospitalar Universitário São João approved this study in November 2018 (ID 201/18). Confidentiality and data anonymity were complied with the guidelines emanating from the Declaration of Helsinki of 1964, revised in Fortaleza, in 2013.

Clinical evaluation

Clinical evaluation included measurement of blood pressure (after 5-minutes rest, sitting position), height and weight. Body mass index ($weigh(kg)/height(m)^2$) and body surface area [$Du\ Bois\ formula$: $0.007184 \times height(cm)^{0.725} \times weight(kg)^{0.425}$] were calculated (215).

Clinical characterization

Maternal cardiovascular health, obstetric and perinatal outcomes, maternal health-related habits, mother's smoking habits, drinking and medical history were systematically collected. For the present pregnancy, obstetric outcomes (especially, gestational diabetes, type of delivery and gestational age at delivery) were also registered.

Echocardiographic assessment

At each evaluation moment, a single operator performed conventional transthoracic echocardiography (TTE) with a 3 MHz phased-array probe (ACUSON SC2000 PRIME™). All images, measurements and variables were obtained from standard views according to the recommendations of the European Society of Cardiology for chamber quantification and

diastolic function evaluation and at least three cardiac cycle images were collected for data analysis (109, 110). TTE was analyzed and validated by an independent cardiologist and discrepancies were settled by discussion and consensus agreement. Structural parameters in mm (interventricular septum in diastole – IVSd and posterior wall thickness in diastole - PWd, left ventricular internal diameter in diastole - LVEDd and systole - LVESd) and their derived parameters (LV mass and relative wall thickness) were obtained from a 2-dimensional parasternal long-axis view. LV mass was estimated using the following equation $0.8 \times (1.04 \times [(LVEDd + PWd + IVSd)^3 – (LVEDd)^3] + 0.6)$.

Left ventricular mass (LVM) was presented according to each previously stated methods: 1) absolute values, without indexing; 2) indexing to the basal (previous pregnancy) BSA; 3) using allometric indexes; or 4) indexing to BSA measured at the same day of cardiac assessment.

Statistical analysis

Continuous variables were reported as median, minimum and maximum, and categorical as absolute values and relative frequencies.

To evaluate cardiac structural changes throughout pregnancy and after delivery, we used the Friedman test (F-statistics, for within-subject assessment) and Kruskall-Wallis test (H-statistics, for between-subject assessment), computed for LV mass, using the 4 indexation methods. Non parametric tests were used because the LV mass values in each time-point period did not reveal a normal distribution (assessed through visual analysis of histograms in tandem with Q-Q plots' examination). The Friedman test effect size (Kendall's W value) and the Kruskall-Wallis effect size $(\eta 2)$ will be estimated as a standardized metric, regardless of the scale or method used to measure the outcome variable – cardiac chambers dimensions – in 1) absolute value or 2) basal BSA indexed (LVM/basal BSA, using Du Bois formula); or 3) using allometric indexes; or 4) BSA indexed according to TTE moment.

For the third method, allometric indexes were estimated using a constant denominator: $height^{1.7}$ or $height^{2.7}$, that have been proposed as an advantage method over indexing to BSA in both normal subjects and obese people, by avoiding within variability (216, 217). We sought to discover whether pregnant population could also benefit from this method and used the two most agreed upon methods in the literature. Mathematically, indexation methods 1), 2) and 3) were expected to produce a similar effect size since there is a common denominator for each one: 1) equals to one (e.g., absolute values); 2) equals to basal BSA (constant across evaluation follow-up); 3) equals to $height^{1,7}$ or $height^{2,7}$ (constant across evaluation follow-up).

The level of significance was 0.05.

Results

Systematic review results

Twenty-seven eligible studies were included in this systematic review (Figure 1), whose characteristics are shown in Tables 1 and 2. All studies had an observational design, with three case control studies (9, 41, 80), six retrospective longitudinal cohort studies (64, 91, 218-221) and 18 prospective longitudinal cohort studies (3, 14-16, 20, 21, 36, 37, 45, 55, 68, 82, 84, 222-225). Four studies did not include echocardiographic assessment during the post-partum period (9, 55, 64, 223). However, in these articles, the investigators performed at least two echocardiographic evaluations during pregnancy. Although all longitudinal studies repeated cardiac assessments during the follow-up period, four articles did not analyse the progression of cardiac remodeling and/or reverse remodeling during pregnancy and after delivery, respectively (64, 80, 218, 219). Regarding echocardiographic variables, namely LA volume, LV volumes or mass, six studies used absolute values without indexing them to BSA (16, 41, 82, 91, 220, 225). LV mass was the most indexed variable to BSA, followed by LA volume. Golinska-Grzybala et al. was the only study to highlight the importance of BSA indexation in analyzing the echocardiographic changes in subjects with distinct body shapes and sizes.

Four studies used a non-pregnant control group (9, 14, 20, 45). Ten articles did not include study group comparisons (eight used consecutive pregnant women recruitment (3, 15, 16, 21, 36, 37, 222, 226) one focused on pregnant women with pulmonary hypertension (221) and one included only women with hypertensive pregnancy disorders (225). Related to the statistical analysis used for longitudinal evaluation of CR and RR, the selection of tests (such as repeated measures ANOVA, linear mixed models, Wilcoxon and paired t test) from within-subjects design was reported in 17 articles (3, 15, 16, 20, 21, 36, 41, 45, 55, 68, 82, 84, 220, 222, 223, 225, 226) and not specified in three manuscripts (reporting only used of student t test (45, 91, 221)). In addition, between-subject design was reported in only three studies to assess the cardiac adaptations during the follow-up period (9, 14, 37).

Figure 1: Flow diagram of the search for eligible studies cardiac remodeling and reverse remodeling during pregnancy and postpartum.

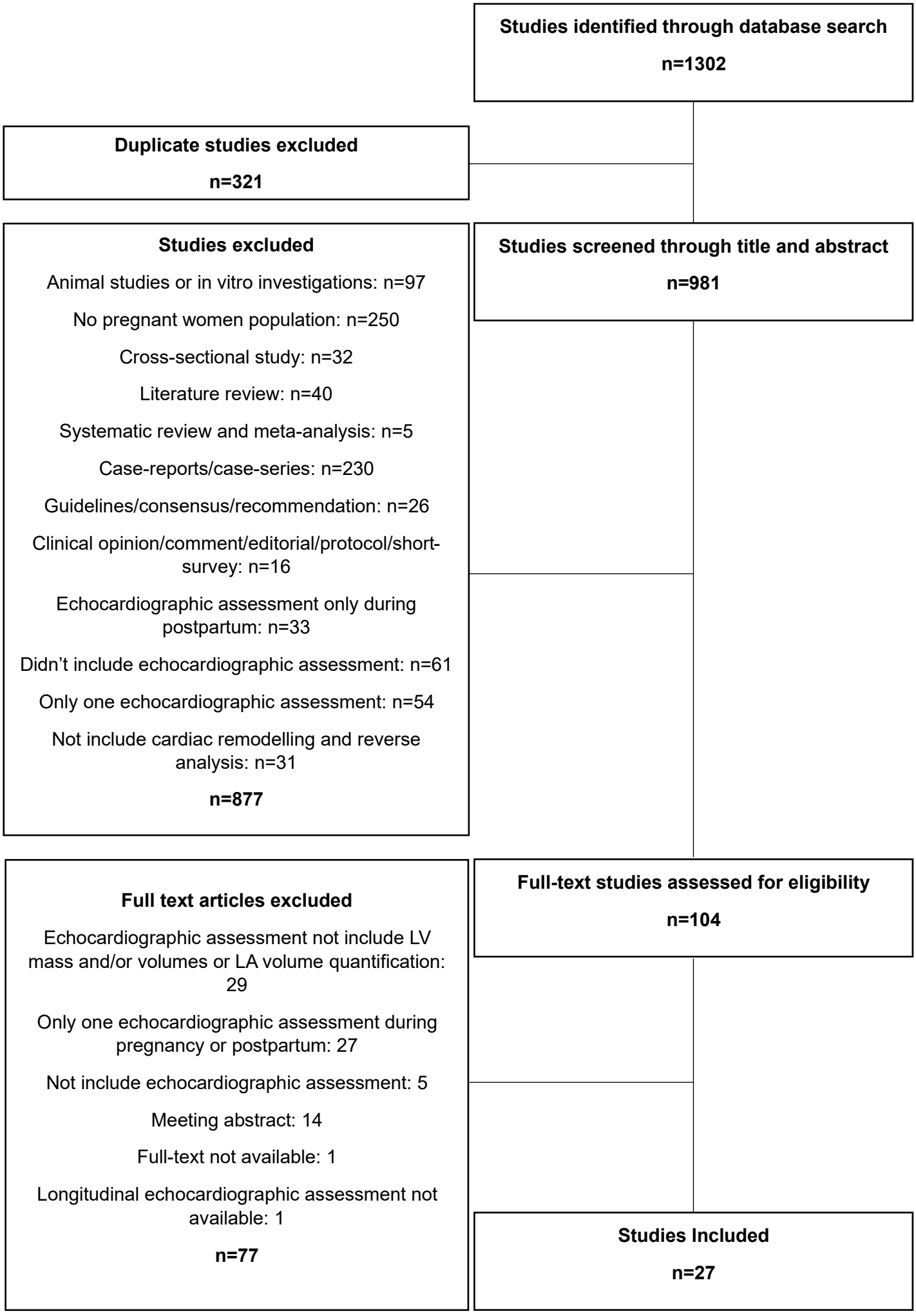

Studies identified through database search
n=1302
Duplicate studies excluded
n=321
Studies screened through title and abstract
n=981
Studies excluded
Animal studies or in vitro investigations: n=97
No pregnant women population: n=250
Cross-sectional study: n=32
Literature review: n=40
Systematic review and meta-analysis: n=5
Case-reports/case-series: n=230
Guidelines/consensus/recommendation: n=26
Clinical opinion/comment/editorial/protocol/short-survey: n=16
Echocardiographic assessment only during postpartum: n=33
Didn't include echocardiographic assessment: n=61
Only one echocardiographic assessment: n=54
Not include cardiac remodelling and reverse analysis: n=31
n=877
Full-text studies assessed for eligibility
n=104
Full text articles excluded
Echocardiographic assessment not include LV mass and/or volumes or LA volume quantification: 29
Only one echocardiographic assessment during pregnancy or postpartum: 27
Not include echocardiographic assessment: 5
Meeting abstract: 14
Full-text not available: 1
Longitudinal echocardiographic assessment not available: 1
n=77
Studies Included
n=27

Table 1: Summary of systematic reviews of echocardiographic assessment during pregnancy and postpartum.

	Study Design	Aim	Country	Recruitment Period	Echocardiographic Assessment During Pregnancy	Echocardiographic Assessment After Delivery
Adeyeye et al., 2016[9]	Case-controlled study	"…assessment of changes in left and right ventricular dimensions (in systole and diastole), left and right atrial dimensions (in diastole), LV systolic and diastolic functions, and LV mass during trimesters of normal pregnancy."	Nigeria	missing	Beginning of the 2nd trimester (15 ± 1 weeks), beginning of the 3rd trimester (28 ± 1 weeks), and middle of the 3rd trimester (35 ± 1 weeks)	n/a
Cong et al., 2016[14]	Prospective study	"…to determine longitudinal strain in each of the three myocardial layers in normal pregnant women according to gestation proceeding."	China	missing	1st trimester (12-14 weeks), 2nd trimester (22-28 weeks) and 3rd trimester (36-40 weeks)	6-9 weeks after delivery
Burlingame, Yamasato, Ahn, Seto, & Tang, 2017[16]	Prospective study	"…to examine BNP and NT-proBNP levels and to correlate them with cardiac structure and function through pregnancy and the postpartum period in normal women."	United States of America	August 2007 to January 2011	1st trimester (6–12 weeks), 2nd trimester (18–24 weeks), 3rd trimester (30–36 weeks) and intrapartum (during labor or immediately prior to delivery)	First 48h postpartum, 6–12 weeks postpartum, and 6–12 months postpartum
Lindley, Conner, Cahill, Novak, & Mann, 2017[218]	Retrospective study	"…the objective of this study was to compare clinical and functional outcomes of peripartum cardiomyopathy patients with pre-eclampsia to those who did not have pre-eclampsia."	United States of America	2004 to 2014	Before delivery	6 to 24 months after diagnosis
Meera, Ando, Pu, Manjappa, & Taub, 2017[64]	Retrospective study	"…to evaluate changes in LV function using speckle tracking echocardiography in patients with gestational diabetes mellitus compared with women with normal pregnancy."	United States of America	2009 to 2014	1st trimester, 2nd trimester (13-26 weeks) and 3rd trimester (27-42 weeks)	n/a
Sato et al., 2017[219]	Retrospective study	"…analyzed gestational hypertension in pregnancies with aortic coarctation and the perinatal changes of echocardiographic parameters."	Japan	Aortic coarctation diagnosis between 1982 and 2015	1st trimester, 2nd trimester and 3rd trimester	After delivery and 1 years after delivery

Sengupta, Bansal, Hofstra, Sengupta, & Narula, 2017[20]	Prospective study	"…to assess serial changes in maternal myocardial contractile function during normal pregnancy and labor and to determine the role of maternal hemodynamic characteristics in these changes."	India	April 2010 to January 2013	1st trimester (10-12 weeks), 2nd trimester (22-26 weeks) and 3rd trimester (30-34 weeks)	Once at labor (first or second stage)
Golińska-Grzybała et al., 2018[222]	Prospective study	"…to evaluate changes in the pulmonary artery diameter (PAD) and their correlations with cardiac remodeling and haemodynamic parameters throughout gestation."	Poland	2014 and 2015	1st trimester (between weeks 10 and 14) and between 2nd and 3rd trimesters (between weeks 25 and 30)	12-38 weeks postpartum
Hieda et al., 2018[82]	Prospective study	"…to compare LV systolic and diastolic function before and during a subsequent pregnancy between women with and without a history of gestational hypertensive disorders."	United States of America	missing	Early (4-8 weeks of gestation) and Late pregnancy (32-36 weeks)	6-10 weeks after delivery
Shahul et al., 2018[80]	Prospective case–control study	"…primary analysis was the association between antepartum activin A levels and postpartum cardiac dysfunction using global peak longitudinal strain. We also performed an exploratory analysis to evaluate the relationship between postpartum activin levels and measurements of diastolic function and mean arterial pressures."	United States of America	July 2013 to November 2016	3rd trimester	12 months after delivery
Umazume et al., 2018[84]	Prospective study	"…to characterize changes in cardiac structures and function in normal pregnancies, …"	Japan	Began in April 2014	1st ($\sim 13^{6/7}$ gestational weeks), 2nd ($14^{0/7}$~$27^{6/7}$ gestational weeks) and 3rd ($28^{0/7}$ gestational weeks) trimesters	within 1 week after childbirth and approximately 1 month postpartum
Umazume et al., 2018[15]	Prospective study	"…to better characterize the heart in hypertensive disorders of pregnancy (HDP) in relation to various cardiac biomarkers, using longitudinally prospectively collected data on simultaneous echocardiography and blood variables from women with HDP.	Japan	April 2014 to March 2016	1st trimester, 2nd trimester and 3rd trimester	1 week and 1 month after delivery

Yu, Zhou, Peng, & Yang, 2018[91]	Retrospective study	"…investigated the LV function of patients with pregnancy-induced hypertension using VVI, combined with NT-pro BNP levels."	China	March 2014 to July 2016	3rd trimester	3 months after delivery
Ghi et al., 2019[223]	Prospective study	"…to compare longitudinal echocardiographic findings in women with uncomplicated monochorionic and dichorionic twin pregnancies."	Italy	2014 to 2016	1st trimester (11-13 weeks), 2nd trimester (20-23 weeks) and 3rd trimester (28-32 weeks)	n/a
Kimura et al., 2019[21]	Prospective study	"…to investigate the values and the changes of brain natriuretic peptide (BNP) and cardiac troponin in pregnant women."	Japan	February 2012 to February 2013	3rd trimester (28–30 weeks gestation)	within 4 days of delivery
Kimura et al., 2019[3]	Prospective study	"…to elucidate the changes in cardiac diastolic function during pregnancy and early after delivery."	Japan	February 2012 to February 2013	3rd trimester (28–30 weeks of gestation)	within 4 days of delivery
Tasar et al., 2019[37]	Prospective study	"…to determine the effects of normal pregnancy on left atrial mechanics using new morphologic and functional echocardiographic parameters while considering left atrial geometry."	Turkey	October 2012 to June 2013	1st trimester, 2nd trimester and 3rd trimester	6-month postpartum
Aguilera et al., 2020[68]	Prospective study	"…to compare maternal cardiac function and structure in women with gestational diabetes mellitus and those with uncomplicated pregnancy at 35 – 36 weeks' gestation and at about 6 months after delivery and assess whether the rate of cardiovascular recovery differs between women with gestational diabetes mellitus and controls."	United Kingdom	missing	35–36 weeks gestation	6 months after delivery
Ambrožič, Lučovnik, Prokšelj, Toplišek, & Cvijić, 2020[41]	Prospective case–control study	"…to assess these time-dependent changes in both hemodynamic, systolic and diastolic function parameters using transthoracic	Slovenia	missing	1 day before delivery	1 and 4 days postdelivery

		echocardiography in women with severe preeclampsia."				
Del Prado Díaz et al., 2020[45]	Prospective study	"…to evaluate and describe changes in RV morphology and function, comparing with LV changes, throughout single gestation pregnancy in a cohort of women without cardiovascular disease."	Spain	missing	2nd trimester (24 ± 2 weeks) and 3rd trimester (32 ± 2 weeks)	From the third month after delivery
Duarte et al., 2020[220]	Retrospective study	"…to characterize changes in LV longitudinal and circumferential strain in women with repaired tetralogy of Fallot, from the preconception to the postpartum period, to help inform counseling of these women regarding the impact of pregnancy on ventricular mechanics."	United States of America	2011 to 2016	Within 18 months prior to conception or in the 1st trimester and 3rd trimester	4-6 weeks postpartum
Herrera, Schell, McIntire, & Cunningham, 2020[221]	Retrospective study	"…to assess the accuracy of measurements of pulmonary artery pressure as estimated by right ventricular systolic pressure using echocardiography as compared with pressures determined directly with right-heart catheterization."	United States of America	2006 to 2017	After 20 weeks of gestation	3 months postpartum
Sonaglioni et al., 2020[36]	Prospective study	"…to evaluate any changes in the common carotid artery intima-media thickness during normal pregnancy and in the postpartum period, and to correlate these changes with neutrophil-to lymphocyte ratio and Red blood cells Distribution Width."	Italy	October 2019 to February 2020	1st trimester (12-14 weeks) and 3rd trimester (36-38 weeks)	6-9 weeks after delivery
Umazume et al., 2020[224]	Prospective study	"…to characterize the cardiac morphofunctional changes in normotensive women with twin or singleton pregnancies and to determine their associations with BNP, NT-proBNP, and hs-TnI levels using longitudinal prospectively collected data."	Japan	April 2014 to March 2016	1st ($\sim 13^{6/7}$ gestational weeks), 2nd ($14^{0/7}$~$27^{6/7}$ gestational weeks) and 3rd ($28^{0/7}$ gestational weeks) trimesters	within 1 week after childbirth and approximately 1month postpartum

Giorgione et al., 2021[225]	Prospective study	"…to compare echocardiographic findings immediately before and after childbirth in women with hypertensive disorders of pregnancy."	United Kingdom	February 2019 to August 2019	4.5 [2-8] days before delivery	3.5 [2-6] days after delivery
Golinska-Grzybala et al., 2021[55]	Prospective study	"…evaluation of the effect of overweight and obesity on myocardial function and parameters of blood flow in the uterine arteries, as well as on birthweight in pregnant women without cardiovascular disorders."	Poland	2014 to 2015	1st trimester (between weeks 10 and 14) and between 2nd and 3rd trimesters (between weeks 25 and 30)	n/a
Sonaglioni, Rigamonti, Nicolosi, Bianchi, & Lombardo, 2021[36]	Prospective study	"…to investigate the influence of chest shape, assessed non-invasively by modified Haller index (MHI), on ventricular-arterial coupling in a population of healthy women with uncomplicated pregnancy."	Italy	October 2019 to June 2020	1st trimester (12-14 weeks) and 3rd trimester (36-38 weeks)	6-9 weeks after delivery

Table 2: Summary of systematic reviews of study groups and echocardiographic variables assessed for cardiac remodeling and reverse remodeling evaluation during pregnancy and postpartum.

	Total Number of Participants	**Study Groups**	**Indexed to BSA** (in each evaluation moment or not)	**LV Mass Indexed**	**LV EDV Indexed**	**LV ESV Indexed**	**LA Volume Indexed**	**Comparison between Study Groups**	**Comparison among Follow-up Period**
Adeyeye et al., 2016[9]	200	Normal pregnant women *versus* Healthy nonpregnant volunteers	In each evaluation moment	yes	n/a	n/a	n/a	yes Independent samples Student's t-test	yes ANOVA
Cong et al., 2016[14]	101	Pregnant women *versus* Nonpregnant women	In each evaluation moment	yes	yes	no	n/a	yes Independent samples Student's t test	yes ANOVA
Burlingame, Yamasato, Ahn, Seto, & Tang, 2017[16]	116	Pregnant women recruited from the community	n/a	no	no	no	no	n/a	yes Linear mixed models (Tukey-Kramer adjustment for multiple comparisons was performed)
Lindley, Conner, Cahill, Novak, & Mann, 2017[218]	39	Pregnant women who developed peripartum cardiomyopathy with pre-eclampsia *versus* Pregnant women who developed peripartum cardiomyopathy without pre-eclampsia	In each evaluation moment	yes	no	no	yes	yes Student's two-sample t test	no

Meera, Ando, Pu, Manjappa, & Taub, 2017[64]	90	Healthy pregnant women *versus* Pregnant women with gestational diabetes	In each evaluation moment	yes	n/a	n/a	n/a	yes Student t test	no
Sato et al., 2017[219]	15	Pregnant women with gestational hypertension *versus* Pregnant women without gestational hypertension	In each evaluation moment	yes	no	no	no	yes Student t-test	no
Sengupta, Bansal, Hofstra, Sengupta, & Narula, 2017[20]	55	Healthy pregnant women *versus* Nulliparous	In each evaluation moment	n/a	no	no	yes	yes Independent samples Student's t-test	yes Linear mixed models
Golińska-Grzybała et al., 2018[222]	69	Consecutive healthy outpatients with a single pregnancy	In each evaluation moment	yes	no	no	yes	yes (not specify the statistical method applied)	yes Repeated measures ANOVA and Bonferroni post hoc correction for multiple comparisons
Hieda et al., 2018[82]	41	Women with a history of gestational hypertensive disorders *versus* Women without such a history	n/a	n/a	no	n/a	n/a	yes Post hoc multiple comparisons 170 were made by Mann-Whitney U test with the use of the Bonferroni correction	yes Repeated measures ANOVA using linear mixed models
Shahul et al., 2018[80]	85	Pregnant women with	In each evaluation moment	yes	no	no	yes	yes	no

		preeclampsia *versus* Pregnant women with gestational or chronic hypertension *versus* Healthy pregnant women						Parametric or nonparametric t-tests as appropriate	
Umazume et al., 2018[15]	51	Healthy pregnant women	In each evaluation moment	yes	n/a	n/a	yes	n/a	yes Wilcoxon's rank sum test and Student's t-test with Bonferroni's correction
Umazume et al., 2018[84]	75	Normotensive pregnant women *versus* hypertensive disorders of pregnancy	In each evaluation moment	yes	n/a	n/a	yes	yes (not specify the statistical method applied)	yes Wilcoxon's rank-sum test and Student's t-test with Bonferroni correction
Yu, Zhou, Peng, & Yang, 2018[91]	82	Pregnant women with gestational hypertension *versus* pregnant women with preeclampsia *versus* Healthy pregnant women	n/a	n/a	no	no	n/a	yes Student t-test	yes Student t test
Ghi et al., 2019[223]	67	Healthy women with twin monochorionic pregnancy *versus* Healthy women with twin	In each evaluation moment	yes	no	no	n/a	yes Independent samples Student's t-test	yes Repeated measures ANOVA

		dichorionic pregnancy							
Kimura et al., 2019[21]	405	Consecutive pregnant women recruitment	In each evaluation moment	n/a	n/a	n/a	yes	n/a	yes Paired t-test
Kimura et al., 2019[3]	397	Consecutive pregnant women recruitment	In each evaluation moment	yes	no	no	yes	n/a	yes Paired t-test
Tasar et al., 2019[37]	47	Only healthy women with singleton first pregnancies	In each evaluation moment	yes	yes	yes	no	n/a	yes ANOVA with the Tukey test
Aguilera et al., 2020[68]	146	Pregnant women with gestational diabetes mellitus *versus* Control with uncomplicated pregnancy	In each evaluation moment	yes	no	no	yes	yes Independent samples Student's t-test or the Mann-Whitney U test	yes Linear mixed models with two random effects (random intercept and random slope) and an unstructured variance–covariance matrix
Ambrožič, Lučovnik, Prokšelj, Toplišek, & Cvijić, 2020[41]	60	Pregnant women with severe preeclampsia *versus* Healthy pregnant women	n/a	no	no	no	no	yes Student t-test or Mann–Whitney U test were used for comparison between two groups depending on the distribution	yes Repeated measures ANOVA or Friedman test depending on the distribution
Del Prado Díaz et al., 2020[45]	133	Healthy pregnant women *versus* Nulliparous	In each evaluation moment	yes	no	no	no	yes Independent samples Student's t-test	yes Linear mixed models (due to longitudinal sample nature with the maximum restricted likelihood

									method. Medium values were estimated and compared by square minimums and multiple comparisons were corrected by Bonferroni method)
Duarte et al., 2020[220]	16	Pregnant women with repaired tetralogy of Fallot, who were enrolled in the Standardized Outcomes in Reproductive Cardiovascular Care (STORCC) registry	n/a	n/a	no	no	n/a	n/a	yes Repeated measures ANOVA
Herrera, Schell, McIntire, & Cunningham, 2020[221]	46	Pregnant women with pulmonary hypertension	In each evaluation moment	yes	n/a	n/a	n/a	yes (not specify the statistical method applied)	yes Student's t-test
Sonaglioni et al., 2020[36]	73	Consecutive healthy pregnant women	In each evaluation moment	yes	yes	yes	no	n/a	yes Repeated measures ANOVA
Umazume et al., 2020[224]	66	Women with singleton pregnancies *versus* Women with twin pregnancies	In each evaluation moment	yes	n/a	n/a	yes	yes (not specify the statistical method applied)	yes Student's t-test with Bonferroni correction

Giorgione et al., 2021[225]	30	Women with hypertensive disorders during pregnancy	n/a	no	no	no	no	n/a	yes Paired t-test or Wilcoxon signed-rank test
Golinska-Grzybala et al., 2021[55]	87	Women with normal weight *versus* Overweight and obese women	In each evaluation moment	yes	yes	yes	yes	yes Mann-Whitney U test	yes Repeated measures ANOVA and Bonferroni post hoc correction for multiple comparisons
Sonaglioni, Rigamonti, Nicolosi, Bianchi, & Lombardo, 2021[226]	59	Healthy pregnant women	In each evaluation moment	yes	yes	yes	yes	n/a	yes Repeated measures ANOVA

Illustrative Example – pregnant women cohort

We enrolled 45 pregnant women with a median age of 33 [25,41] years, of whom 40% were multiparous. Only three participants developed gestational diabetes. Regarding smoking habits, five pregnant women maintained smoking during gestation, three stopped smoking at the beginning of pregnancy, and ten were former smokers. The variation of body mass index and BSA during pregnancy and postpartum are represented in Table 3. Data were analyzed considering four indexation methodologies and using a within and a between design.

Table 3: Variation of body mass index and BSA during pregnancy and postpartum.

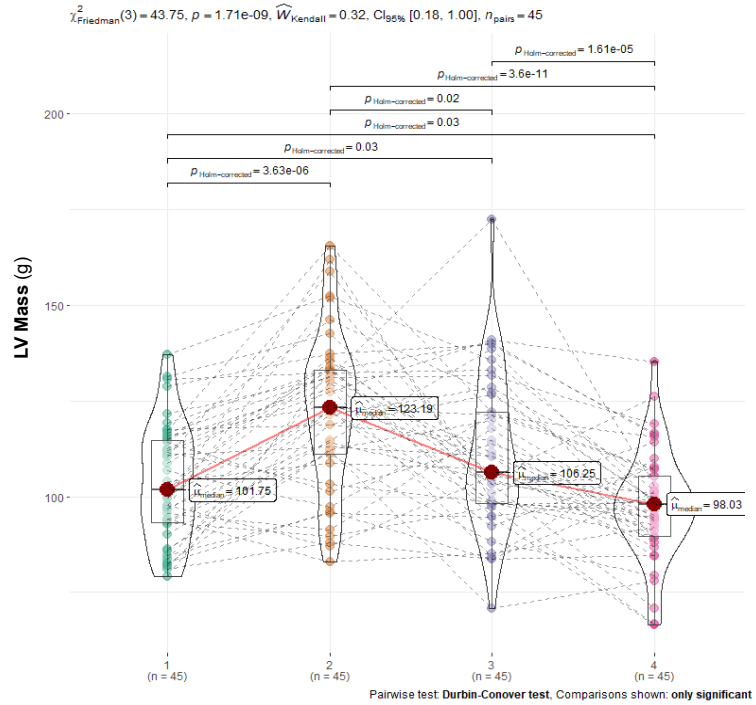
	Prior to Pregnancy	**1st Trimester**	**3rd Trimester**	**1 Month After Delivery**	**6 Months After Delivery**
Body Mass Index (kg/m^2)	22.66 [17.91; 29.37]	23.31 [18.67; 29.55]	27.13 [21.15; 33.23]	24.57 [18.29; 30.49]	23.44 [17.53; 31.51]
Body Surface Area (m^2)	1.68 [1.48; 1.92]	1.71 [1.50; 1.94]	1.80 [1.58; 2.02]	1.71 [1.50; 1.99]	1.69 [1.46; 2.01]

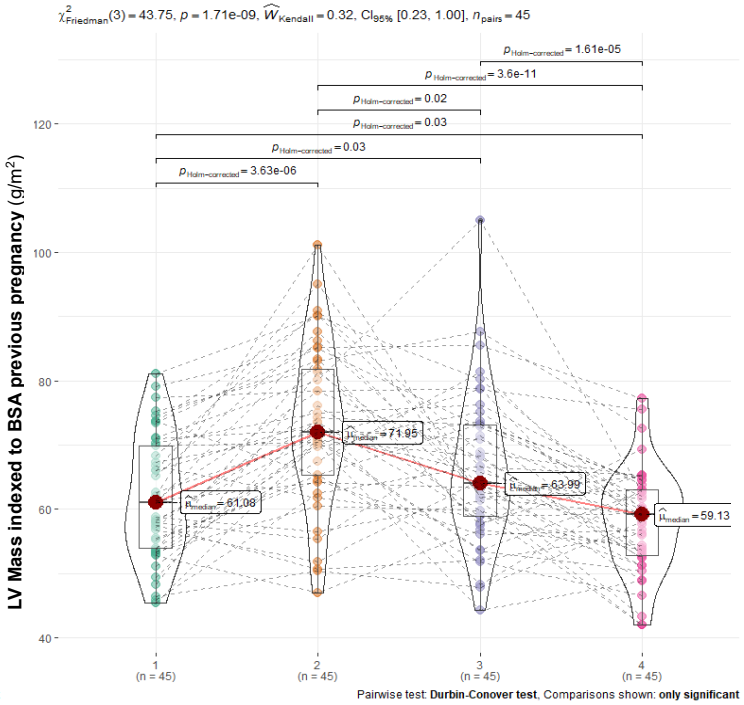
Values reported through median [minimum; maximum].

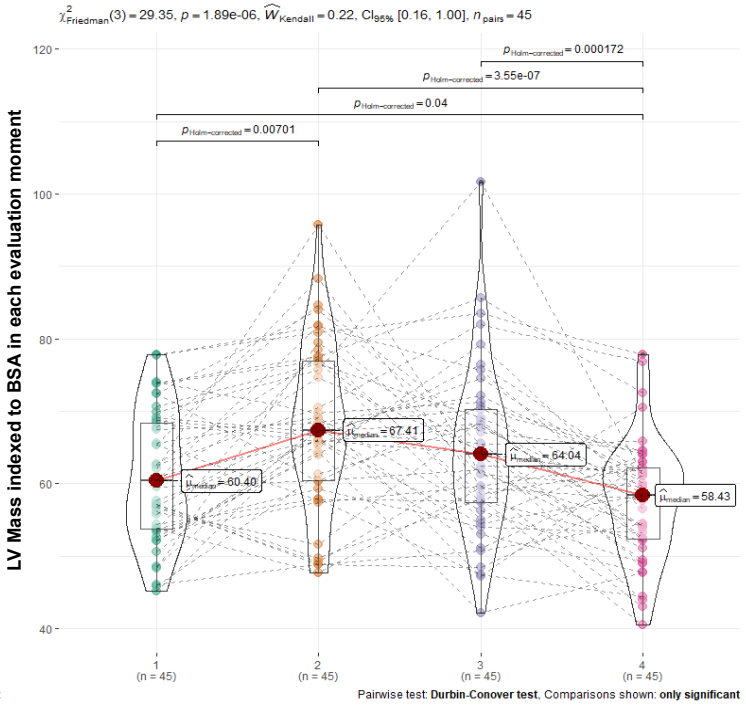
We focused on the longitudinal variation of LVM to analyse the CR and RR during pregnancy and postpartum, respectively. Figures 2 and 3 show the variation of LVM, using Friedman and Kruskal-Wallis tests, respectively. In these plots, each point represents one pregnant woman, and a dashed connective line depicts the change between evaluation times.

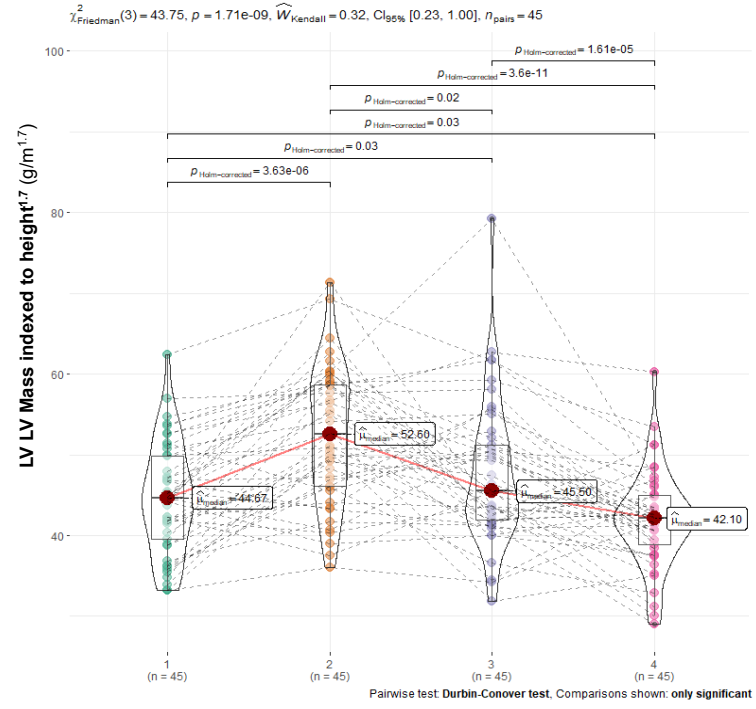
Related to LVM progression, a significant increase was found from 1st to 3rd trimesters followed by a significant decrease from the 3rd trimester to 6 months postpartum regardless of the indexation method used (Figures 2 and 3). However, when LVM was indexed to BSA at each time-point, the LVM regression from the 3rd trimester to 1st month after delivery lost statistical significance, contrasting with results from other indexation methodologies (Figures 2 and 3).

Figure 2: Longitudinal analysis of LVM variation of LVM using Friedman test.


$\chi^2_{Friedman}(3) = 43.75$, $p = 1.71e\text{-}09$, $\widehat{W}_{Kendall} = 0.32$, CI$_{95\%}$ [0.18, 1.00], $n_{pairs} = 45$
LV Mass (g)
$p_{Holm\text{-}corrected} = 1.61e\text{-}05$
$p_{Holm\text{-}corrected} = 3.6e\text{-}11$
$p_{Holm\text{-}corrected} = 0.02$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 3.63e\text{-}06$
$\hat{\mu}_{median} = 101.75$
$\hat{\mu}_{median} = 123.19$
$\hat{\mu}_{median} = 106.25$
$\hat{\mu}_{median} = 98.03$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Durbin-Conover test**, Comparisons shown: **only significant**


$\chi^2_{Friedman}(3) = 43.75$, $p = 1.71e\text{-}09$, $\widehat{W}_{Kendall} = 0.32$, CI$_{95\%}$ [0.23, 1.00], $n_{pairs} = 45$
LV Mass indexed to BSA previous pregnancy (g/m^2)
$p_{Holm\text{-}corrected} = 1.61e\text{-}05$
$p_{Holm\text{-}corrected} = 3.6e\text{-}11$
$p_{Holm\text{-}corrected} = 0.02$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 3.63e\text{-}06$
$\hat{\mu}_{median} = 61.08$
$\hat{\mu}_{median} = 71.95$
$\hat{\mu}_{median} = 63.99$
$\hat{\mu}_{median} = 59.13$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Durbin-Conover test**, Comparisons shown: **only significant**


$\chi^2_{Friedman}(3) = 29.35$, $p = 1.89e\text{-}06$, $\widehat{W}_{Kendall} = 0.22$, CI$_{95\%}$ [0.16, 1.00], $n_{pairs} = 45$
LV Mass indexed to BSA in each evaluation moment
$p_{Holm\text{-}corrected} = 0.000172$
$p_{Holm\text{-}corrected} = 3.55e\text{-}07$
$p_{Holm\text{-}corrected} = 0.04$
$p_{Holm\text{-}corrected} = 0.00701$
$\hat{\mu}_{median} = 60.40$
$\hat{\mu}_{median} = 67.41$
$\hat{\mu}_{median} = 64.04$
$\hat{\mu}_{median} = 58.43$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Durbin-Conover test**, Comparisons shown: **only significant**


$\chi^2_{Friedman}(3) = 43.75$, $p = 1.71e\text{-}09$, $\widehat{W}_{Kendall} = 0.32$, CI$_{95\%}$ [0.23, 1.00], $n_{pairs} = 45$
LV LV Mass indexed to height$^{1.7}$ (g/m$^{1.7}$)
$p_{Holm\text{-}corrected} = 1.61e\text{-}05$
$p_{Holm\text{-}corrected} = 3.6e\text{-}11$
$p_{Holm\text{-}corrected} = 0.02$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 3.63e\text{-}06$
$\hat{\mu}_{median} = 44.67$
$\hat{\mu}_{median} = 52.60$
$\hat{\mu}_{median} = 45.50$
$\hat{\mu}_{median} = 42.10$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Durbin-Conover test**, Comparisons shown: **only significant**

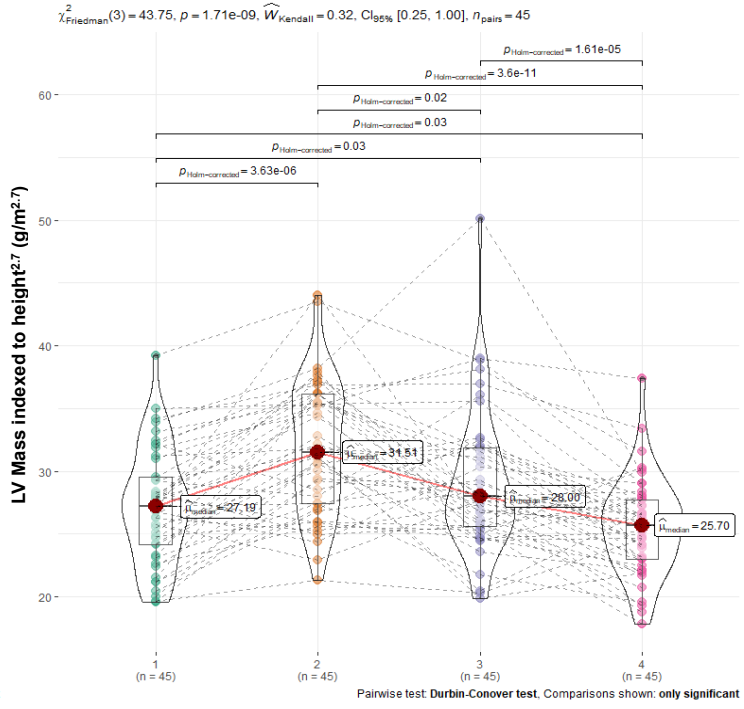
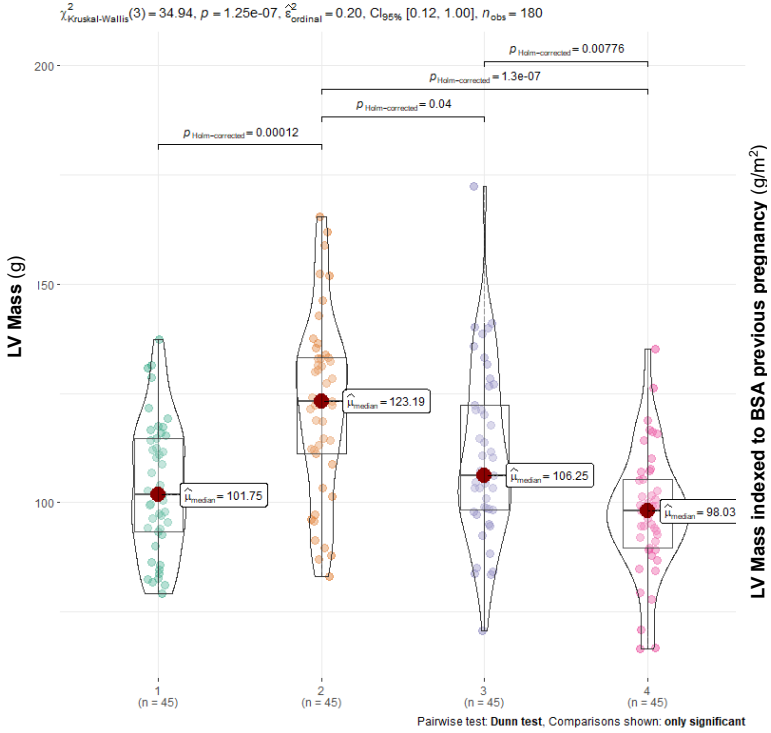
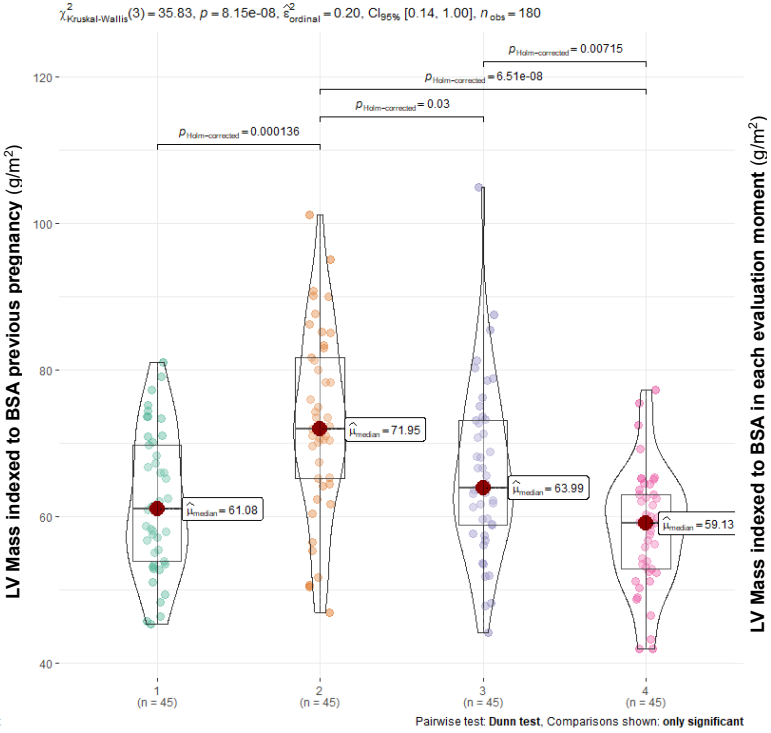
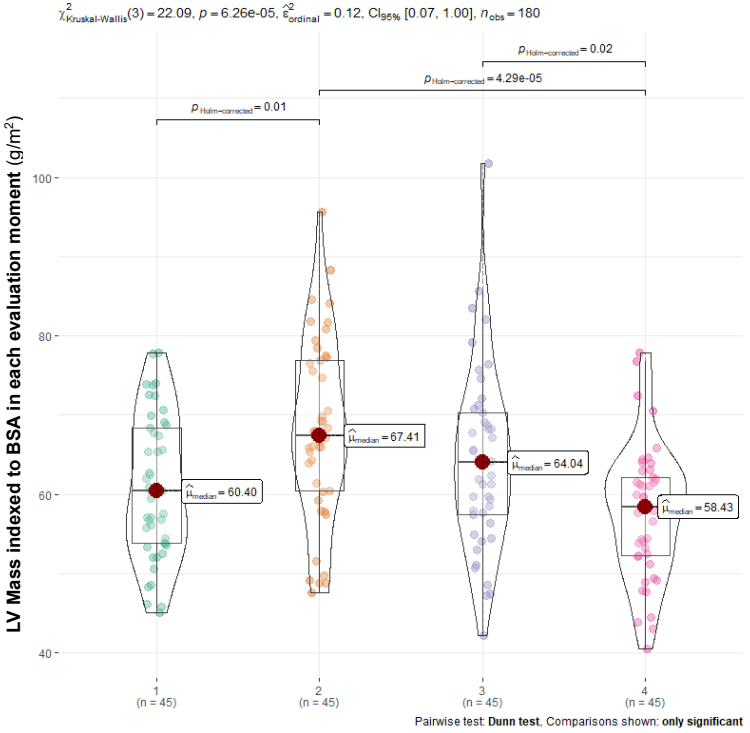
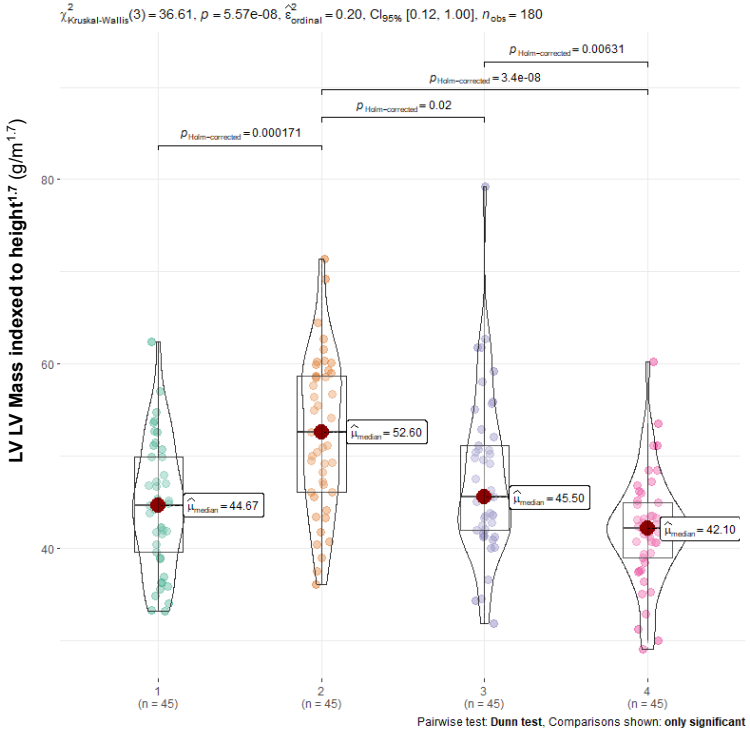

$\chi^2_{Friedman}(3) = 43.75$, $p = 1.71e\text{-}09$, $\widehat{W}_{Kendall} = 0.32$, CI$_{95\%}$ [0.25, 1.00], $n_{pairs} = 45$
LV Mass indexed to height$^{2.7}$ (g/m$^{2.7}$)
$p_{Holm\text{-}corrected} = 1.61e\text{-}05$
$p_{Holm\text{-}corrected} = 3.6e\text{-}11$
$p_{Holm\text{-}corrected} = 0.02$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 3.63e\text{-}06$
$\hat{\mu}_{median} = 27.19$
$\hat{\mu}_{median} = 31.51$
$\hat{\mu}_{median} = 28.00$
$\hat{\mu}_{median} = 25.70$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Durbin-Conover test**, Comparisons shown: **only significant**

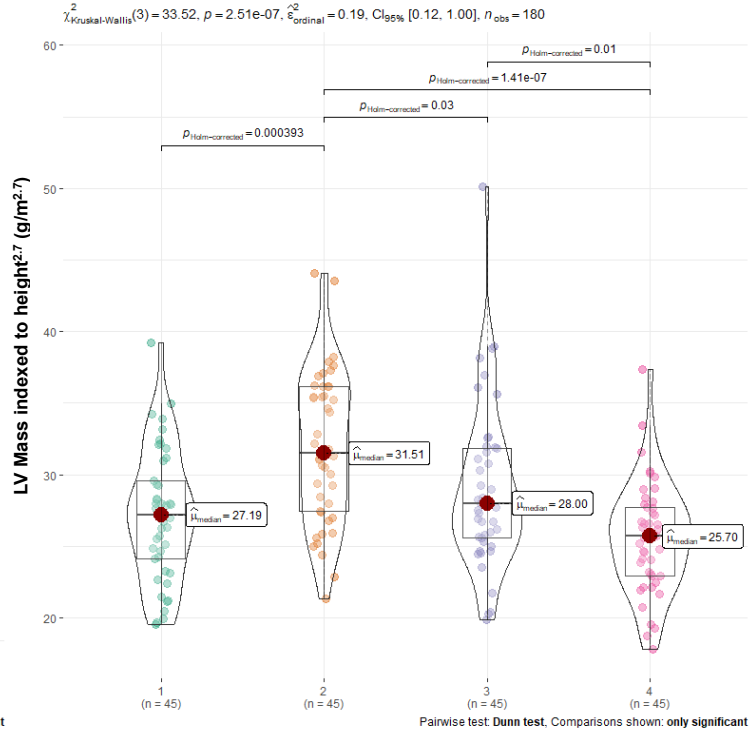
Figure 3: Longitudinal analysis of LVM variation of LVM using Kruskal-Wallis test.


$\chi^2_{Kruskal\text{-}Wallis}(3) = 34.94$, $p = 1.25e\text{-}07$, $\hat{\epsilon}^2_{ordinal} = 0.20$, CI$_{95\%}$ [0.12, 1.00], $n_{obs} = 180$
LV Mass (g)
$p_{Holm\text{-}corrected} = 0.00012$
$p_{Holm\text{-}corrected} = 0.04$
$p_{Holm\text{-}corrected} = 1.3e\text{-}07$
$p_{Holm\text{-}corrected} = 0.00776$
$\hat{\mu}_{median} = 101.75$
$\hat{\mu}_{median} = 123.19$
$\hat{\mu}_{median} = 106.25$
$\hat{\mu}_{median} = 98.03$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Dunn test**, Comparisons shown: **only significant**


$\chi^2_{Kruskal\text{-}Wallis}(3) = 35.83$, $p = 8.15e\text{-}08$, $\hat{\epsilon}^2_{ordinal} = 0.20$, CI$_{95\%}$ [0.14, 1.00], $n_{obs} = 180$
LV Mass indexed to BSA previous pregnancy (g/m^2)
$p_{Holm\text{-}corrected} = 0.000136$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 6.51e\text{-}08$
$p_{Holm\text{-}corrected} = 0.00715$
$\hat{\mu}_{median} = 61.08$
$\hat{\mu}_{median} = 71.95$
$\hat{\mu}_{median} = 63.99$
$\hat{\mu}_{median} = 59.13$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Dunn test**, Comparisons shown: **only significant**


$\chi^2_{Kruskal\text{-}Wallis}(3) = 22.09$, $p = 6.26e\text{-}05$, $\hat{\epsilon}^2_{ordinal} = 0.12$, CI$_{95\%}$ [0.07, 1.00], $n_{obs} = 180$
LV Mass indexed to BSA in each evaluation moment (g/m^2)
$p_{Holm\text{-}corrected} = 0.01$
$p_{Holm\text{-}corrected} = 4.29e\text{-}05$
$p_{Holm\text{-}corrected} = 0.02$
$\hat{\mu}_{median} = 60.40$
$\hat{\mu}_{median} = 67.41$
$\hat{\mu}_{median} = 64.04$
$\hat{\mu}_{median} = 58.43$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Dunn test**, Comparisons shown: **only significant**


$\chi^2_{Kruskal\text{-}Wallis}(3) = 36.61$, $p = 5.57e\text{-}08$, $\hat{\epsilon}^2_{ordinal} = 0.20$, CI$_{95\%}$ [0.12, 1.00], $n_{obs} = 180$
LV LV Mass indexed to height$^{1.7}$ (g/m$^{1.7}$)
$p_{Holm\text{-}corrected} = 0.000171$
$p_{Holm\text{-}corrected} = 0.02$
$p_{Holm\text{-}corrected} = 3.4e\text{-}08$
$p_{Holm\text{-}corrected} = 0.00631$
$\hat{\mu}_{median} = 44.67$
$\hat{\mu}_{median} = 52.60$
$\hat{\mu}_{median} = 45.50$
$\hat{\mu}_{median} = 42.10$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Dunn test**, Comparisons shown: **only significant**


$\chi^2_{Kruskal\text{-}Wallis}(3) = 33.52$, $p = 2.51e\text{-}07$, $\hat{\epsilon}^2_{ordinal} = 0.19$, CI$_{95\%}$ [0.12, 1.00], $n_{obs} = 180$
LV Mass indexed to height$^{2.7}$ (g/m$^{2.7}$)
$p_{Holm\text{-}corrected} = 0.000393$
$p_{Holm\text{-}corrected} = 0.03$
$p_{Holm\text{-}corrected} = 1.41e\text{-}07$
$p_{Holm\text{-}corrected} = 0.01$
$\hat{\mu}_{median} = 27.19$
$\hat{\mu}_{median} = 31.51$
$\hat{\mu}_{median} = 28.00$
$\hat{\mu}_{median} = 25.70$
1 (n = 45)
2 (n = 45)
3 (n = 45)
4 (n = 45)
Pairwise test: **Dunn test**, Comparisons shown: **only significant**

Comparison among different methods of indexation using a within or a between design

The effect size can be used as a standardized metric to compare the magnitude of reported effects for LVM indexation (LVMi), using four different normalization methods 1) absolute values, without indexing; 2) indexing to pre-pregnancy BSA; 3) allometric indexing or 4) indexing to BSA measured at the same day of cardiac assessment).

Regarding the within-subjects design, the effect size of the variation of LVM analysis was similar when indexed to pre-pregnancy BSA, without indexation or using allometric indexation (Table 4). However, the effect size was smaller when indexed to BSA computed at each time-point (Table 4). The comparisons made by the within-subjects design were all statistically significant (p-value<0.05).

Concerning the between-subjects design, the effect size of the LVM comparison among four time points was similar using pre-pregnancy BSA indexation, allometric indexes or without any indexation method (Table 4). The effect size reported in LVM indexed to BSA calculated for each time-point of cardiac assessment was lower (Table 4). The comparisons made by the between-subjects design were all statistically significant (p-value<0.05).

Focusing on comparisons between within and between-subjects designs, the first one displayed higher effect size values in all indexation methods (Table 4). Additionally, we found that the effect size calculated from LVM progression indexed to BSA in each evaluation through within-subject design was similar to the effect size quantified in the between-subjects design when indexed to pre-pregnancy BSA, without indexation or using allometric indexes.

Table 4: Comparison among different methods of indexation using a within or a between design.

	F statistic	p-value	Effect Size	Confidence Interval
Within-Subjects Design				
LV Mass (g)	43.75	1.71E-09	0.32	[0.18;1.00]
LV Mass indexed to BSA previous pregnancy (g/m^2)	43.75	1.71E-09	0.32	[0.23;1.00]
LV Mass indexed to height$^{1.7}$ ($g/m^{1.7}$)	43.75	1.71E-09	0.32	[0.23;1.00]
LV Mass indexed to height$^{2.7}$ ($g/m^{2.7}$)	43.75	1.71E-09	0.32	[0.25;1.00]
LV Mass indexed to BSA in each evaluation moment (g/m^2)	29.35	1.89E-06	0.22	[0.16;1.00]
Between-Subjects Design	**H statistics**	**P-value**	**Effect Size**	**Confidence Interval**
LV Mass (g)	34.94	1.25E-07	0.20	[0.12;1.00]
LV Mass indexed to BSA previous pregnancy (g/m^2)	35.83	8.15E-08	0.20	[0.14;1.00]
LV Mass indexed to height$^{1.7}$ ($g/m^{1.7}$)	36.61	5.57E-08	0.20	[0.12;1.00]
LV Mass indexed to height$^{2.7}$ ($g/m^{2.7}$)	33.52	2.51E-07	0.19	[0.12;1.00]
LV Mass indexed to BSA in each evaluation moment (g/m^2)	22.09	6.26E-05	0.12	[0.07;1.00]

Discussion

We performed a systematic review to explore which indexation methods were applied most frequently in literature to evaluate cardiac remodeling and reverse remodeling during pregnancy and postpartum, respectively. Current evidence suggested that echocardiographic parameters were mostly indexed to BSA measured at each evaluation moment(3, 9, 14, 15, 20, 21, 36, 37, 45, 55, 64, 68, 80, 84, 218, 221-224, 226, 227). Additionally, we found that indexation was most commonly performed to evaluate LV mass and LA volume (3, 9, 14, 15, 20, 21, 36, 37, 45, 55, 64, 68, 80, 84, 218, 221-224, 226, 227). Most studies selected within-subjects design to analyse the longitudinal cardiac remodeling and reverse remodeling processes(3, 15, 16, 20, 21, 36, 41, 45, 55, 68, 82, 84, 220, 222, 223, 225, 226).

In our illustrative echocardiographic example, focused on LVM assessment, we gauged alternative scaling methods, 1) using absolute values without indexing; 2) indexing to pre-pregnancy BSA; 3) using allometric indexes (indexes 1.7 and 2.7) or 4) indexing to BSA measured on the same day of cardiac assessment). The indexation method used conditioned the effect magnitude of the LVM variation during pregnancy and postpartum. Considering the progressive increase of BSA during pregnancy due to fetus development, maternal weight gain, and the potential incomplete weight recovery after delivery, the assessment of CR and RR exclusively related to volume overload is difficult to estimate. Indeed, indexing with pre-pregnancy BSA and allometric indexes end up dismissing the contribution of the fetus development. Indeed, indexing to BSA measured at each evaluation could underestimate the impact of the CR process, since changes in LVM could be neutralized by the increased BSA across gestation. In addition, the selection of statistical tests from within versus between-subjects design is critical and depends on the research question and the experimental design study. In within-subjects analysis, the effect sizes were higher in all indexation methods selected compared to between-subject analysis. The within-subjects design considers two sources of variability: 1) differences across individuals and 2) the variability due to within-subjects, while the between-subjects design only considers the first source of variability. When using the BSA indexation for each moment in the within-subjects design, one is reducing the second source of variability by handing a new and changeable variable contributing to a small effect size like the ones obtained by the between-subjects analysis without longitudinal BSA variation. However, the effect size comparisons were not statistically assessed. To our knowledge, this is the first work to evaluate the impact of indexation for the longitudinal analysis of cardiac adaptation, specifically LVM, during pregnancy and 1 to 6 months after delivery, as well as to discuss the potential statistical methods applied for evaluation purposes.

In the literature review, we found that most studies used a within-subjects design to analyse longitudinal cardiovascular adaptations during pregnancy and postpartum statistically. We also discovered that the echocardiographic parameters were mostly indexed to BSA measured at each evaluation moment. According to the illustrative example, this was associated with lower effect size values compared to the lack of normalization method, using absolute values. Only three of twenty-five research articles selected between-subjects design to explore cardiovascular remodeling and RR, all of them using BSA indexation measured at each evaluation moment, which we revealed to result in the lowest effect size (9, 14, 37).

Interestingly, even in publications that included a comparison of independent study groups (for example, pregnant women with repaired tetralogy of Fallot versus nulliparous women; or, pregnant women with gestational hypertension versus pregnant women with preeclampsia versus healthy pregnant women; or pregnant women with severe preeclampsia versus healthy pregnant women), some authors did not index echocardiographic variables to analyse cardiac adaptations during the follow-up period between study groups (41, 91, 220). Only Golinska-Grzybala et al. reported the importance of indexing echocardiographic parameters among study groups that differ significantly in body shapes or size (55). In contrast, the vast majority authors did not give any reason or justification for their choice of indexation method (9, 14, 20, 45, 64, 68, 80, 84, 218, 219, 223, 224). A longitudinal analysis comparing study groups was not the purpose of our illustrative example.

Some studies, especially in overweight and obese subjects, suggested height-based indexing as more advantageous method than indexing to BSA for detection of left ventricular hypertrophy (211, 217, 228). However, the selection of a better allometric coefficient is not agreed upon and may vary according to study sample age (for example, three arose as the optimal height exponent for indexing LVM in juvenil population) (229, 230). The allometric coeffient 1.7 was reported as more sensitive to classify obesity-related LV hypertrophy than BSA indexation, being consistently correlated with cardiovascular events and all-cause mortality (217). In addition, LVM/height$^{2.7}$ showed an increased capacity to diagnose LV hypertrophy and to detect increased (~ two-fold) population-attributable risk for fatal and nonfatal cardiovascular outcomes compared to BSA (231). In pregnancy context, this method has not been explored yet.

The present study presented some limitations: small sample size including only a single tertiary center; lack of statistical evaluation of the effect size between within and between-subjects designs; the findings from our illustrative example only apply to LVM assessment, limiting the potential inferences for other cardiac metrics. In addition, the results about effect size

differences could not transpose into clinically relevant findings, and there was no longitudinal outcomes to compare the performance of different indexation methods on clinical results.

Conclusion

Our literature review showed different approaches and methodologies concerning indexation of echocardiographic parameters and statistical analysis for the study of CR and RR progression in pregnancy. Although there was a most common approach: indexing to BSA at the time of evaluation (twenty-one out of twenty-seven studies), this topic needs further discussion and consensus among the scientific community. Regarding the longitudinal progression of LVM, we tested two additional alternative indexation methods, indexing to the pre-pregnancy BSA or using allometric indexes, which yielded a higher magnitude of effect compared to the already used indexation (BSA measured at each evaluation), and an equal effect to not indexing, using within-subjects design. Additionally, we trust it is relevant to explore the effect of BSA variation in LV remodeling and RR assessment through generalized mixed model, application adjusting to baseline BSA or weight gain/loss during pregnancy/postpartum, without any normalization of echocardiographic parameters. In summary, we have to seek the best-performing monitoring index.

Data Accessibility

Data sharing is not applicable to this article.

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Not applicable

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Competing interests

The authors have no competing interests to declare.

Authors' contribution

Conceived the ideas or experimental design of the study	Francisca Saraiva
Performed experiments/data collection	Ana Filipa Ferreira; Francisca Saraiva
Data analysis and interpretation	Ana Filipa Ferreira; Francisca Saraiva; Sílvia Diaz; António Barros
Primary author	Ana Filipa Ferreira
Provided revisions to scientific content of manuscript	Francisca Saraiva; Maria João Azevedo; Sílvia Diaz; António Barros; Inês Falcão-Pires; Carla Sousa; Benedita Sampaio-Maia; Carla Ramalho
Provided funding	Inês Falcão-Pires; Adelino Leite-Moreira
Provided access to crucial research component: equipment and lab	Adelino Leite-Moreira
Provided access to crucial research component: recruitment process	Carla Ramalho
Provided support to crucial research component: echocardiographic assessment	Carla Sousa
Provided support to crucial research component: statistical analysis	Francisca Saraiva; Sílvia Diaz; António Barros
Principal investigator	Inês Falcão-Pires

Table S1: Searched query

#1 – population	(pregnancy OR gestation OR pregnancies OR “pregnant women” OR “pregnant woman”)
#2 – intervention	(echocardiography OR “transthoracic echocardiography” OR echocardio* OR “cardiac function”)
#3 – study period	(2016-08-01 to 2021-08-01)
#4 – language	(portuguese OR english OR spanish OR french)
#5 – restrictions	(fetal OR fetus OR neonatal OR infant OR newborns OR “infants newborn” OR “newborn infant” OR “newborn infants” OR newborn OR neonate OR neonates OR infant OR offspring OR childhood)
#6 – study type	(systematic review OR editorial OR letter OR review OR meta-analysis OR cross-sectional).
Final query	#1 AND #2 AND #3 AND #4 NOT #5 NOT #6

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Chapter V: Discussion and Conclusions

The PERIMYR project represents the first study designed to include a population of pregnant women to be used as a model to study cardiac remodelling and reverse remodelling (RR) allowing to dissect the impact of comorbidities and to uncover potential biomarkers of these remodelling stages. PERIMYR also served to characterise the response to an acute myocardial stretch in conditions of volume overload and physiologic hypertrophy. To achieve these goals, the enrolled pregnant women underwent cardiovascular evaluation during pregnancy, in the 1st and 3rd trimesters, and during the postpartum, at 6-8 weeks, 6-7 months and 1 year. Figure 2 overviews and summarizes an of the main aims, achievements and outputs of this thesis.

The use of pregnant women as a study model stems from the fact that pregnancy represents an excellent and unique human model to investigate cardiovascular remodelling and RR under physiological (pregnancy-induced remodelling and postpartum associated RR) and pathological conditions, i.e., in the presence of arterial hypertension, gestational diabetes or obesity. Thus, PERIMYR can provide invaluable knowledge translatable to the context of heart failure (HF). On the one hand, using pregnant women as a model provides several advantages by reducing several confounding risk factors present in the pathologic context of HF and outperforming the information derived from animal models. On the other hand, pregnancy has been assigned as an opportunity to screen subclinical cardiovascular disease and, consequently, to assess the propensity of these women to develop HF in the long-term (140). Indeed, women who have experienced adverse pregnancy outcomes revealed a higher risk of developing cardiovascular disease, largely explained by the interim development of traditional risk factors, such as arterial hypertension and diabetes (114, 115, 140).

Figure 2: Overview and summary of the main aims, achievements and outputs of this thesis.

	Chapter III	Chapter IV Subchapter i	Chapter IV Subchapter ii	Chapter VI Subchapter iii	Chapter VI Subchapter iv	Chapter VI Subchapter v
Aims	To describe the PERIMYR prospective cohort study protocol, which enrolls pregnant women as a model to understand the mechanism of cardiovascular diseases.	To evaluate cardiovascular remodelling during pregnancy and to identify CVR factors and plasma biomarkers that might predict cardiac and vascular reverse remodelling after delivery.	To profile the urinary proteome in pregnant women with or without CVR factors and identify putative proteins associated with cardiac postpartum reverse remodelling.	To characterize the response to stretch induce-compliance mechanism in pregnant women with and without CVR factors.	To characterize the right cardiovascular (reverse) remodelling induced by pregnancy and postpartum respectively and to explore the impact of CVR factors in this process.	To identify the most common indexing methodologies in the literature of pregnant population through a systematic review; and, to compare 4 indexation methods applied to PERIMYR's cohort.
Achievements	**1)** Description of PERIMYR's protocol which comprises a prospective cohort study to characterize the cardiovascular (reverse) remodelling induced by pregnancy and postpartum to explore the impact of cardiovascular risk factors in these processes and clarify the stretch-induced compliance mechanism.	**1)** Characterization of cardiovascular reverse remodelling until one year after delivery. **2)** Identification of CVR factors associated with worse postpartum hypertrophy reversal. **3)** Identification of plasma biomarkers associated with left ventricular mass during pregnancy and postpartum. **4)** Description of CVR factors associated with pulse wave velocity, used to assess the vascular (reverse) remodelling.	**1)** Comparison of cardiovascular reverse remodelling between pregnant women with and without CVR factors from 3rd trimester to 6 months after delivery. **2)** Identify proteins associated with left ventricular mass, used to assess the cardiac reverse remodelling. **3)** Description of common pathways that involved the identified proteins.	**1)** Echocardiographic and plasmatic characterization of the LV response immediately after passive leg elevation and following 15 minutes in this position in the 1st and 3rd trimesters. **2)** Description of the impact of CVR factors, parity, and age in stretch-induced compliance in pregnancy context.	**1)** Characterization of right cardiovascular remodelling and reverse remodelling from 1st trimester to one year after delivery. **2)** Comparison of right cardiovascular remodelling and reverse remodelling between women with and without CVR factors.	**1)** Identification of the most common indexing methodology for longitudinal cardiac assessment in pregnant population, through systematic review. **2)** Comparison 4 indexing methods to display cardiac remodelling during pregnancy, puerperium and postpartum periods: a) to use absolute values, without indexing; b) indexing to the baseline BSA (before pregnancy); c) using allometric indexes; or d) indexing to BSA measured at the same day of cardiac assessment in an illustrative example.
Outputs	***The PERInatal MYocardial Remodelling (PERIMYR) cohort study protocol: a prospective study of cardiac remodelling and "recovery" in pregnancy as a model to understand the impact of comorbidities in cardiac remodelling and reverse remodelling.*** Revista Portuguesa de Cardiologia (2023) doi:10.1016/j.repc.2022.08.015	***Cardiovascular risk factors during pregnancy impact the postpartum cardiac and vascular reverse remodeling.*** American Journal of Physiology Heart and Circulatory Physiology (2023) doi:10.1152/ajpheart.00200.2023	***The extent of postpartum cardiac reverse remodeling is reflected in urine proteome.*** Under second review at Journal of Proteome Research	***Stretch-induced compliance mechanism: response of physiological cardiac hypertrophy induced by Pregnancy.*** Submitted to Acta Physiologica	***Cardiovascular remodelling and reverse remodelling during pregnancy and postpartum: looking at the right side of the heart.*** Ready to submit	***The Impact of Echocardiographic Indexation to Evaluate Cardiac Reverse Remodelling throughout Pregnancy and Postpartum.*** Revista Portuguesa de Cardiologia (2023) doi: 10.1016/j.repc.2023.04.014

In our cohort study, cardiovascular risk factors presented a major impact on pregnancy-induced cardiac (reverse) remodelling, less evident in the right ventricle. Indeed, women with cardiovascular risk factors have demonstrated more dramatic cardiovascular adaptations during gestation despite optimal management of glucose, blood pressure and weight gain. In the present thesis, arterial hypertension arose as a shared predictor of cardiac and vascular remodelling and RR, being associated with lower LV mass regression and higher aortic stiffness after delivery. The persistence of this RR pattern may lead concentric LV remodelling or hypertrophy development at the long-term, which, combined with a subsequent increase of LV filling pressures, reveals a higher propensity to HFpEF diagnosis (2). Interestingly, in the PERIMYR cohort, C-reactive protein and ST2/IL33-receptor were identified as potential biomarkers of postpartum hypertrophy reversal, similarly to what has been shown for HFpEF (232, 233). This finding is in line with our previous transcriptomic study that showed an up-regulation of inflammatory pathways in aortic debanded mice with a worse pattern of RR (234). Some urinary proteins (namely, complement C3, fibronectin, and serotransferrin) identified in our cohort sample were also associated with worse LV mass regression after delivery, which is involved in the regulation of insulin-like growth factor transport and uptake by IGF binding proteins pathway. Consistently, the concentration of IGF-1 in urine samples was associated with low LVM regression after delivery. This pathway has also been described as critical in HFpEF (235-237). Indeed, Faxén *et al.* reported increased concentrations of insulin-like growth factor 1 and IGF-binding protein 1 in HFpEF patients' serum (235).

The longitudinal analysis of postpartum cardiovascular RR evidenced that the evaluated functional and anatomical parameters recovered to pre-pregnancy values between 6 to 12 months postpartum, despite their significant regression as soon as 1 month after delivery. These findings were supported by the significant reduction of some plasmatic biomarkers, such as troponin I, ST2/IL33 receptor, C-reactive protein, procollagen I C terminal propeptide, relaxin-2 and plasminogen activator inhibitor type 1 from 3rd trimester to 6 months after delivery.

Regarding the mechanism of stretch-induced compliance (SIC) evaluated in a sample of PERIMYR, we trust that the presence of higher LV stiffness in 3rd trimester, supported by elevated procollagen I C terminal propeptide levels and diastolic impairment, may have limited the additional expansion of LV end-diastolic volume 15 minutes after acute preload increase. Concomitantly, a significant reduction of E/e' (less than reported at the rest) was observed, leading to a stroke volume increase and, consequently, LV elastance reduction. Indeed, SIC-responsed seemed to be exacerbated in 3rd trimester when compared to 1st trimester. The

simultaneous absence of significant variation of natriuretic peptides after 15 minutes of passive leg elevation suggested that it may not be a crucial player in cardiac response to AVO. Pregnancy-derive neuroendocrine players, such as estrogen, progesterone, relaxin and oxytocin, whose diuretic, vasodilator and direct myocardial effects may surpass those induced by natriuretic peptides (11, 205-207). Indeed, the stretch-induced compliance mechanism was not compromised in physiological cardiac hypertrophy induced by pregnancy, being the LV response in 3rd trimester distinct to the 1st trimester and influenced by cardiovascular risk factors.

Considering the longitudinal nature of the PERIMYR cohort study and echocardiography guidelines that suggested normalizing LVM for body size, we questioned ourselves about the best indexation method to compare PERIMYR's groups. By using a within-subjects design, we showed that indexing echocardiographic parameters to the pre-pregnancy body surface area or the use of allometric indexes yielded a higher magnitude of effect compared to the indexation methods currently used (index to body surface area measured at each evaluation moment) and an equal effect to not indexing. Indeed, some authors have been using the allometric coefficient 2.7 to index LV mass because it is more sensitive for the identification of LV hypertrophy, allowing to study LVM progression longitudinally during normal pregnancy as well as to compare pregnant-women with different cardiovascular risk backgrounds (33, 63).

Main Conclusions

The main conclusions of the present thesis are:

- The cardiac reverse remodelling was characterized by significant regression of LV hypertrophy and improvement of diastolic function associated with reduced left atrial volume as soon as 1 month after delivery. In parallel, a significant increase in arterial stiffness and systemic vascular resistance was observed. Although this significant recovery occurred in early postpartum, the echocardiographic parameters only returned to baseline values between 6 to 12 months after delivery.
- The cardiovascular risk factors significantly affect cardiac and vascular remodelling and RR. Indeed, arterial hypertension was a shared predictor of cardiac and vascular remodelling and RR, evidencing lower LV hypertrophy regression after delivery. This fact highlights the importance of extending the follow-up after delivery in women with cardiovascular risk factors, in line with the latest American Heart Association Recommendations (2021).
- Significant impact of cardiovascular risk factors was also displayed in SIC-response following acute volume overload (AVO). In addition, a distinct functional response to SIC

was observed between 1st and 3rd trimesters. Despite the left ventricle of 3rd trimester pregnant women showing a structural limitation to dilate and accommodate increased volume upon AVO, its physiological hypertrophy did not compromise the SIC-mechanism, suggesting being exacerbated.

- Curiously, the cardiovascular risk factors (including arterial hypertension, gestational diabetes, obesity, and smoking habits) seemed to have a minor impact on right cardiovascular remodelling and reverse remodelling. Cardiovascular risk factors imposed a delayed recovery of right atrial and ventricular strain, combined with reduced values of E/A ratio, suggesting a diastolic function impairement, although all the measures parameters were within the physiologic interval.
- Regarding plasmatic biomarkers, low ST2/IL33 receptor and high C-reactive protein levels were associated with worse LV mass regression.
- Urinary proteins involved in pathways, such as regulation of insulin-like growth factor transport and uptake by IGF binding proteins, platelet activation, signalling and aggregation and immune system, were also independently associated with LV mass regression. Indeed, the concentration of IGF-1 in urine samples was associated with low LVM regression after delivery.
- Considering that echocardiography guidelines suggest normalizing LVM for body size, we verified that the indexation to the pre-pregnancy body surface area (BSA) or use of allometric indexes yielded a higher magnitude of effect compared to the already used indexation (BSA measured at each evaluation) and an equal effect to not indexing.

Limitations and future prespectives

The limitations of the studies presented in this thesis were the small sample size and the higher non-attendance rates of women with cardiovascular risk factors compared to healthy participants. We cannot neglect the negative impact of COVID-19 pandemic on our cohort, which led to a considerable number of participants dropping out the follow-up evaluations, due to circulation restrictions, recommendations from the Directorate General of Health and participant anxiety.

However, further updates of these studies can be published considering our effort to prolong the recruitment and expand PERIMYR cohort and follow-ups. Thus, future studies are warranted to clarify:

- The impact of each cardiovascular risk factor, taking into account its severity in cardiac remodelling and RR, as well as the influence of pharmacological therapy administered

in these processes (i.e., insulin versus metformin versus both in case of gestational diabetes);

- Exploring the mechanisms of the most relevant biological pathways associated with the proteins identified in urine as being associated with LVM regression;
- Exploring oxytocin effect in cardiac remodelling and RR.
- The impact of lifestyle factors, such as physical exercise, nutrition and chronic stress, in cardiac remodelling and RR.

In addition, we trust that by increasing the number of participants with cardiovascular risk factors, mainly with chronic or gestational arterial hypertension, and keeping the cardiovascular assessments long-term, repeating them in intervals of 10 years, we can measure the incidence of HFpEF in the PERIMYR prospective cohort and consequently explore the potential echocardiographic, plasmatic or proteome predictors from cardiovascular remodelling during pregnancy and early reverse remodelling processes after delivery. This may allow us to find the predictive pattern of cardiovascular remodelling in pregnancy for HFpEF.

Chapter VI: Bibliography

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Supplement I

Mechanisms of myocardial reverse remodelling and its clinical significance: a scientific statement of the ESC Working Group on Myocardial Function

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Abstract

Cardiovascular disease (CVD) is the leading cause of morbimortality in Europe and worldwide. CVD imposes a heterogeneous spectrum of cardiac remodelling, depending on the insult nature, i.e., pressure or volume overload, ischaemia, arrhythmias, infection, pathogenic gene variant or cardiotoxicity. Moreover, the progression of CVD-induced remodelling is influenced by sex, age, genetic background and comorbidities, impacting on patients' outcomes and prognosis.

Cardiac reverse remodelling (RR) is defined as any normative improvement in cardiac geometry and function that rarely occurs spontaneously or is driven by therapeutic intervention. While RR is the outcome desired for most CVD treatments, often, they only slow/halt its progression or modify risk factors, calling for novel and more timely RR approaches. Interventions that trigger RR depend on the myocardial insult and include drugs (RAASi, β-blockers, diuretics and SGLT2i), devices (CRTs, VADs), surgery (valve replacement, CABG) or physiological responses (deconditioning, postpartum). Subsequently, cardiac RR is inferred from the degree of normalisation of left ventricular (LV) mass, ejection fraction (EF) and end-diastolic/end-systolic volumes. However, strategies to achieve cardiac recovery, predictive models of RR extent or even clinical endpoints that allow to distinguish complete from incomplete RR or adverse remodelling objectively remain limited and controversial.

This scientific statement aims to define RR, clarify its underlying (patho)physiologic mechanisms and address (non)pharmacological options and promising strategies to promote RR, focusing on the left heart. We highlight RR and cardiac recovery predictors and review the prognostic significance/impact of incomplete RR/adverse remodelling. Lastly, we present RR animal models and potential future strategies under preclinical evaluation.

Keywords: myocardial remodelling, reverse remodelling, adverse remodelling, cardiac recovery, physiologic remodelling, heart failure

Introduction

Over 6 million new cases of cardiovascular disease (CVD) are reported every year, leading to a prevalence of 11 million patients in Europe(238). Cardiac diseases lead to remodelling, a combination of geometric and functional alterations imposed by pathophysiologic stimuli (ischaemia, haemodynamic load, neurohumoral activation and others)(239, 240), which, considering the limited regenerative capacity of this organ, may progress to heart failure (HF). Cardiac remodelling is a multicellular process that involves different myocardial cell types and matrix (ECM) and encompasses molecular to organ level adaptations. As the heart remodels, important cellular (e.g., hypertrophy, excitation-contraction coupling, inflammation, cell-survival signalling and mitochondrial disturbances) and ECM modifications (e.g., fibrosis) impact remodelling progress and severity(239).

The term "reverse remodelling" (RR) is defined as the result of any intervention that can chronically reverse myocardial remodelling, either by pharmacological treatment(241), interventional/surgical (e.g., ventricular assist devices (VADs), revascularisation, resynchronisation or valve surgery)(242) or after certain physiologic events (partum or significant weight-loss)(243).

An intriguing aspect of RR is the broad spectrum of ventricular responses to a given intervention. While some patients progress towards complete RR or cardiac recovery, others present incomplete RR (limited functional and structural recovery) or further worsening of cardiac function regardless of the intervention (adverse remodelling, AR). The underlying reasons for such diverse outcomes often remain unclear. Indeed, different factors influence the extent of the RR and patients' prognosis, including hypertension, coronary artery disease (CAD), obesity, diabetes mellitus (DM), ageing, sex, genetic risk, lifestyle (smoking habits, alcoholism, nutrition and sedentarism) as well as the degree of systolic and/or diastolic dysfunction, hypertrophy and fibrosis before intervention(244, 245). In clinical practice, changes in ejection fraction (EF), LV end-diastolic/end-systolic volumes, mass and sphericity index are often used as surrogates for remodelling or RR(243, 246-248). "Myocardial recovery" is the desirable goal of RR and describes a sustained, favourable clinical response associated with lower long-term morbimortality, normalization of cardiac biomarkers, increased exercise tolerance(242) and no prospect of future HF(249) (**Figure 1**).

This scientific statement aims to: 1) harmonise the definition of RR and its underlying mechanisms; 2) discuss RR pharmacological options; 3) pinpoint predictors of RR and cardiac

recovery; 4) highlight the prognostic significance/impact of incomplete/adverse RR and 5) review animal models of RR.

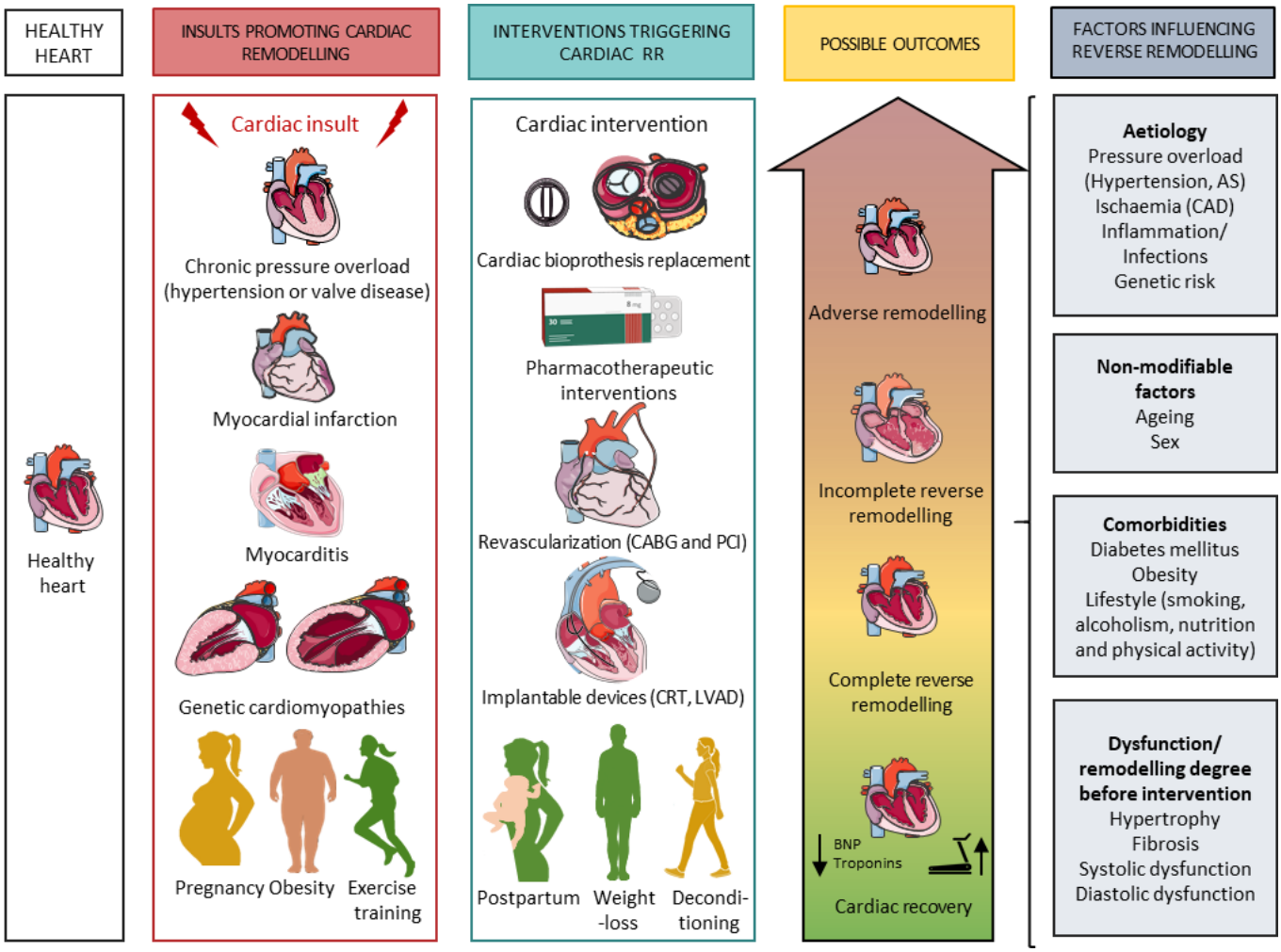

Figure 1: Graphical abstract representing pathologic and physiologic cardiac remodelling triggers, interventions that may lead to reverse remodelling, and factors impacting its trajectory.

1. Interventions that trigger reverse remodelling

The current knowledge on the extent of cardiac (reverse) remodelling is mostly derived from imaging techniques, such as echocardiography and cardiac magnetic resonance (CMR). They measure cardiac chamber dimensions, left ventricle mass (LVM) and functional parameters, such as LVEF and myocardial strain. Compared to echocardiography, CMR provides higher accuracy, reproducibility, spatiotemporal resolution, and tissue characterisation. For instance, T1 mapping estimates interstitial fibrosis by quantifying extracellular volume fraction, while contrast-enhanced CMR with late gadolinium enhancement (LGE) is able to measure focal fibrosis (scarred

myocardium)(250-252). Importantly, cardiac imaging parameters may predict RR/AR after an intervention/surgery alone or combined with other baseline variables, such as circulating biomarkers of cardiac stress or myocardial remodelling (reviewed elsewhere(253, 254)), that can improve the accuracy of predictive models. The clinical significance of predictors of RR and its assessment will be discussed in the following subsections.

1.1. Pressure overload relief-induced RR

Aortic valve stenosis (AS) remains the primary valve disease requiring intervention (transcatheter aortic valve replacement - TAVR) or surgical aortic valve replacement (SAVR) in Europe(255). Restricted aortic valve opening increases LV afterload and triggers myocardial remodelling, characterised by hypertrophy, reactivation of the foetal gene programme and interstitial fibrosis(256). Aortic valve replacement (AVR, hereupon used interchangeably for TAVR/SAVR) promotes myocardial RR, including normalising hypertrophy, diastolic function and molecular remodelling. Diastolic dysfunction, amelioration depends on decreasing/delaying the progression of interstitial fibrosis (matrix metalloproteinases, MMPs, overcoming the activity of tissue inhibitors of metalloproteinases, TIMPs), collagen isoform switch (I, stiffer, to III, more compliant) and the restoration of the active relaxation mechanisms (higher expression of SERCA2a and other calcium handling proteins)(257-259). The extent of post-AVR RR largely depends on how far the remodelling has advanced. A complete correction of LV afterload at the valve and arterial levels is critical(260, 261). Indeed, uncontrolled hypertension, cardiac amyloidosis or prosthesis/patient mismatch increases the risk of incomplete-RR/AR(262, 263). Even for similar afterload, hypertensive AS patients show worse RR as assessed by valvulo-arterial impedance (measuring the cumulative valvular and arterial overload), highlighting the role of neurohumoral activation(263).

Pre-existing DM was found to be an independent predictor of increased stiffness and hypertrophy one year after AVR(263, 264), as demonstrated by inadequate LVM regression and higher cardiomyocyte passive force and interstitial fibrosis in diabetic AS patients(265). In turn, obesity increases the risk of prevalent post-AVR LV hypertrophy, potentially induced by cardiac steatosis(266). Notwithstanding, studies reporting the association between obesity and post-AVR survival are conflicting, with some advocating that patients with more severe obesity display higher survival (the so-called "obesity paradox")(267), others claiming that overweight, but not obese, patients are more protected(268) and others rebutting any protection(269). Concerning CAD, non-revascularized patients with moderate coronary atherosclerosis present poorer RR 3

years after AVR, as shown by lower reduction of LVM, LV diastolic dimension, posterior wall and interventricular septum thicknesses(270). Besides the slower regression of the LVM, the mechanical performance of AS+CAD subpopulations is overall ~50% lower than that of AS subjects, as assessed by CMR-tracked intramyocardial circumferential strain(271). Age and sex have a controversial impact on RR. While a systematic study found no influence on LVM regression(272), there is evidence that hypertrophy regression can be faster in women(273) and post-AVR survival is expectedly lower in older patients(274).

As in AS, the long-term exposure to haemodynamic stress induced by arterial hypertension leads to changes in LV shape, size or function(275, 276) and eventually HF(275, 277, 278). These modifications are more dramatic when combined with other risk factors, usually resulting in additive hypertrophic effects(279, 280). Specific drugs impact the long-term RR and prognosis of hypertensive patients. Such drugs induce RR resembling that caused by AVR by acting at three different levels: preventing cardiomyocyte hypertrophy and death for preserving cardiac function, reversing interstitial alterations (inflammation and fibrosis) to reduce stiffness, and boosting coronary angiogenesis to improve oxygen and nutrition supply(276, 281, 282) (**Table 1**). For instance, the antifibrotic effect of beta-blockers in hypertensive patients is controversial(283, 284), in part related to the type of beta-blocker used(285). Reports on renal sympathetic denervation show a beneficial impact on abnormal proteolytic activity, cardiovascular remodelling and ECM turnover in refractory hypertension(286-288).

For AS or systemic hypertension, LVM regression is the preferred RR endpoint evaluated. Higher baseline LVM has repeatedly proved to independently predict LVM regression after AVR(263, 289, 290), reflecting a higher potential to reverse hypertrophy despite existing comorbidities known as negative predictors of RR(263, 266, 291). In AS, pre-AVR fibrosis and valvulo-arterial impedance correlate and predict LVM regression(260, 261). A slower normalisation of LV geometry and worse diastolic function is observed in patients with more fibrosis(292), and LVM regression is greater when fibrosis is lower (no LGE)(293). Diastolic dysfunction triggers left atrial (LA) remodelling with increased intracavitary pressure, being considered an independent predictor of post-AVR systolic dysfunction(294) and worse global peak atrial longitudinal strain 3 months after AVR(295). Vice-versa, a reduced baseline atrial strain may also jeopardise diastolic function normalisation, leading to higher post-AVR left atrial pressure(296). Interestingly, myocardial strain in any orientation (radial, circumferential, and longitudinal global) predicts LVM regression after AVR(297). The usefulness of molecular markers to predict post-AVR RR has been shown, for instance, by García *et al*., who reported plasma miR-133a as a positive predictor of LVM reduction 1-year post-AVR(298).

Early identification of patients with limited post-AVR RR, i.e., presenting with marked residual hypertrophy, LA enlargement and diastolic dysfunction, is critical due to their worse outcome (hospitalisation and death). For instance, post-AVR residual hypertrophy is associated with non-fatal HF-induced hospitalisation, valve reintervention, myocardial infarction (MI), complete atrioventricular block and LV outflow tract obstruction(246, 299). When sided with LA dilation, LV hypertrophy 1-year after surgery was associated with major adverse cardiac and cerebrovascular events and higher mortality at 3-years(300). A larger and longer (>9-years follow-up) study using the Japan registry J-PROVE-Retro confirmed that patients with increased hypertrophy and LA dilation 1-year after AVR were more likely to be hospitalised due to HF or at higher risk of cardiac death(301).

1.2. Post-ischemic RR and cardiac rehabilitation

Ischaemia may promote cardiac remodelling via different mechanisms. First, chronic ischaemic disease without acute myocardial infarction (AMI) promotes ischaemic but viable cardiac tissue with chronically depressed contraction, known as hibernating myocardium(302). In this case, depending on the number of viable segments, myocardial revascularisation can improve regional and global LV function, including reverse LV dilation(303), lower the incidence of cardiac events(304) and improve symptoms, LV geometry and EF. However, trials investigating RR and the outcome after revascularization of CAD ischemic patients led to disappointing results(305). Secondly, acute ischaemia in AMI induces myocardial necrosis and stunning(306), i.e., viable myocardium slowly recovering from ischaemia and characterised by reversible contractile and biochemical dysfunction(302) (307). The mechanisms underlying reperfusion injury include oxidative stress and reduced calcium responsiveness. This energy deficit results in transient mechanical dysfunction(302) that usually resolves spontaneously, resulting in global LV function recovery 3 months post-AMI(308). Yet, the temporary dysfunction associated with stunning can result in life-threatening post-AMI events. Therefore, in addition to the fastest-possible revascularisation, different drugs have shown potential to accelerate, recover or prevent myocardial stunning (**Table 1**). Thirdly, the substantial loss of viable myocardium after AMI can lead to AR, almost invariably progressing to HF(303). It involves cardiomyocyte hypertrophy, replacement (scarring) and interstitial fibrosis, and variable degrees of LV dilation of both infarct and remote zones, with consequent dysfunction and bad prognosis(248, 309). Drugs and devices (e.g., LV-assist devices) have been evaluated for their efficacy in preventing or reversing AR after MI(310, 311) (**Tables 1 and 2**). Particularly, combined therapies of ARNI or ACEi/ARBs, β-Blockers,

MRA or SGLT2 inhibitors) are the preferred strategy to restrain LV AR(303, 310-312). Notwithstanding, these drugs are non-specific for mechanisms underlying postischemic RR and their selection might change depending on the RR stage. Later, scar maturation and contraction set the stage for remodelling where remote, viable myocardium has to maintain cardiac output and adapt to altered loading conditions. In each of these stages, different mechanisms contribute to a very dynamic remodelling. Thus, targeted and phase-specific therapies may be needed. E.g., early anti-inflammatory treatments to address wound healing and scar maturation (**Table 2**) in addition to standard HF therapies (**Table 3)**.

The presence of a transmural ST-segment elevation myocardial infarction (STEMI), a large infarct or myocardial-damaged area, microvascular obstruction, intramyocardial haemorrhage and advanced age are the main risk factors for subsequent higher LV systolic volume(313) and AR(247), without impact of sex(309). **Figure 2** depicts the predictors of RR or AR. Identifying patients with RR/AR after percutaneous coronary intervention (PCI) is important due to the different outcomes. A sub-analysis of the PRESERVATION I trial demonstrated that STEMI patients with increased LVEF (RR) one month after PCI have lower rates of recurrent MI, hospitalisation and death 1-year post-intervention(314). In contrast, STEMI patients showing post-PCI AR (≥20% increase in LVEDV) have higher HF-hospitalization(315). Furthermore, when combined with LVEF impairment, a post-PCI AR at 6-months (≥20% increase in LVEDV) reduced survival and event-free survival rates in a median follow-up of 76 months(316). Circulating non-coding RNAs also bear prognostic value(253, 254), such as miR-1254, which predicts LV volume and EF changes at 6-months after PCI, even after adjustment for clinical, imaging variables, hs-cTNT and NT-proBNP(317).

Cardiac rehabilitation programs based on exercise training, the fifth pillar of HF management, have been shown to induce LV RR, characterised by reduced LV volumes and increased EF in patients after AMI, preventing HF progression(318-320). The impact of exercise depends on its type, duration and timely onset(321). Aerobic training is known to improve diastolic function and Ca^{2+}-handling(321). It promotes cardiac mitochondrial biogenesis and metabolic remodelling by upregulating AMPK-PGC-1α pathway(322), favouring aerobic glycolysis and fatty acids utilisation, reducing lipid deposition and cardiac lactic acid accumulation(322).

High-intensity training improves cardiac function by activating circulating progenitor stem-cells and the proliferation and differentiation of resident tissue-specific cardiac stem-cells, inducing neoangeogenesis, cardiomyocyte hyperplasia and reducing myocardial wall stress(323). Compared to moderate-intensity continuous training, high-intensity interval training (HIIT)

shows controversial results. On the one hand, it improves echocardiographic parameters of systolic/diastolic function, myocardial work efficiency, global longitudinal LV and LA strain and reduces LVEDD(324). On the other hand, the HIIT-EARLY Randomized Controlled Trial documented a long-term worsening of the global longitudinal strain of optimally-treated patients subjected to HIIT early after acute STEMI, without significant differences in cardiac RR when compared with moderate-intensity continuous training(325). Indeed, moderate-intensity exercise training confers substantial benefits, as shown by an increase in exercise capacity without fluctuations of LVEDD independently of the baseline LVEF of patients who started a 3-months programme after AMI(326). Analogous results were found in patients with reduced ventricular function after MI who performed cycling training for 2 months(327). Despite the absence of LV volume regression, post-MI patients subjected to moderate-intensity treadmill training after a successful primary PCI revealed an improvement of LV global and regional systolic function and exercise capacity(328). Finally, low-intensity training led to a smaller LVEF decline and a significant NT-proBNP decrease in AMI patients submitted to PCI(329).

The low risk of cardiovascular events documented in high, moderate and low-intensity aerobic exercise demonstrated the safety of these cardiovascular rehabilitation programs(330). Still, enlarged LV cavity and augmented percentage of abnormal wall motion in MI after cardiac rehabilitation remained a predictor of unfavourable long-term prognosis associated with LV dilation(331).

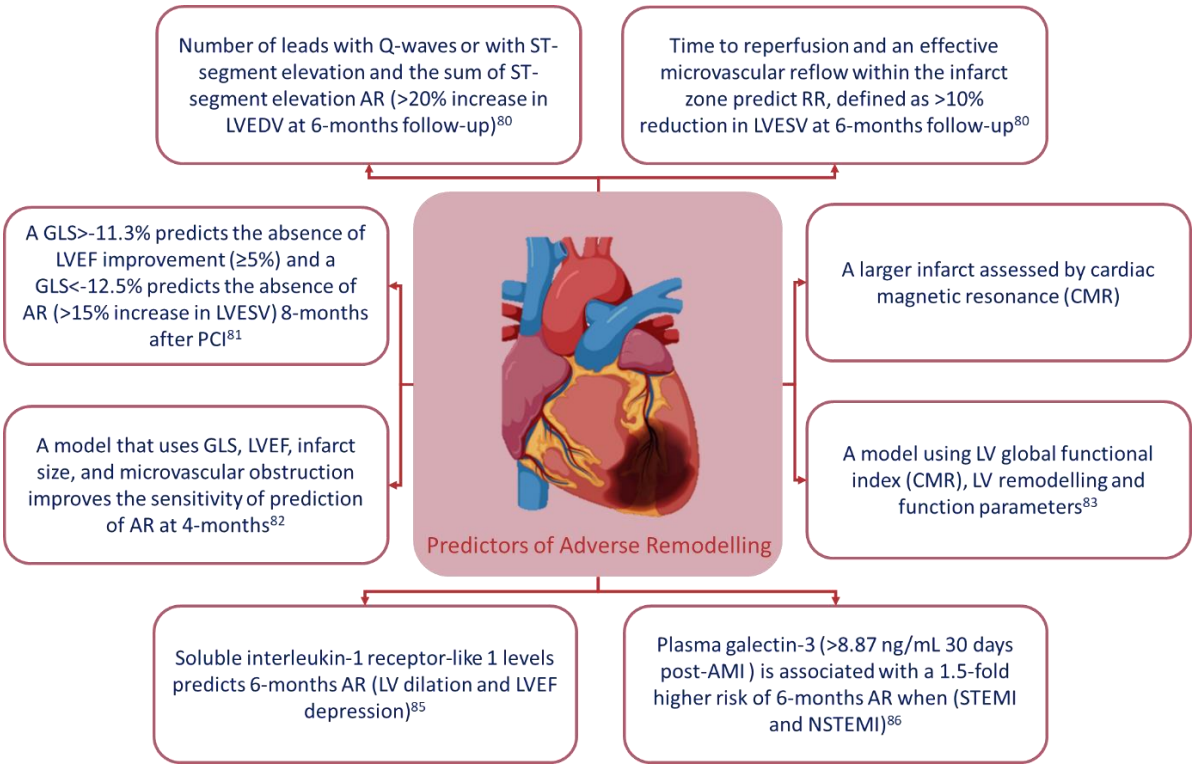

Figure 2. Predictors of post-ischemic adverse remodelling.

1.3. Immunosuppressive therapy-induced RR in myocarditis and inflammatory conditions

Myocardial inflammatory infiltration may lead to acute myocarditis (AM) and/or chronic inflammatory cardiomyopathy (CIM), a multifactorial process by which predisposition (e.g., genetic and autoimmune conditions) and other triggering factors, such as pathogens or cardiotoxic drugs, ethyl or recreational drugs can contribute to tissue inflammation and injury(332). Pathogens, such as viruses, may directly infect cardiomyocytes (enteroviruses) or indirectly promote myocardial dysfunction via infection of non-cardiomyocyte cells (e.g., Parvovirus B19 infection of endothelial cells)(333). Acute and massive inflammation lead to cardiomyocyte damage (e.g., in giant cell- or lymphocytic myocarditis), a Dallas criteria used to diagnose myocarditis histologically. These conditions require prompt and potent treatment that leads to a fast recovery but impose a dismal prognosis if left untreated. More gradually developing chronic inflammatory cardiomyopathy may still respond to immunosuppressive therapies with varying outcomes. As revised in Lake Louise criteria(334), imaging (CMR with LGE and T1 and T2 mapping) and endomyocardial biopsy analysis (histology, immunohistochemistry, pathogen detection) are required to stratify patients and select appropriate patients for immunosuppressive therapies. However, even gold-standard imaging approaches (CMR) have limited sensitivity for early detection before overt dysfunction or morphological remodelling is

observed, as in the case of an immune checkpoint inhibitor (ICI)-induced myocarditis, where EF assessed by echocardiography or CMR may be normal and tissue characterisation unremarkable in early stages(335). According to previous registries, AM is a cardiac condition affecting mostly young patients (30-45 years-old) and men (60-80%)(336). Regarding the predictors of RR outcomes, an extensive compilation has been described elsewhere(336), but reduced EF, presence of LGE/oedema, higher NYHA class, type of myocarditis (e.g. GCM, lymphocytic, eosinophilic), genetic predisposition and signs of myocardial inflammation on histology are generally accepted as independent predictors of AR.

1.4. Reverse remodelling in genetic cardiomyopathies

Genetic cardiomyopathies encompass a heterogeneous group of heart muscle diseases and represent a common cause of cardiac dysfunction, acute cardiac arrest and HF. While dilated (DCM) and hypertrophic cardiomyopathy (HCM) are the most prevalent conditions (up to 1:250-1:1000)(337), arrhythmogenic right ventricular (ARVC), non-dilated left ventricular (NDLVC) and restrictive (RCM) cardiomyopathies are rare(338). Targeted therapies for cardiomyopathies are slowly entering the clinic but are mostly in the experimental stage. Most patients receive standard HF treatment, including RR-inducing classical HF drugs, such as β-blockers (**Table 2**), which induce RR in some patients, while others respond poorly, requiring a pacemaker or an implantable cardioverter *defibrillator (*ICD). The predictors, impact of comorbidities, age and sex of LVAD-related RR pattern are detailed in section 2.5. Myocarditis with increased levels of hsTn and arrhythmias but normal EF may also occur during the incompletely understood “hot phase” of desmoplakin cardiomyopathy(339). Approximately 30% of patients with NDLVC (arrhythmogenic) present the potentially pathogenic desmoplakin (gene), which is associated with a constellation of chest pain, troponin release, and ECG abnormalities with coronary arteries(340).

Besides classical HF pharmacotherapy(341, 342), drugs targeting myosin have shown promising results in cardiomyopathies (**Table 3**). Myosin activators, such as omecamtiv mecarbil, have shown the potential to increase contractility in DCM. Conversely, myosin inhibitors, such as mavacamten, have demonstrated beneficial effects in obstructive HCM(343). Both have been approved or are close to approval in many countries. Other strategies are still in the experimental phase. For instance, proteasome inhibition remains an exciting option for certain genetic cardiomyopathies(344-346), although long-term RR effects and its efficacy in pre-symptomatic mutation carriers remain unproven. Recent reports have uncovered pathomechanisms through

gene editing to correct specific genetic variants and thereby prevent/treat cardiomyopathy. CRISPR/Cas9 or antisense oligonucleotides can target cardiac function (HCM-induced by sarcomere gene mutations)(347, 348) or mitochondrial metabolism(337, 349), preventing pathological remodelling or promoting RR.

1.5. Device-induced RR

Despite the aetiology, when AR progresses towards end-stage HF due to genetic cardiomyopathies, myocarditis or ischaemia, heart transplantation is the final option(336), with device therapy (LVADs) becoming an increasingly common alternative to induce RR (**Table 1**). While LVADs have been used in advanced HF patients with significant ventricular dilation and dysfunction, CRTs are useful in HFrEF patients with dyssynchrony. Currently, the criteria used to assess RR or cardiac recovery after CRT or LVAD are predominantly based on LVEF improvement, LV dilation (LVEDD) reduction, mass and cardiomyocyte size normalisation and increased myocardial contractility.

LVADs are used in end-stage HF as destination therapy or bridge to transplant, and complete recovery in this setting is rare (350). Only a small proportion of advanced HF patients achieve cardiac recovery after LVAD implantation leading to successful explantation, the majority present incomplete RR and remain stable(351). Until now, despite LVEF strongly depending on LVAD flow, the clinical differentiation between complete (recovered) and incomplete RR is based on LVEF changes. Nevertheless, several cellular and structural changes have been reported(352, 353). While improved calcium handling is reported during LVAD-induced RR, regardless of an ischaemic/non-ischaemic aetiology, the regression of cardiomyocyte hypertrophy and ECM turnover is not consistently described(354). In response to LVAD support, the collagen turnover and ECM volume present a slow, biphasic pattern(355). Klotz et al. showed an increase in type I and III collagen and the I/III ratio even 4-6 months after LVAD implantation, favouring myocardial stiffness(356). Additionally, decreased MMP-1/TIMP-1 and MMP-1 and MMP-9 levels combined with increased angiotensin I and II suggests ECM preservation after LVAD therapy(356).

The implications of post-LVAD RR in terms of patient outcomes are less explored compared to other interventions. The duration or type of LVAD support, age and HF aetiology (ischaemic or idiopathic cardiomyopathy) all influence the RR process(357). For instance, patients submitted to mechanical support for shorter periods and experiencing maximal cardiac recovery had a lower probability of HF relapses(358-360). Conversely, prolonged mechanical support may lead to cardiac function deterioration(358-360). Furthermore, the duration of HF history and its

aetiology were deemed the main predictors of myocardial recovery(352). In this sense, the INTERMACS Cardiac Recovery Score (I-CARS) was probably the largest study (>14,000 patients) aiming to build a predictive model of myocardial recovery, only observed in 1.3% of all patients. With an excellent performance (AUC=0.94) in the validation phase, this model predicts myocardial recovery (LVAD explantation) based on the combination of 6 independent variables: age <50 years, non-ischaemic aetiology, recent diagnosis (<2 years), absence of implantable cardioverter defibrillator, creatinine ≤1.2mg/dL, and LVEDD <6.5cm(360). However, follow-up of "recovered" patients was not available in 89% of the cohort. Molecular marker-based models have also been described, such as a 2-cytokine model (IFN-? and TNF-α), which predicts "responders" to LVAD (final LVEF≥40% or between 35%-40% when LVEF improvement ≥50%) with an AUC>0.90(361).

Predicting the response to LVAD may help anticipating adverse events and the need for coadjuvant therapies. Patients with LVEF improvement of ≥40% within 2-years of the implantation had a lower incidence of a composite endpoint which included HF-driven hospitalisation and death(362). Survival was also superior in LVAD patients with pVO_2 of 12-14 mL/min/kg and VE/VCO_2 slope <35 on cardiopulmonary exercise testing(363). Previously, others showed that incomplete left heart unloading (higher LA pressure and shorter mitral deceleration index) and right ventricular dysfunction (lower tricuspid lateral annulus velocity) measured 1-month post-LVAD were associated with a worse mid-term (90-days) outcome, characterised by NYHA class ≥III, HF-hospitalisation, and death(364).

In contrast with LVADs, CRT devices are implanted to achieve RR. The extent of myocardial RR is often measured as the reduction in LV volume. Maximal cardiac recovery should occur within the first 6 months, but it depends on the preoperative myocardial state. For instance, advanced myocardial AR marked by LV and/or LA dilation reduces the chances of a "super-response" to CRT: a combination of NYHA class reduction with preserved systolic function(365, 366). Moreover, a longer HF history may also preclude the success of post-CRT RR. Conversely, while the global contractile reserve is preserved, a response to CRT is expected (LVEF improvement and/or decrease in LVESV), provided that the myocardial substrate is electrical stimuli-responsive(367). Many factors can predict the response to CRT, like the combination of baseline LVEDD ≤71mm and QRS ≥170ms, which predict a response defined by a 6-months improvement of LVEF ≥5% and NYHA class ≥1 (AUC>0.83)(351). In larger studies, female sex, non-ischaemic aetiology, higher baseline LVEF, and longer QRS were all found to be independent predictors of CRT super-responders, defined by LVEF>50% at follow-up (median of 2.8 years)(368). Conversely, prior episodes of ventricular tachyarrhythmias, HF-hospitalisation and non-left bundle branch

block intrinsic QRS pattern are considered predictors of incomplete RR(369). As opposed to LVAD-induced RR, for CRT, age seems not to be a determinant factor(370, 371).

A limitation for RR models' generalisation, and thereby outcomes' prediction after device implantation, is that the extent of RR (fraction of CRT responders and non-responders) depends on the RR evaluation timepoint. Indeed, super-responder patients show lower rate of major adverse cardiac events, including the implantation of cardioverter-defibrillators, HF-hospitalisation or cardiac death(368). Among these, 15% should be classified as "brief responders", as LVESV reduction (≥15%) is temporarily observed at 6-months but not at 1-2 years follow-up. Moreover, a reduction in LVESV after CRT is associated with a lower risk of cardiovascular or all-cause death(372), and the larger the reduction, the lower the mortality, according to a retrospective analysis of the PREDICT-CRT(373). The improvement of GLS, another feature of post-CRT RR, attributed to the recruitment of contractile reserve, is associated with better survival alone or combined with a ≥15% reduction of LVESV(374). A LVEF >35% 1-year after CRT marks a reduction in the risk of sudden cardiac death or the need for ventricular fibrillation treatment(372). Finally, correcting mechanical dyssynchrony at 1-year after CRT is also associated with improved survival(373).

The prediction of a patient's response to CRT might improve the current management of HF. In addition, new innovative modalities in CRT may help improve outcomes. These include: 1) multipoint left ventricular pacing that shows higher impact in cardiac volume regression and LVEF increase compared to biventricular CRT(375, 376); and 2) His-optimized CRT, which can lead to a significant narrowing and often normalization of LBBB, improved LVEF and NYHA class associated with pronounced reduction of LV volumes at least up to 12 months after device implantation(377-379).

Table 1. Device-induced reverse remodelling.

Pathologic condition/ cardiac insult	**Device**	**Mechanism of action**	**Reverse remodelling pattern**	**Refs.**
Moderate-to-severe HF Ischaemic and non-ischaemic dilated cardiomyopathy	Cardiac Resynchronization Therapy (CRT)	Contraction recoordination Optimisation of atrioventricular delay	↑ Ejection fraction ↓ LV end-systolic and end-diastolic diameters ↓ LVESV and LVEDV ↓ LV mass ↓ LA volume	(380-388)

			↓ Mitral regurgitation severity ↓ NYHA class	
Advanced/ end-stage HF Ischaemic and non-ischaemic dilated cardiomyopathy	LVAD	↓ LV load (mechanically) ↓ Neuroendocrine activation (epinephrine, norepinephrine, angiotensin II, and arginine vasopressin levels in plasma) ↓ Myocyte hypertrophy	↑ Ejection fraction ↓ LV end-systolic and end-diastolic diameters ↓ NT-proBNP ↓ Cardiomyocyte hypertrophy - Diastolic amelioration ↓ Indexed left atrial volume ↓ LVESV and LVEDV ↓ Mitral regurgitation severity - Modification of cytoskeletal proteins ↓ Collagen content	(352, 357, 389-396)

Abbreviations: CRT, cardiac resynchronization therapy; HF, heart failure; LA, left atrial; LAVI, LA volume index; LVAD, left ventricular assist device; LV, left ventricle; LVEDP, LV end-diastolic pressure; LVEDV, NYHA, New York Heart Association.

1.6. RR induced by metabolic unloading, weight loss and SGLT2 inhibitors

Obesity and DM are primarily associated with vascular complications that will promote myocardial ischaemia but also with an intrinsic cardiac dysfunction called diabetic cardiomyopathy(397). The prevalence of diabetic cardiomyopathy is higher in women and characterised by worse structural and functional impairment (398, 399). Diabetic patients present two different cardiac phenotypes. A concentric LV remodelling associated with diastolic dysfunction will lead to HFpEF, whereas eccentric LV dilation with systolic dysfunction will lead to HFrEF(400). Both phenotypes are characterised by interstitial and perivascular fibrosis, cardiomyocyte hypertrophy and cell loss. Amongst the complex multifactorial stimuli involved in the development of DCM, cardiac metabolic over-fuelling is critical, leading to the loss of metabolic flexibility, lipotoxicity, glucotoxicity, impaired mitochondrial respiration and insulin resistance(401, 402).

Rapid weight loss resulting from bariatric surgery is associated with reduced cardiovascular mortality in HF and non-HF patients(403, 404). Weight loss is associated with a linear regression of LV hypertrophy independent of age, gender and cardiometabolic risk(405). The decrease in LVM parallels the improvement of LV geometry and diastolic function(406). The improvement appears to be mostly caused by wall thickness reduction without modifications of the end-

diastolic volume(407). However, in a recent study, RR promoted by weight loss after bariatric surgery was reported as incomplete, considering a slight decrease in LVEF and worse diastolic function despite increased LV GLS(407). Besides bariatric surgery, caloric restriction and pharmacological therapies using SGLT2 inhibitors have shown their merits in promoting RR in diabetic HF (**Table 2**).

Caloric restriction, particularly efficient for losing weight, has been used in an animal model of metabolic syndrome to evaluate its action on RR. It attenuates remodelling and diastolic dysfunction in line with reduced fibrosis and oxidative stress(408, 409). When started after myocardial infarction-mediated HF, caloric restriction improves cardiac dysfunction and inotropic reserve in non-diabetic animals(410).

To summarise, the goal of these different clinical, behavioural and pharmacological interventions is to limit cardiac overfuelling that eventually leads to insulin resistance, metabolic inflexibility and mitochondrial and contractile dysfunction, i.e., to metabolically unload the heart(411).

2. Drugs to promote reverse remodelling

2.1. Guideline-recommended drugs

Several guideline-recommended drugs are able to induce reverse remodelling in different pathophysiologic conditions (Table 2).

Table 2. Class I drugs that induce reverse remodelling.

Guideline-recommended drugs	Mechanism of action	Pathologic condition/ cardiac insult	Effect on reverse remodelling	Refs.
AT1 blocker	RAAS inhibition (angiotensin II receptor antagonism; blood pressure reduction)	HFrEF Hypertension STEMI	↓ Hypertrophy ↓ Fibrosis ↑ Coronary flow reserve	(412-417)
Angiotensin receptor neprilysin inhibitor (ARNI)[a]	RAAS inhibition (angiotensin II receptor antagonism; blood pressure reduction) + natriuretic peptides degradation inhibition (neprilysin inhibition) + improving cGMP signalling (neprilysin inhibition)	HFrEF	↓ NT-proBNP ↑ LVEF ↓ Hypertrophy ↓ LVEDVI, LVESVI ↑ Diastolic function (↓ LAVI, ↓ E/e') ↓ NYHA class ↓ Hospitalisation	(418-421)

			↓ Mortality risk	
Angiotensin-converting enzyme inhibitor	RAAS inhibition (the conversion of angiotensin I to angiotensin II is prevented; blood pressure reduction)	HFrEF Hypertension STEMI	↓ Hypertrophy ↓ LVESV and LVEDV ↑ Ejection fraction ↓ Hospitalisation ↓ Mortality risk	(416, 417, 422-427)
β-blockers	β-adrenergic receptors inhibition (inotropic/chronotropic effects, blood pressure reduction, depending on selectivity)	HFrEF Hypertension STEMI Ischaemic and non-ischaemic cardiomyopathy Atrial fibrillation (ventricular rate control)	↓ LVM ↓ Hypertrophy ↓ LVEDV and LVESV ↑ Diastolic function ↑ Ejection fraction ↑ Exercise capacity ↑ Diastolic coronary blood flow time ↑ Myocardial oxygen supply/demand ↓ Adverse events ↓ Mortality risk	(283, 428-445)
Calcium channel blockers	L-type calcium channel inhibition (antiarrhythmic)	Hypertension Hypertrophic cardiomyopathy Atrial fibrillation	↓ LVEDP ↑ dP/dT max ↓ Infarct size ↓ Fibrosis ↑ Septal thickness	(446, 447)
Diuretics	Renal-mediated increase of water and electrolyte excretion	HFrEF HFmrEF HFpEF (congestion) Hypertension	↓ LVMI ↓ LVEDV and LVESV ↓ LAVI ↑ E-wave deceleration time ↓ E/e' ↑ Ejection fraction ↑ Exercise capacity	(448-452)
Mineralocorticoid receptor antagonists	Aldosterone antagonism at its receptors	HFrEF HFpEF STEMI Hypertension (resistant)	↓ LVMI	(453)
Sodium-glucose co-transporter 2 inhibitors	Reduction of the reabsorption of filtered glucose (glycemia control) and sodium + anti-	Type 2 diabetes mellitus HFrEF and HFpEF	↓ NT-proBNP ↑ LVEF ↑ Diastolic function (↓ E/e')	(310, 454-457)

	inflammatory, antioxidative effects, endothelial function improvement, modulation of neurohormonal pathways		↓ LVESV, LVEDV ↓ LVM ↓ LAVI ↓ E wave deceleration time	

[a] May be beneficial in HFpEF patients. Meta-analysis showed that ARNI induced a significant improvement in LVMI and LAV in HFpEF. NT-proBNP was also more reduced when HFpEF patients were treated with LCZ696 than with valsartan. **Abbreviations:** AT1, angiotensin II receptor type 1; BNP, brain natriuretic peptide; dP/dT, rate of pressure development; E/e', ratio of transmitral early filling velocity to early diastolic tissue velocity; HFmrEF, Heart failure with mildly reduced ejection fraction; HFrEF, Heart failure with reduced ejection fraction; HFpEF, Heart failure with preserved ejection fraction; LA, left atrial; LAVI, LA volume index; LV, left ventricle; LVEDP, LV end-diastolic pressure; LVEDV, LV end-diastolic volume; LVEDVI, LVEDV index; LVESV, LV end-systolic volume; LVESVI, LVESV index; LVEF, LV ejection fraction; LVM, LV mass; LVMI, LVM index; NT-proBNP, N-terminal pro-BNP; NYHA, New York Heart Association; RAAS, renin-angiotensin-aldosterone system.

2.2. Preclinical/early clinical evidence for novel therapies directed at reverse remodelling

Promising novel approaches have focused on tackling the hallmarks of AR and eventually inducing RR. Such is the case of RNA-based, anti-inflammatory, myofilamentary or mitochondria-targeting drugs, as depicted in Table 3.

Table 3: Promising new therapeutic options to achieve myocardial recovery in animal models of phase I clinical trials (CT)

	Potential novel targets for RR and preclinical trials	**Mechanism of action**	**Pathologic condition/cardiac insult/animal model**	**Effect on Reverse Remodelling**	**Refs**
Pressure-overload conditions	Treatment with regulatory T cells	Induction of immunosuppressive T-regulatory activity or reduction of proinflammatory T effector lymphocytes.	Mice with aortic banding and angiotensin II-dependent hypertensive mice	↓ Cardiac fibrosis by decreased TGF-β1 activity. ↑ Coronary arteriolar endothelium-dependent relaxation.	(458-460)

	Phosphodiesterase 9A inhibitor (PF-9613)	Inhibition of cGMP hydrolysis (mainly that generated by natriuretic peptide signalling, less than that caused by NO signalling, as for PDE5 inhibitors)	Pressure overload (hypertension and/or aortic valve stenosis mimic)	↓ Hypertrophy ↓ LVESD ↑ Fractional shortening	(461)
	Apelin receptor agonist (BMS-986224)	Activation of the apelin receptor pathway (Gαi activation and β-arrestin inhibition, ERK activation)	Renal hypertension-induced cardiac hypertrophy	↑ Stroke volume ↑ Cardiac output ↔ Hypertrophy ↔ Fibrosis	(462)
	Mocetinostat (MGCD0103)	Inhibition of histone deacetylase class I or IV (broad positive transcriptional effect)	Pressure overload (hypertension and/or aortic valve stenosis mimic)	↓ Hypertrophy ↓ Fibrosis	(463)
	MitoTEMPO	Mitochondrial superoxide and peroxyl scavenger (superoxide dismutase mimetic)	Pressure overload + isoproterenol continuous administration (Non-ischaemic HF)	↓ Cardiac dilation ↑ Fractional shortening	(464)
	JQ1 (Bromodomain and extraterminal inhibitor)	Blockage of the transactivation of specific genes	Pressure overload (hypertension and/or aortic valve stenosis mimic) Acute myocardial infarction (without reperfusion)	↓ Hypertrophy ↓ Fibrosis ↑ LVEF	(465)
Acute myocardial	Galectin-1	Modulation of cell survival and proliferation, control	Acute myocardial infarction	↓ LVESD, LVEDD ↑ Fractional shortening	(466)

		of inflammation and neovascularisation			
	Doxycycline	Bacteriostatic antibiotic which prevents bacteria growth by inhibiting protein synthesis	Acute myocardial infarction (STEMI) and LV dysfunction	↓ LVEDVI ↑ LVEF ↓ Infarct size and severity	(467)
	STING inhibitor	Inhibition of STING palmitoylation and multimerisation, essential for TANK-binding kinase 1 phosphorylation and subsequent type I interferon gene expression	Acute myocardial infarction	↓ Infarct size ↓ Hypertrophy ↑ LV systolic function (fractional area change)	(468)
	NLRP3 inflammasome inhibitor (16673-34-0)	Inhibition of the cell danger-sensor of intracellular (e.g., bacterial or viral infective agent) or extracellular signals (e.g., ischaemia), ultimately preventing cell death	Acute myocardial infarction Doxorubicin cardiotoxicity	↓ Infarct size (reperfusion only) ↑ LV systolic function (fractional shortening) ↓ Fibrosis (doxorubicin only)	(469)
	MicroRNA-144	Modulation of local inflammation (among others)	Acute myocardial infarction (without reperfusion)	↓ Infarct size ↑ LV systolic function (LVEF, fractional shortening) ↓ LVESV, LVEDV ↑ dP/dT_{max}	(470)
	Ischaemic Postconditioning	Mediated mainly through PI3K–PKB/Akt signalling pathway (reducing	Acute myocardial infarction	↓Infarct size	(471-478)

		reactive oxygen species, lipid peroxidation, intracellular and mitochondrial calcium concentrations)			
	Levosimendan	Positive inotropism (Calcium-sensitizing, ATP-dependent potassium channel opener)	Acute myocardial infarction Acute HF Cardiogenic shock	Restoration of ventriculo-arterial coupling, ↑Tissue perfusion Anti-stunning and anti-inflammatory effects	(479, 480)
	Antisense oligonucleotide (GapmeR) for *Wisper*	*Wisper* silencing	Acute myocardial infarction	↓ Hypertrophy ↓ Fibrosis ↓ LVIDd ↑ LV systolic function (LVEF, fractional shortening)	(481)
	Antisense oligonucleotide for miR-132 (antimiR-132, CDR132L)	miR-132 silencing	Acute myocardial infarction Chronic (ischaemic) HF[a]	↓ NT-proBNP ↑ LVEF ↓ LVESVI, LVEDVI ↓ LAVI ↑ dP/dT_{max} ↓ dP/dT_{min} ↓ Hypertrophy ↓ Fibrosis QRS narrowing	(482-484)
Myocardial infarction and Heart Failure with reduced	Neuregulin-1	Activation of the Neuregulin-1/ErbB pathway, inducing multiple protective effects (cardiomyocyte proliferation and	Ischaemic cardiomyopathy HFrEF	↑ LVEF ↓ LVEDV and LVESV	(485, 486)

		hypertrophy, enhanced contractility, reduced apoptosis, angiogenesis)			
	Sodium/hydrogen exchange inhibitor (EMD-87580)	Prevention of intracellular sodium and calcium accumulation and pH regulation	Myocardial infarction	↔ Infarct size ↓ Hypertrophy ↓ LVEDP ↓ Plasma ANP	(487)
	βARKct peptide (gene transfer by adenovirus)	Inhibition of the membrane translocation-activation of GRK2, normalising β-adrenergic receptor signalling	Myocardial infarction	↓ BNP ↓ Hypertrophy ↑ dP/dT_{max} ↓ Fibrosis	(488)
	S100A1 protein (gene transfer by adenovirus)	Regulation of calcium cycling, improving contractility (systolic and diastolic performance) and increase of mitochondrial ATP production	Myocardial infarction and ischaemic HF	↓ Hypertrophy ↑ Ejection fraction ↑ dP/dT_{max} ↓ P/dT_{min} ↓ LVEDP	(489)
	Ataciguat	NO-independent activation of the soluble guanylate cyclase, inducing multiple effects (e.g., vasodilation)	Chronic myocardial infarction and HFrEF	↓ Hypertrophy ↓ LVEDV and LVESV ↑ Ejection fraction ↑ dP/dT_{max} ↓ dP/dT_{min} ↓ LVEDP ↑ Angiogenesis ↓ Fibrosis	(490)

	Ferric Carboxymaltose	Improves iron deficiency	HFrEF with CRT therapy	↑ LVEF ↓ LVESV ↑ Exercise capacity	(371)
	Omecamtiv mecarbil	Positive inotropism (Myotrope: increases cardiac systolic force by selectively binding to myosin and increasing the number of active cross-bridges)	HFrEF	↑ Systolic ejection time (while dP/dt remained unchanged)	(491)
	Danicamtiv (MYK-491)	Positive inotropism (Myotrope)	HFrEF	↑ Stroke volume ↑ Global longitudinal and circumferential strain ↓ LAVI ↑ LA function index	(492)
Hypertrophic cardiomyopathy	Mavacamten (MYK-461)	Negative inotropism (reduces contractility by decreasing the ATPase activity of the cardiac myosin heavy chain)	Hypertrophic cardiomyopathy Obstructive hypertrophic cardiomyopathy	↓ Fractional shortening ↓ Hypertrophy ↓ Fibrosis	(343, 493, 494)
	Aficamten (CK-274)	Negative inotropism (cardiac myosin inhibitor that decreases myosin ATPase activity)	Hypertrophic cardiomyopathy	↓ Fractional shortening ↓ LVIDs ↓ IVRT	(495, 496)
DCM	Antisense oligonucleotide for titin gene	Exon skipping to correct titin reading frame	Dilated cardiomyopathy	Improvement of sarcomere formation ↑ Contractile performance ↑ LVEF	(497)

	CRISPR/Cas9 gene editing of titin	Correction of a titin truncating mutation restoring wildtype titin levels	Dilated cardiomyopathy[b]	↑ Sarcomere number ↑ Contractile performance	(497)
Myocarditis	Interleukin-1β antibody	Interleukin-1β neutralisation to prevent inflammation activation	Coxsackievirus B3 myocarditis	↓ Inflammation ↓ Fibrosis	(498)

[a] A phase 2, multicentre, randomised, parallel, 3-arm, placebo-controlled study to assess the efficacy and safety of CDR132L in HFrEF after MI is ongoing (NCT05350969); [b] probably various cardiomyopathies caused by truncated titin. **Abbreviations:** AMI, acute myocardial infarction; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; dP/dT, rate of pressure development; ERK, extracellular signal-regulated kinase; GRK2, G protein-coupled receptor kinase 2; HFrEF, Heart failure with reduced ejection fraction; IVRT, isovolumic relaxation time; LA, left atrial; LAVI, LA volume index; LV, left ventricle; LVEDD, LV end-diastolic dimension; LVEDP, LV end-diastolic pressure; LVEDV, LV end-diastolic volume; LVEDVI, LVEDV index; LVESD, LV end-systolic dimension; LVESV, LV end-systolic volume; LVESVI, LVESV index; LVEF, LV ejection fraction; LVIDd, LV internal dimension at diastole; LVIDs, LV internal dimension at systole; LVOT, LV outflow tract; NLRP3, NACHT, LRR and PYD domains-containing protein 3; NT-proBNP, N-terminal pro-BNP; NYHA, New York Heart Association; PDE5, phosphodiesterase 5; STEMI, ST-segment elevation myocardial infarction; STING, stimulator of interferon genes; TANK, TRAF family member-associated NF-kappa-B activator.

3. Lessons from reverse remodelling in physiologic conditions

Despite significant differences between pathologic and physiologic cardiac remodelling, "human models" such as the athletes' LV RR after a deconditioning period and the postpartum RR upon delivery are useful to study specific aspects of RR, becoming an appealing animal-free alternative.

3.1. Deconditioning-induced RR

The impact of exercise on the cardiovascular system depends on its frequency, intensity, duration, modality and conditioning volume(499). Prolonged (>6 months), regular and intensive exercise induces physiological cardiac hypertrophy with cardiac chamber enlargement, enhanced myocardial contractility and stroke volume(499). For instance, the athletes' heart rate

declines and diastolic function improves due to increased filling time and venous return(500). It is commonly accepted that endurance and resistance training are associated with eccentric remodelling and concentric hypertrophy despite the similar thickness of the interventricular septum and the posterior wall(500, 501). Sex influences this remodelling, as female athletes show greater enlargement of LV chambers, and males develop higher LV wall thickness and mass(502). Exercise-induced hypertrophy is characterised by cardiomyocyte growth, fibrosis and apoptosis reduction, improved calcium handling, activation of resident cardiac stem cells, increased NO production, angiogenesis, improved endothelial function and antioxidative protection, resulting in preserved heart structure and function(499, 500, 503). The insulin-like growth factor-1 (IGF-1)-PI3K(p110α)-Akt pathway is responsible for this cardiac remodelling course(499, 503). Exercise training also increases the mitochondrial-to-myofibril volume ratio, promoting a more energy-efficient Frank-Starling mechanism(500).

Maron et al. first recognised decreased LV maximum wall thickness and mass after a deconditioning period of Olympic athletes(504). Furthermore, four weeks after marathon completion, LV mass and wall thickness regressed with unchanged chamber volumes and function following (blood) volume unloading(505). This RR was not evident eight weeks after the marathon(505). The delay between the acute blood volume reduction and the Frank-Starling adaptation is possibly explained by weight gain after deconditioning(505). After a deconditioning period of about 5 years, athletes showed decreased LV cavity dimensions and increased chronotropy(506). The duration and the level of the previous exercise practice also modulate the long-term RR, mainly if athletes exceed the upper limits of ventricular morphology for physiological cardiac hypertrophy(500). For instance, after 38 years of deconditioning, former professional cyclists showed slightly larger LVEDV and LVM index than golfers, despite similar interventricular septum thickness(507).

3.2. Postpartum RR

The progressively increased demand of the growing foetus imposes a physiological haemodynamic load on the mother during pregnancy. The preload alterations, combined with increased peripheral resistance and myocardial contractility, induce mild LV hypertrophy to reduce wall stress while maintaining function(508-512). Recent literature suggests physiological concentric rather than eccentric hypertrophy during gestation(17, 58). This seems to be influenced by oestrogens, resulting in Kv4.3 channel downregulation, increased PI3K/Akt activation and stretch-responsive kinase c-Src phosphorylation(22). The latter is also downregulated during AS-induced hypertrophy, being responsible for increased intracellular

calcium and activation of the calcineurin-nuclear factor of activated T-cells (NFAT) pathway(22). NFAT is silenced in pregnancy, preventing a maladaptive hypertrophic response(22). Several hormonal-induced ECM changes are described during pregnancy and postpartum, namely some MMPs/TIMPs(513, 514) and a shift in LV expression of collagens I and III at the late stage of pregnancy(31, 513), whose mechanisms remain unclear(31).

RR begins immediately after delivery, leading to a full recovery of women's hearts, which is characterised by normalisation of global and segmental myocardial performance to its pre-gravid structure, approximately 6 months postpartum(37, 40). The increased levels of BNP and hs-TnI immediately after delivery have been considered the best predictors of LA volume and LVM index, respectively, during postpartum RR(15, 21). Cardiovascular risk factors, such as arterial hypertension, DM and overweight interfere with both pregnancy-induced remodelling and postpartum RR, increasing the risk of future CVD and mortality(72, 84, 113, 115, 118). In the third trimester, obese pregnant women exhibit reduced myocardial performance and increased LVM (concentric remodelling) and lower EF(62), being at risk of developing diastolic and systolic dysfunction(52, 56, 57). Also, in hypertensive pregnant women, a 5-fold rise in relative wall thickness, without a proportional enlargement of LVEDD, predisposes to concentric remodelling(17, 74, 75), which can be aggravated by environmental, genetic, inflammatory and placental factors(75). Despite an apparent preservation of systolic function, diastolic dysfunction is a common finding in gestational hypertension, usually manifested after 20 weeks of gestation and resolved up to 42 days after delivery(74, 118, 143). During RR, diastolic dysfunction usually resolves within two months postpartum(143). *Vasapollo et al.* identified diastolic dysfunction (increased E/e') and concentric hypertrophy before pregnancy(78) and postpartum as the strongest predictors of incomplete RR and early-to-long-term postpartum complications in women with pre-gestational hypertension. Simmons *et al.* disclosed diastolic and systolic dysfunction in women with preeclampsia(88), while normotensive women showed complete RR until three months postpartum(43). In fact, women who develop hypertensive disorders during pregnancy revealed incomplete RR postpartum that includes subclinical cardiac dysfunction, hypertrophy, reduced LV relaxation, and increased peripheral vascular resistance(41, 72, 80, 84, 91).

Gestational diabetes could also interfere with cardiac remodelling and long-term RR. Pregnant women with gestational diabetes, even under optimal glucose management, showed impaired diastolic relaxation and lower LV strain combined with increased LVM compared to healthy pregnant women; despite an improvement, these changes persisted for at least 6 months after delivery(64, 65, 68). Indeed, in the 20-years follow-up CARDIA study, gestational diabetes was significantly associated with impaired diastolic function, depressed longitudinal and

circumferential strain and increased LVM, although the hypertrophic pattern remains debatable(65, 66). Gestational diabetes is also associated with a higher risk of developing type 2 DM and cardiovascular risk after delivery(69-71).

4. Lessons learned from animal models of RR

The difficulty of accessing human myocardial tissue during RR demands the use of animal models to clarify its underlying mechanisms and predictors of the extent of RR. Animal models of RR need to recapitulate, firstly, cardiac remodelling induced by a stressor and, secondly, the RR process after a given intervention(515, 516). In theory, every animal with cardiac injury subjected to treatment is a suitable model to study RR. However, when it comes to studying cardiac hypertrophy, fibrosis, angiogenesis, and oxidative stress recovery, two surgical experimental models are primarily used; namely, the aortic banding followed by debanding and the left anterior descending (LAD) coronary ligation followed by its removal(515) in small(517, 518) and large animal models(519, 520) (**Figure 3**). The former is associated with chronic pressure overload and its subsequent relief, mimicking conditions like AS and hypertension before and after surgical/therapeutic intervention. Heterotopic heart transplantation can also be replicated in aortic banding and other animal models to promote reverse remodelling. LAD-ligation and its removal mimic an ischaemic event followed by myocardial reperfusion(515). Combining both models was recently shown to replicate two clinically relevant comorbidities, i.e., ischemic heart disease and arterial pressure overload. The aortic constriction removal two weeks after LAD ligation and transverse aortic constriction surgery allow for elucidating the impact of mechanical unloading (e.g., hypertensive therapy) in cardiac RR(521). The main advantage of these models is the longitudinal collection of biofluids and heart tissue at different time points of the RR process, allowing the identification of potential biomarkers of the degree of cardiac disease reversal and the use of genetic gain-/loss-of-function models. To replicate comorbidities impacting RR might be challenging(522), but this can usually be added on top of the above-mentioned models. Regarding age, landmark studies have linked cellular senescence to the release of senescence-associated secretory phenotype (SASP) in many age-associated cardiac pathologies, including HF(523). Senescent cardiomyocytes secrete growth factors that promote cardiac fibroblast activation, inducing pathological cardiac remodelling(524-527). Among the rare experiments in aged animals, ageing impaired RR in mice after β-adrenergic-induced cardiomyopathy. Indeed, 22-month-old elderly female animals displayed persistent cardiac hypertrophy, fibrosis and dysfunction upon β-adrenergic withdrawal. In contrast, young females

(10-month-old) could recover from cardiac remodelling and HF after the release of the primary stimulus(527). Additional mechanistic studies in aged animals are required to understand better the impact of ageing on RR.

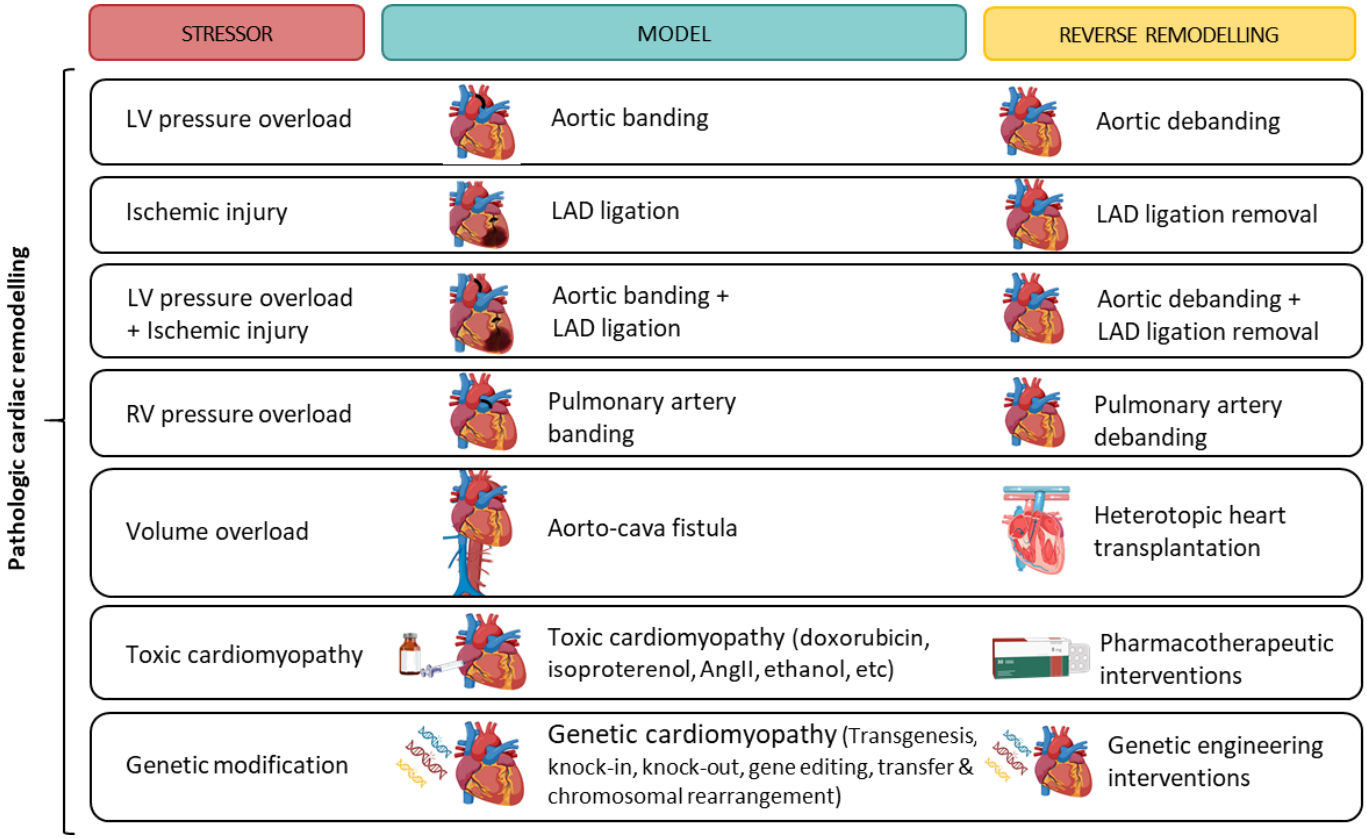

Figure 3. Models to achieve cardiac remodelling and reverse remodelling in animal models.

5. Conclusion and future perspectives

Despite the tremendous value of advanced imaging techniques, such as CMR, these are not widely available. Therefore, establishing models to predict the extent of RR will be facilitated in the following years by integrating circulating molecular biomarkers with more advanced yet widely accessible echocardiographic parameters, such as those providing a more precise evaluation of myocardial function than the typical LVEF. This includes strain parameters, as we have discussed, and the emerging "myocardial work", which has the additional advantage of accounting for LV afterload. For instance, a global wasted myocardial work of ≤200 mmHg% improved the prediction of non-responders to CRT when added to a baseline model containing, for example, QRS duration, LBBB, atrial fibrillation, or myocardial scar(528). Moreover, RR prediction may be improved with machine learning approaches. Recently, several linear and non-

linear models were able to predict a 1-year increase in LVEF≥10% among 752 patients undergoing CRT with more significant performance (AUC>0.72)(529) than that established by the guidelines (AUC<0.54)(530). In addition, LBBB morphology, basal LVESD and PCI history (ischaemic aetiology) repeatedly showed high importance in the five best models(529). Another important application of these models is that they could help establish the best timing for (non)-pharmacological intervention in patients. Of note, a finer assessment of myocardial tissue characteristics through radiomics analysis of ECV shows potential as a tool for AR prediction(531). Altogether, these recent studies point to novel predictive models of RR that include classical and novel molecular markers and more advanced imaging techniques. Apart from these conventional markers, the integration of personalized multiscale information (genes, metabolism, inflammation etc), potentially with the help of artificial intelligence, will better predict individual responses to therapy and RR potential. Those read-outs are urgently needed for guideline integration and clinical practice.

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