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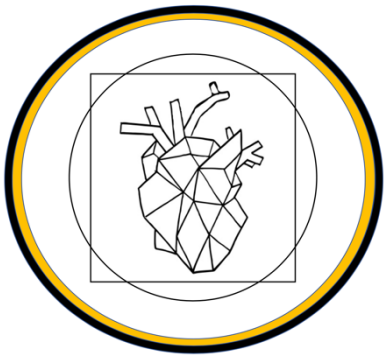

Cardiovascular Impact of Metabolic Syndrome: From Mechanisms to New Treatment Targets

O Impacto Cardiovascular da Síndrome Metabólica: Dos Mecanismos a Novos Alvos Terapêuticos

Orientador: Prof. Doutor Ricardo Fontes-Carvalho

Coorientadores: Prof. Doutor Adelino Leite-Moreira
Prof. Doutor Nuno Bettencourt

Ricardo Ladeiras-Lopes
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1. Ricardo Ladeiras-Lopes, Francisco Sampaio, Sara Leite, Diogo Santos-Ferreira, Eduardo Vilela, Adelino Leite-Moreira, Nuno Bettencourt, Vasco Gama, Pedro Braga, Ricardo Fontes-Carvalho. **Metformin in non-diabetic patients with metabolic syndrome and diastolic dysfunction: the MET-DIME randomized trial.** (Submitted)

2. Ricardo Ladeiras-Lopes, Pedro Teixeira, Ana Azevedo, Adelino Leite-Moreira, Nuno Bettencourt, Ricardo Fontes-Carvalho. **Metabolic syndrome severity score is associated with diastolic dysfunction and low-grade inflammation in a community-based cohort.** Eur J Prev Cardiol 2019; in press (doi: 10.1177/2047487319895400).

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   Fator de impacto (JCR® 2014): 3.189

Aos Meus Pais,

Aos Meus Avós,

Aos Meus Amigos,

Aos Gigantes da Frase:
"If I Have Seen Further Than Others, It Is By Standing Upon The Shoulders Of Giants"

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INTRODUÇÃO

A síndrome metabólica (SM) é uma epidemia global que afeta aproximadamente um terço da população adulta mundial e representa um conjunto de fatores de risco de doença cardiovascular e diabetes *mellitus* tipo 2 (DMT2) que surgem frequentemente associados. Esta síndrome está associada a uma alta taxa de utilização de recursos de saúde, com elevados custos financeiros. Apesar da sua importância, a SM representa ainda um desafio clínico, a necessitar de uma identificação mais precoce dos doentes que preencham os critérios de diagnóstico e uma gestão terapêutica mais dirigida dos seus fatores de risco. Para ultrapassar algumas destas dificuldades, a SM foi recentemente incluída num novo modelo de doença crónica de causa cardiometabólica, que foi proposto para promover a saúde cardiometabólica e mitigar o desenvolvimento de doença cardiovascular.

As principais alterações metabólicas que caracterizam a SM (adiposidade e disglicemia), juntamente com o seu mecanismo fisiopatológico central, a insulinorresistência, justificam grande parte do seu impacto cardiovascular. Adicionalmente, o excesso de tecido adiposo, a insulinorresistência e a disglicemia têm uma associação forte com a disfunção diastólica do ventrículo esquerdo (DDVE) e com a insuficiência cardíaca com fração de ejeção preservada (ICFEP). A presença de DDVE está associada a um aumento do risco de insuficiência cardíaca e parece estar também associada a um aumento do risco de morte. Assim, precisamos de perceber a sua associação com os fatores de risco cardiometabólico e avaliar o potencial de novas intervenções terapêuticas que possam contribuir para diminuir a morbimortalidade desta patologia.

OBJETIVOS

Este projeto de investigação teve como objetivo explorar os fatores de risco cardiometabólico e a sua associação com a DDVE como precipitante de aumento do risco de morbimortalidade, fornecendo novas perspetivas sobre os componentes da SM, da sua fisiopatologia, e da quantificação do seu impacto cardiovascular.

Foram objetivos específicos deste trabalho: a) avaliar a relação entre a SM (e a insulinorresistência) e o risco de DDVE; b) determinar se a gordura abdominal visceral e subcutânea está associada a um aumento do risco de eventos cardiovasculares e de morte; c) avaliar se as diferentes categorias de valores de pressão arterial, nomeadamente a pré-hipertensão, estão associadas à DDVE; d) avaliar se um *score* contínuo de gravidade de SM está associado à DDVE e a biomarcadores de inflamação e insulinorresistência; e) determinar se a metformina pode melhorar a função diastólica de doentes não-

diabéticos com SM; f) analisar e quantificar a associação entre a DDVE e a incidência de eventos cardiovasculares *major* e morte.

POPULAÇÃO E MÉTODOS

Para avaliar a relação entre a SM (e a insulinorresistência) e o risco de DDVE foi analisado um grupo de 1,582 indivíduos da coorte MESA (*Multi-Ehtnic Study of Atherosclerosis*), com fração de ejeção do ventrículo esquerdo igual ou superior a 50% e sem história de eventos cardiovasculares. A função diastólica foi avaliada por ressonância magnética cardíaca através do *strain rate* telediastólico e do *strain relaxation index*, e foi realizada a quantificação do volume extracelular para avaliação da matriz extracelular miocárdica.

Com o objetivo de perceber se a gordura abdominal visceral e subcutânea estava associada à mortalidade de todas as causas e a eventos cardiovasculares, procedemos a um estudo de coorte retrospetivo baseado num registo que incluiu 713 doentes referenciados para angiografia coronária por tomografia computorizada (TC) por suspeita de doença arterial coronária. Foi recolhida informação sobre as áreas de gordura abdominal visceral e subcutânea e do *score* de cálcio coronário, tendo sido também calculado o rácio de gordura abdominal visceral-subcutânea. O *endpoint* primário combinado incluiu mortalidade de todas as causas, enfarte agudo do miocárdio e revascularização miocárdica realizada pelo menos 1 mês após a coronariografia por TC.

Uma coorte comunitária de 925 indivíduos assintomáticos sem doença cardiovascular conhecida e que participaram num avaliação ecocardiográfica detalhada foi utilizada para explorar a associação entre pré-hipertensão e DDVE e para avaliar a relação entre um *score* contínuo de gravidade de SM, a presença de DDVE e biomarcadores plasmáticos de inflamação e insulinorresistência. Todos os participantes foram submetidos a exame clínico e analítico (incluindo quantificação dos níveis de insulina, adiponectina, leptina e proteína C reativa de alta sensisibilidade). A função diastólica foi avaliada através das velocidades *e'* e do rácio *E/e'*.

Para avaliar se a metformina tem um efeito benéfico sobre a função diastólica de doentes não diabéticos com SM, foi realizado um ensaio clínico randomizado, não controlado por placebo e com ocultação de *endpoints* (estudo MET-DIME). Foram randomizados 54 adultos não diabéticos com SM e DDVE (avaliada por ecocardiografia) para: grupo controlo, intervenção para alteração do estilo de vida; grupo ativo, intervenção para alteração do estilo de vida e terapêutica com metformina. O *endpoint* primário for a variação da velocidade *e'* aos 6, 12 e 24 meses. Os *endpoints* secundários incluíram alterações na insulinorresistência, capacidade funcional e qualidade de vida.

Finalmente, para avaliar a associação entre DDVE e a incidência de eventos cardiovasculares major e morte, realizámos uma revisão sistemática (19 estudos) e meta-análise (9 estudos) que incluiu

estudos de coorte que avaliassem a função diastólica de adultos na comunidade e que fornecessem dados sobre a ocorrência de eventos cardiovasculares ou morte. As estimativas de risco relativo comparando indivíduos com e sem disfunção diastólica foram combinadas usando um modelo de *random effects*.

PRINCIPAIS RESULTADOS

Utilizando o *tagging* miocárdico para avaliar a função diastólica e a quantificação do volume extracelular pelo T1 *mapping* como indicador de fibrose intersticial miocárdica, demonstrámos que o aumento da insulinorresistência e a presença de SM, mesmo sem o diagnóstico de DMT2, estão associados a alterações da função diastólica (aumento do *strain relaxation index* e diminuição do *strain rate* telediastólico). Este achado revelou-se independente do interstício miocárdico (fibrose), portanto as alterações funcionais encontradas parecem depender intrinsecamente do cardiomiócito. Assim, as alterações cardíacas iniciais na SM parecem ter um impacto preferencial no relaxamento miocárdico e na tensão do cardiomiócito em repouso.

O aumento do rácio entre as gorduras abdominais visceral e subcutânea está associado a um aumento da mortalidade total e da incidência de eventos cardiovasculares major. Esta associação revelou-se independente dos fatores de risco cardiometabólicos tradicionais e do cálcio coronário, sugerindo que uma acumulação preferencial de gordura no componente visceral abdominal poderá promover um estado inflamatório crónico, de baixo grau, aumentando o risco de vulnerabilidade de placa, rotura e trombose.

Apesar das alterações mais significativas da função diastólica estarem presentes em indivíduos hipertensos, também estão já presentes em indivíduos pré-hipertensos, refletindo lesão de órgão subclínica nesta população. Para além disso, o aumento do *score* de gravidade de SM está associado a um aumento da insulinorresistência, de biomarcadores inflamatórios e a um perfil de citocinas metabolicamente desfavorável (aumento da leptina e diminuição da adiponectina). Assim, este *score* funciona como um índice integrado de disfunção metabólica. A sua elevação associou-se a uma diminuição da velocidade *e'* (perturbação do relaxamento miocárdico) e a um aumento do rácio *E/e'* (aumento das pressões de enchimento do ventrículo esquerdo), e os doentes com disfunção diastólica apresentaram um *score* de gravidade de SM superior.

Na revisão sistemática e meta-análise que avaliou a associação entre a DDVE e eventos cardiovasculares e morte, incluímos dados de 19 estudos com aproximadamente 63,000 indivíduos. Os critérios de diagnóstico e a classificação da disfunção diastólica encontrados foram muito diferentes entre os estudos. A maioria (17 estudos em 19) mostraram que a DDVE foi um preditor significativo de eventos cardiovasculares e morte. Na estimativa ponderada, a DDVE associou-se a um aumento de 3.53 vezes do risco de evento cardiovascular *major* ou morte e a um aumento de 3.13 vezes do risco de morte.

Finalmente, no ensaio clínico randomizado MET-DIME demonstrámos que o tratamento com metformina de doentes não diabéticos com SM está associada a uma melhoria da função diastólica (aumento da velocidade *e'*), independente de alterações da insulinorresistência. Esta melhoria não se associou a variações significativas da capacidade funcional ou qualidade de vida relacionada com a saúde.

CONCLUSÕES

Alinhado com uma visão centrada no doente e interpretando a SM no contexto do modelo de doença crónica de causa cardiometabólica, neste projeto propomos uma nova perspetiva para os fatores de risco tradicionais, com o objetivo de otimizar a avaliação do risco cardiovascular. Adicionalmente, o *score* contínuo de gravidade da SM está associado a DDVE, esta última um preditor significativo de doença cardiovascular e morte. Assim, em doentes com fatores de risco cardiometabólico, sugerimos a avaliação sistemática da função diastólica por ecocardiografia como parte de uma estratégia intensiva para identificação de casos de DDVE pré-clínica. Nesses doentes, a alteração do estilo de vida é muito importante, mas a metformina também parece ser útil para melhorar a função diastólica, independentemente da presença de DMT2.

BACKGROUND

Metabolic syndrome (MetS) is a global epidemic, affecting approximately one-third of the adult world population. MetS represents a clustering of risk factors for cardiovascular disease (CVD) and type 2 diabetes (T2DM). It is associated with higher utilization of healthcare resources and health costs. In spite of its importance, MetS is still a clinical challenge which needs an earlier identification of patients fulfilling its diagnostic criteria and a more focused management of their risk factors. To overcome some of those difficulties, more recently, MetS was included in a new cardiometabolic-based chronic disease model that was proposed to promote cardiometabolic health and mitigate the development of CVD.

The primary metabolic drivers of MetS, adiposity and dysglycaemia, and its key pathophysiological mechanism, insulin resistance, mediate most of its deleterious cardiovascular impact. We also started to understand that adiposity, insulin resistance and dysglycaemia have an exquisite association with left ventricular diastolic dysfunction (LVDD) and heart failure with preserved ejection fraction (HFpEF). In addition, the presence of LVDD is associated with an increased risk of incident heart failure and seems to be associated with increased risk of death. Therefore, we need to understand its association with cardiometabolic risk factors and evaluate potential therapeutic interventions that can reduce patients' morbidity and mortality.

PURPOSE

This research project aimed to explore the cardiometabolic risk factors and its association with LVDD as a key driver of morbidity and mortality, putting into perspective the components of MetS, its pathophysiology, and assessing different ways of gauging its cardiometabolic impact. The specific aims were: a) to evaluate the relationship between MetS (and insulin resistance) and the risk of LVDD; b) to assess if visceral and subcutaneous abdominal fat is associated with all-cause mortality and cardiovascular events; c) to detail if different categories of blood pressure, especially pre-hypertension, are already associated with subclinical diastolic dysfunction ; d) to evaluate if the continuous MetS severity score is associated with LVDD and inflammatory and insulin-resistance biomarkers; e) to assess if metformin can improve diastolic function in non-diabetic patients with MetS; f) to evaluate the association between LVDD and the incidence of major adverse cardiovascular events and death, and quantify the strength of this association.

POPULATION AND METHODS

For the evaluation of the relationship between MetS (and insulin resistance) and the risk of LVDD, we conducted a cross-sectional study including 1,582 individuals from the Multi-Ethnic Study of Atherosclerosis (MESA) with left ventricular ejection fraction of at least 50% and no history of cardiac

events. Diastolic function was evaluated with end-diastolic strain rate and strain relaxation index using tagged cardiac magnetic resonance imaging, and the quantification of extracellular volume was performed for the evaluation of myocardial extracellular matrix.

To assess if visceral and subcutaneous abdominal fat is associated with all-cause mortality and cardiovascular events, we performed a registry-based retrospective cohort study including 713 patients referred to coronary computed tomography (CT) angiography for suspected coronary artery disease. Information on visceral and subcutaneous abdominal fat areas and calcium score was collected, and the visceral-to-subcutaneous abdominal fat ratio was calculated. The combined primary endpoint included all-cause mortality, myocardial infarction or revascularization procedure at least 1 month after coronary CT angiography.

A community-based cohort of 925 asymptomatic individuals without known cardiovascular disease that underwent a detailed echocardiographic examination was used to explore the association between different categories of blood pressure, especially pre-hypertension, and subclinical LVDD, and to evaluate if the continuous MetS severity score was associated with LVDD and inflammatory and insulin-resistance biomarkers. All participants underwent clinical and analytical (insulin, adiponectin, leptin and high-sensitivity C-reactive protein) examination. Diastolic function was evaluated by echocardiography using *e'* velocities and *E/e'* ratio.

To assess if metformin can improve diastolic function in non-diabetic patients with MetS we conducted a prospective, randomised, open-label, blinded-endpoint trial (MET-DIME trial). Fifty-four non-diabetic adults with MetS and LVDD (as assessed by echocardiography) were randomised to lifestyle counseling (control group) or to lifestyle counseling plus metformin (active group). The primary endpoint was the change in mean e' velocity at 6, 12 and 24 months. Secondary endpoints were improvements in insulin resistance, functional capacity and quality of life.

Finally, to evaluate the association between LVDD and the incidence of major adverse cardiovascular events and death, we performed a systematic review (19 studies) and meta-analysis (9 studies) including cohort studies that assessed diastolic function in adults in the community and provided some information regarding the occurrence of any cardiovascular event or mortality. Relative risk estimates comparing individuals with versus without diastolic dysfunction were combined using a random effects model.

MAIN RESULTS

Firstly, relying on myocardial tagging to assess diastolic function, and on extracellular volume using T1 mapping as a surrogate to interstitial myocardial fibrosis, we showed that both higher insulin resistance and the presence of MetS, even without T2DM, were associated with impaired diastolic

function (higher strain relaxation index and lower end-diastolic strain rate). This finding was independent of the myocardial interstitium (including fibrosis), and therefore the functional myocardial changes seemed to result from intrinsic cardiomyocyte alterations. The earliest changes in the heart of patients with MetS seem to have a preferential impact upon myocardial relaxation and myocyte resting tension.

Secondly, we observed that increased visceral to subcutaneous abdominal fat ratio was associated with higher total mortality and incidence of major adverse cardiovascular events. The association was independent of traditional cardiometabolic risk factors and coronary calcium, suggesting that preferential accumulation of fat in the abdominal visceral compartment might promote chronic, low-grade systemic inflammation, increasing the risk of plaque vulnerability, rupture and thrombosis.

In addition, diastolic function impairment was found to be more pronounced in hypertensive individuals, but changes in diastolic function were already present in prehypertensive individuals, reflecting subclinical organ damage in this population. Moreover, increasing MetS severity score was associated with higher insulin resistance, increased inflammatory biomarkers and metabolically dysfunctional adipokines profile (high leptin and low adiponectin). Altogether, this implies that this score works as an integrated index of metabolic dysfunction. Higher MetS score was associated with decreased *e'* velocity (impaired relaxation) and increased *E/e'* ratio (higher LV filling pressures) and patients with diastolic dysfunction showed higher MetS score.

In the systematic review and meta-analysis evaluating the association between LVDD and cardiovascular events and mortality, we included data from nineteen studies and approximately 63,000 individuals. Diagnostic criteria and classification of diastolic dysfunction differed substantially between studies. Most studies (17 studies out of 19) showed LVDD as a significant predictor of cardiovascular events and death. LVDD was associated with a 3.53-fold higher risk of MACE or death and 3.13-fold increased risk of death.

Finally, in the MET-DIME trial we showed that treatment with metformin of non-diabetic adults with MetS was associated with improved diastolic function (increase in *e'* velocity), independently of changes in insulin resistance. This improvement was not associated with a significant impact on functional capacity or health-related quality of life.

CONCLUSIONS

In line with a patient-centric viewpoint and facing MetS in the context of a cardiometabolic-based chronic disease model, in this project we propose an updated perspective of traditional risk factors in order to optimize our evaluation of cardiovascular risk. In addition, a continuous score of MetS severity is also associated with diastolic dysfunction, and the latter is a significant predictor of CVD and CV death. Therefore, systematic evaluation of diastolic function by echocardiography in patients with

cardiometabolic risk factors is suggested as part of an intensive case finding strategy to detect pre-clinical LVDD. In those patients, intensive lifestyle change is paramount, and metformin might be useful to improve diastolic dysfunction, irrespective of the presence of T2DM.

INTRODUCTION

INTRODUCTION

1. METABOLIC SYNDROME

1.1. Definition

The metabolic syndrome (MetS) is a global epidemic and represents a clustering of risk factors for cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM)[1]. Its five components, commonly occurring together, are: impaired glucose homeostasis, raised blood pressure, elevated triglyceride levels, low high-density lipoprotein cholesterol (HDL-C) levels and obesity (especially "central" obesity). MetS is a chronic and progressive pathophysiological state, associated with serious comorbidity, which is clinically under-recognized[2].

Historically, MetS was originally devised by Haller and Hanefeld in 1975[3], but proposed to the general public by Gerald M. Reaven in 1988 during the American Diabetes Association Meeting, with the name "Syndrome X" [4]. At that time, the term illustrated the unknown significance of the clustering of these risk factors and their importance to the pathophysiology of CVD, but with a potential common denominator – insulin resistance. The first global and formal definition of MetS was provided in 1998 by the World Health Organization (WHO)[5], requiring evidence of insulin resistance to establish the diagnosis. Since then, other organisations [International Diabetes Federation (IDF), American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI)], provided revised definitions of the diagnostic criteria, highlighting discrepancies around the need of an obligatory component (insulin resistance or central obesity) and cut-offs for central obesity and impaired glucose homeostasis[6, 7]. In 2009, a unified set of criteria for diagnosis of MetS was provided in a Joint Interim Statement coming from the WHO, AHA/NHLBI, IDF, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity[1], removing insulin resistance as an obligatory component and introducing population- and country-specific cut-offs to define central obesity.

There have been criticisms about the clinical utility of diagnosing MetS, on top of the additive effect of each one of the individual risk factors[8]. However, accruing evidence suggest that the diagnosis of MetS is clinically meaningful. As an example, in a meta-analysis including 43 cohorts and more than 170,000 individuals, MetS was associated with a 54% increased risk of CV events or death, even after controlling for its component risk factors[9]. This wave of thinking led in 2015 to the development of a "patient care model" for MetS, defining it as a complex pathophysiological state comprised of a cluster of clinically measured and typically unmeasured risk factors, progressive in its course, and associated with serious and extensive comorbidity[2]. Recently, MetS was included in a new cardiometabolic-based chronic

disease model that was proposed to promote cardiometabolic health and mitigate the development of CVD[10, 11]. This model integrates upstream clinical primary and metabolic drivers of MetS that are affected by modifiable risk factors, representing interventional targets to prevent downstream CVD.

1.2. Pathophysiology

There is a strong link between MetS and the obesity epidemic. More than half of the European population is overweight or obese[12]. Increased waist circumference (WC) is one of the diagnostic criteria of MetS, accounting for the "obesity component". However, obesity "per se", as defined by simple anthropometric measurements such as the body mass index (BMI) or WC, seems not to be a sufficient predictor of its association with increased risk of CVD[13]. Intra-abdominal fat, especially its visceral compartment, may represent the primary driver of the cardiometabolic implications of obesity, promoting a highly lipolytic state, with proinflammatory cytokine release and favouring insulin resistance and endothelial dysfunction. This contrasts to increased WC due to preferential accumulation of subcutaneous fat, that is associated with lower risk of insulin resistance[14].

MetS is also closely related to prediabetes and T2DM. Prediabetes is defined as an intermediate state of impaired glucose homeostasis (impaired fasting glucose, impaired glucose tolerance or raised haemoglobin A_{1c}) that does not meet criteria for T2DM[15]. Therefore, this should be regarded as one of the components of MetS (impaired glucose homeostasis). On the other hand, while MetS is associated with an increased risk of T2DM, the hyperglycaemia in the diabetic range is not excluded from the MetS diagnostic criteria, and therefore most patients with T2DM will have also criteria for MetS. Considering the close link between MetS, prediabetes and T2DM, it is not surprising that insulin resistance plays a pivotal role in the pathophysiology of MetS and it worsens in severity across added components of the syndrome[16].

Insulin resistance is associated with the typical "atherogenic dyslipidaemia" found in MetS patients (high triglycerides and low HDL-C)[17], a proinflammatory and prothrombotic state characterized by increased levels of C-reactive protein, interleukin-6 and plasminogen activator inhibitor-1[17], endothelial dysfunction[18], and increased sympathetic system activity promoting sodium retention and increased blood pressure levels above optimal values[18] [the risk of cardiovascular events is directly related to systolic BP (SBP) and diastolic BP (DBP) values, with a progressively higher risk for BP levels over 115 mmHg of SBP and 75 mmHg of DBP[19]]. Overall, insulin resistance mediates the complex interplay between abnormal adiposity and dysglycaemia, unifying the pathophysiological relationships between MetS, obesity, T2DM and CVD, as suggested in the recently described cardiometabolic-based chronic disease model[10].

Interestingly, in line with the perspective of a continuum of cardiometabolic risk, in the last decade a new approach has been described to gauge the severity of MetS[20]. Gender and race might be associated with a different contribution of the same risk factor to the overall risk of CVD and T2DM. In this regard, a continuous gender and race/ethnicity-specific MetS severity score was described and validated, representing a continuous measure of MetS for potential use in identifying adults at higher risk for MetS-related diseases and following changes within individuals over time. Interestingly, this MetS severity score was associated with future coronary heart disease independently of individual MetS components, and showed superior predictive and discrimination ability than NCEP-ATPIII criteria[21].

1.3. Epidemiology

MetS is a global epidemic, with a strong link to sedentary lifestyle and obesity. Its true prevalence is hard to determine due to different diagnostic criteria used in the past. By 2012, approximately a third of all US adults met the diagnostic criteria of MetS[22]. Data from the MARE (Metabolic syndrome and Artery Research) Consortium published in 2015, including more than 30,000 subjects from several cohorts worldwide (Europe and US), suggested a prevalence of 24%[23]. In Portugal, the PORMETS study was a cross-sectional (2007-2009) evaluation of approximately 4,000 non-institutionalized adults[24]. A diagnosis of MetS was present in 43% of the individuals and MetS was more frequent in non-urban areas, women and older individuals.

MetS is associated with higher utilization of healthcare and costs[25]. Overall, total costs increase by 24% per additional risk factor. In addition, the primary metabolic drivers of MetS, adiposity and dysglycaemia, represent a major burden to healthcare resources. Overweight and related conditions represent approximately 9% of healthcare expenditure of the Organisation for Economic Co-operation and Development and the number increases to 14% in the United States[26]. Furthermore, the IDF estimates the annual global health expenditure on diabetes at 760 billion US dollars, with an additional 35% increase coming from "indirect" costs: premature death and disability and other health complications[27].

Overall, MetS represents a public health problem, asking for counselling targeting the general public to adopt a healthy lifestyle. In addition, it is also a clinical challenge, in order to identify patients fulfilling the diagnostic criteria and needing focused management of their risk factors.

1.4. Therapy

The non-pharmacological integrative intervention to manage MetS is based on intensive lifestyle change, by promoting healthy eating patterns, aerobic physical activity, tobacco cessation, sleep hygiene

and alcohol moderation[11]. In the Diabetes Prevention Program (DPP), including more than 3,200 individuals with pre-diabetes, a lifestyle intervention (greater than 7% weight reduction on a low-calorie, low-fat diet, and moderate intensity physical activity of at least 150 min per week) reduced the incidence of T2DM by 58%, whilst metformin was associated with a reduction of 31%[28]. Healthy diet is indeed a powerful weapon against MetS, as was highlighted by a meta-analysis including 50 original research studies, showing that adherence to a Mediterranean diet is associated with a 31% reduced risk of MetS, with a favourable and significant impact in each of its components[29].

Regarding the pharmacological treatment of MetS, and excluding the adequate guideline-directed medical therapy to address each of its full-blown components (T2DM, hypertension, dyslipidaemia), the two main strategies that might be adopted target obesity or insulin resistance. Several drugs (e.g., orlistat, liraglutide, phentermine-topiramate, naltrexone-bupropion) are approved for the treatment of obesity and are associated with improvement in glycaemia, lipid profile and blood pressure, as well as with reducing the risk of T2DM in pre-diabetic individuals[30]. A pharmacological approach to modulate insulin resistance is also very attractive in MetS patients, and metformin might play a key role. As previously stated, in the DPP study, metformin was associated with reduced incidence of T2DM in patients with pre-diabetes[28], and it is considered for almost 2 decades as a potential treatment for non-diabetic patients with MetS[31]. Interestingly, novel therapeutic targets for metformin have been described, including a cardioprotective action especially in ischaemic heart disease and heart failure, that might represent an asset to manage MetS patients in the cardiometabolic chronic disease continuum (a detailed description of the novel therapeutic targets of metformin focused on MetS and CVD is provided in the review article in the following pages)[32].

Review

EXPERT OPINION

Novel therapeutic targets of metformin: metabolic syndrome and cardiovascular disease

Ricardo Ladeiras-Lopes†, Ricardo Fontes-Carvalho, Nuno Bettencourt, Francisco Sampaio, Vasco Gama & Adelino Leite-Moreira
†*Cardiovascular Research and Development Centre, Faculty of Medicine of the University of Porto, Porto, Portugal*

Introduction: Metformin is a widely used drug in the treatment of type 2 diabetes mellitus (T2DM). However, it is becoming an attractive drug to manage patients with pre-diabetes and to possibly prevent cardiac remodeling and fibrosis and heart failure.

Areas covered: In this review, we highlight the novel therapeutic targets of metformin with a special emphasis on cardiovascular disease. We discuss its key mechanisms of action and new signaling pathways that could partially account for its effect. Furthermore, metformin's role in the management of patients with metabolic syndrome is debated, emphasizing its potential to prevent diabetic heart disease. On the other hand, intense research is ongoing to clarify if metformin will be a future drug to target ischemia-reperfusion injury in the setting of myocardial ischemia.

Expert opinion: In the following years, one should look carefully at basic science results to successfully design and conduct clinical trials, emphasizing patients without full-blown T2DM, but who otherwise might have increased insulin resistance. Topics such as the prevention of cardiac fibrosis and heart failure with preserved ejection fraction, the attenuation of ischemia-reperfusion injury on an acute coronary syndrome and the post-myocardial infarction left ventricle remodeling surely deserve a special interest and should be faced as potential therapeutic targets for metformin.

Keywords: cardiovascular disease, heart failure, ischemic heart disease, metabolic syndrome, metformin

Expert Opin. Ther. Targets [Early Online]

1. Introduction

Metformin is a biguanide used for the treatment of type 2 diabetes mellitus (T2DM), mainly because of its insulin-sensitizing effect [1]. It is a widely used drug with proven efficacy, safety and overall good tolerability. The mechanistic exploration of its pharmacodynamics revealed that metformin decreases insulin resistance not only by AMP kinase (AMPK)-dependent pathways but also by AMPK-independent pathways, including mitochondrial effects [2].

Insulin resistance plays a key role in the pathophysiology of metabolic syndrome (MetS), a constellation of cardiovascular risk factors, and T2DM [3]. Those states of insulin resistance increase the risk of cardiovascular disease (CVD), a leading cause of morbidity and mortality worldwide [4]. In the past years there has been a growing body of evidence suggesting that metformin might also have an important therapeutic role in MetS [5] and might exert a cardioprotective effect, especially in ischemic heart disease [6] and heart failure [7].

This review aims to provide a clinical viewpoint of the role of metformin not only in the treatment of MetS and prevention of CVD but also in the management of the

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Article highlights.

- Metformin already has a role in the management of non-diabetic patients, such as patients with metabolic syndrome.
- One of its key subcellular effects is mitochondrial complex I inhibition, changing the cell's energetic balance. Furthermore, downstream signaling includes AMP kinase-dependent and -independent pathways.
- In the recently published GIPS-III trial, metformin did not improve left ventricle function after a STEMI in non-diabetic patients.
- Its cardioprotective role may include the prevention of post-myocardial infarction remodeling and heart failure, the regression of cardiac fibrosis caused by states of pressure overload or insulin resistance and the improvement of ischemia-reperfusion injury.
- The design of randomized Phase II and Phase III clinical trials to address new potential indications for metformin in a non-diabetic population is of uttermost importance, in order to fully understand the potential of this widely available, inexpensive drug.

This box summarizes key points contained in the article.

insulin-resistant patient who already faced a cardiovascular event, emphasizing its direct cardioprotective action.

2. Metformin: mechanisms of action

The overall antihyperglycemic effect of metformin is driven by the suppression of hepatic gluconeogenesis (**Figure 1**). However, a comprehensive understanding of metformin's mechanism of action is still lacking. Its antihyperglycemic effect is largely accounted for by the inhibition of the mitochondrial electron transport chain (complex I) [8]. Furthermore, AMPK, a leading cellular energy sensor [9], is indirectly stimulated by metformin through an increase in the AMP:ATP and ADP:ATP ratios [10]. However, this finding has been challenged in experiments with hepatocytes lacking either AMPK or its upstream activating enzyme LKB1 [11]. In this way, it seems plausible that part of metformin's effect is mediated by AMPK-independent pathways. Indeed, metformin leads to the accumulation of AMP and related nucleotides, inhibiting adenylate cyclase and thereby reducing cyclic AMP levels and downstream signaling via protein kinase A, suppressing glucagon-dependent hepatic gluconeogenesis [12].

Despite its effects on glucose metabolism, it is important to highlight that the long-term beneficial pleiotropic effects of metformin may depend not only on these previously described mechanisms but also on other signaling pathways and mediators not characterized yet (**Figure 1**). For example, the protective role of metformin on the vascular endothelium seems to be partially mediated by increased nitric oxide (NO) production due to AMPK activation of endothelial NO synthase (eNOS) [13] and decreased reactive oxygen species through inhibition of mitochondrial complex I [14].

3. MetS and diabetic cardiac disease – the diabetic continuum

The MetS is a constellation of cardiovascular risk factors that have reached epidemic proportions during the past two decades [15]. It is also known as 'pre-diabetes', including patients who share some cardiovascular risk factors that have a central pathophysiology mechanism, insulin resistance, but who do not have criteria for T2DM diagnosis. It is well established that insulin resistance is central to its pathophysiology, being associated with a proinflammatory, prothrombotic and oxidative state that increases the risk of CVD, with its microvascular and macrovascular complications [3]. According to the current recommendations for the management of non-diabetic patients with MetS, lifestyle changes are mandatory, and metformin is an option to these patients [5]. Indeed, in patients at risk of T2DM, metformin is associated with weight loss and improved lipid profile and insulin resistance, decreasing the incidence of T2DM by 40% [16].

The previously described metabolic dysfunctional status is associated with deterioration of cardiac structure and function. Indeed, myocardial fibrosis plays a pivotal role in cardiac dysfunction in hypertensive and diabetic heart disease [17] and is also present in patients with MetS [18]. Furthermore, experimental studies have shown that diabetic patients have changes in myocardial substrate utilization, impaired calcium homeostasis, mitochondrial dysfunction, increased oxidative stress, activation of the renin-angiotensin-aldosterone and of the sympathetic nervous systems and deposition of advanced glycation end products [19,20]. These neurohumoral and subcellular mechanisms lead to structural and functional changes in the heart, such as increased myocardial fibrosis [21], myocardial steatosis [22], left ventricular hypertrophy [23] and changes in systolic and diastolic function [24]. In humans, diastolic dysfunction is considered the earliest manifestation of myocardial involvement in T2DM [25] and a key component of diabetic cardiomyopathy [19].

Considering the dominant role of insulin resistance in the pathophysiology of the MetS and its cardiac deleterious effects, it seems reasonable to consider that an increase in insulin sensitivity might be associated with a global improvement in the structure and function of the heart. In the past years, it was demonstrated in animal models of insulin resistance and arterial hypertension that metformin prevented cardiac remodeling and progression to heart failure by several mechanisms [26,27]. On the one hand, metformin inhibited cardiac fibrosis in pressure-overloaded mice and collagen synthesis in cardiac fibroblasts by blocking the TGF-β1-Smad3 signaling pathway independently of AMPK activation [28]. On the other hand, metformin attenuated ventricular hypertrophy in rat model of pressure overload, via activation of AMPK, a downstream signaling involving

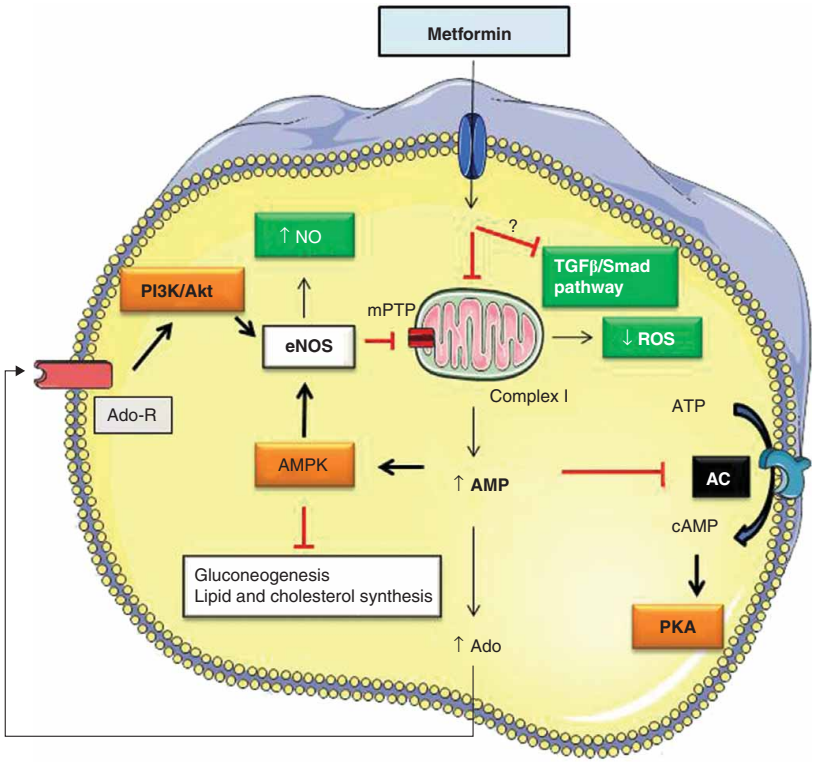

Figure 1. An overview of the mechanisms of action of metformin is shown. After entering the cell, metformin inhibits the mitochondrial respiratory chain (complex I), eliciting a decrease in energy production. Therefore, AMP concentration increases, representing a central mechanism of its intracellular action. The most important mechanisms potentially responsible for its cardiovascular action include: a decrease in ROS production through inhibition of mitochondrial respiratory chain, an increase in NO production due to activation of AMPK and inhibition of TGF-β/Smad pathway (still unknown mechanism).

This figure was produced using Servier Medical Art [65].

AC: Adenylate cyclase; Ado: Adenosine; Ado-R: Adenosine receptor; AMPK: AMP kinase; eNOS: Endothelial nitric oxide synthase; mPTP: Mitochondrial permeability transition pore; NO: Nitric oxide; PI3K: Phosphoinositide-3-kinase; PKA: Protein kinase A; ROS: Reactive oxygen species.

eNOS–NO [29]. Therefore, cardiac beneficial effects of metformin seem to depend on several signaling pathways, suggesting that long-term therapy with this drug may exert a cardioprotective action.

There is considerable interest in understanding if the administration of drugs acting in an earlier phase of the diabetic continuum can improve myocardial structure and function, especially diastolic dysfunction. Considering that changes in diastolic function are already present before the onset of T2DM [30], our group is now conducting a single-center, Phase II, randomized clinical trial to evaluate if the administration of metformin can improve diastolic function in patients with MetS and left ventricular diastolic dysfunction [31].

4. 'Obesity cardiomyopathy': adipokines, insulin resistance and metformin

Obesity has reached global epidemic proportions worldwide, being nowadays a public health concern [32]. Furthermore, recent studies have shown that obesity can directly induce changes in cardiac structure and function, particularly left ventricular hypertrophy and subclinical diastolic dysfunction [33,34]. Several mechanisms may be involved in the obesity cardiomyopathy, such as increased circulating volume and cardiac output [35], induction of a systemic proinflammatory state and secretion of several adipokines [36,37].

The obesity-inflammation continuum is associated with an increase in proinflammatory adipokines (e.g., leptin, resistin,

TNF) and reduced plasma concentration of 'protective' adipokines, especially adiponectin [38]. Adiponectin is a 30 kDa protein that is predominantly secreted by adipose tissue [39], although its levels are inversely correlated with the volume of adipose tissue [40]. Therefore, hypoadiponectinemia is a marker of adipose tissue dysfunction and MetS [41].

After binding to its transmembrane receptors, AdipoR1 and AdipoR2, adiponectin elicits an intracellular cascade that includes activation of AMPK and PPAR-α [42]. The normal intracellular transduction of signal is partially dependent on an adaptor protein, adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1 (APPL1) [43]. Adiponectin exerts a vasodilatory effect that is mediated by an increase in NO production [44]. This vascular effect is impaired in diabetic rats, not only due to hypoadiponectinemia but also through downregulation of APPL1 and subsequent adiponectin resistance.

Metformin might be able to positively interfere and partially correct the 'adiponectin resistance' present in obesity and MetS. For example, administration of metformin to diabetic rats restored adiponectin levels and APPL1 expression, although it did not have any impact on adiponectin-induced vasodilation. The latter may be explained by downregulated eNOS and adiponectin receptors [45]. In humans, metformin treatment of obese adolescents with insulin resistance improved inflammatory activity by preventing a decrease in adiponectin concentration and eliciting a decrease in the TNF-α concentration [46].

5. Ischemic heart disease

Data from the UK Prospective Diabetes Study (UKPDS) 34 trial showed that metformin decreased diabetes-related clinical events and all-cause mortality not only when comparing to non-pharmacological therapy but also in patients taking insulin or sulfonylureas [47]. Since then metformin is considered the first-line oral drug in the therapy of T2DM. Interestingly, metformin was superior to insulin and sulfonylureas, despite equal reductions in hemoglobin A_{1c}, already suggesting additional cardioprotective actions besides the antihyperglycemic effect.

Animal experiments performed in the past decade revealed that metformin exerted a protective effect against ischemia-reperfusion (I/R) injury in murine hearts [48], attenuated cardiac remodeling and heart failure after myocardial infarction (MI) and even improved survival [49]. Furthermore, diabetic patients already taking metformin when admitted for a ST-segment elevation MI have smaller MI sizes, when compared to diabetics not taking this drug [50].

Metformin exerts its protective effect against I/R injury through several mechanisms. Its administration is associated with the activation of the phosphatidylinositol-3-kinase and Akt, kinases belonging to the reperfusion injury salvage kinase (RISK) pathway [51]. This pathway prevents the opening of the mitochondrial permeability transition pore, which is a pivotal mechanism of I/R injury during early reperfusion [52]. Furthermore, the activation of AMPK and downstream stimulation of eNOS activity may also contribute to the cardioprotective effect of metformin [48]. Interestingly, it was demonstrated in a rat model of I/R injury that metformin-induced reduction in infarct size was critically dependent on adenosine receptor stimulation [53], suggesting that adenosine might be a key mediator of metformin's cardioprotective action. Considering that insulin is known as a cardioprotective agent capable of activating the RISK pathway [54] and recruiting its downstream targets including the phosphorylation of p70S6K, Bcl-2-associated death promoter and eNOS [55,56], metformin might be able to circumvent the loss of this effect in the insulin-resistant myocardium and boost this protective cascade on an ischemic insult.

The enthusiasm for the cardioprotective role of metformin was recently dampened when the Glycometabolic Intervention as Adjunct to Primary Percutaneous Coronary Intervention in ST-Segment Elevation MI (GIPS-III) study showed that when administered for 4 months to non-diabetic patients after a ST-segment elevation MI, metformin was not associated with short-term improved left ventricular function [57]. Future clinical studies should help clarify the importance of metformin's cardioprotective effect against ischemic myocardial injury.

6. Pleiotropic vascular effects

Accumulated evidence shows that metformin exerts several beneficial effects besides those on insulin resistance and cardioprotection. For example, endothelial dysfunction, a key player of the atherosclerosis-inflammation continuum, is ameliorated even with short-term administration of metformin through increased availability of NO and improved endothelium-dependent vasodilation [58]. In this trial including young women with polycystic ovarian syndrome, metformin also reduced arterial stiffness, thereby globally improving vascular function.

Metformin treatment is associated with an improved atherothrombotic and inflammatory blood profile, including reduced levels of plasminogen activator inhibitor type 1, TNF-α and C-reactive protein, as well as increased concentration of adiponectin [59]. Its effect on platelets is still poorly characterized, with studies suggesting decreased platelet aggregation [60], whereas others found no effect on platelet function [61].

The putative antihypertensive effect of metformin also deserves further research. Although older studies suggested that metformin decreased blood pressure [62,63], a recent randomized trial including obese hypertensive individuals without T2DM showed no change in blood pressure in metformin-treated patients [64].

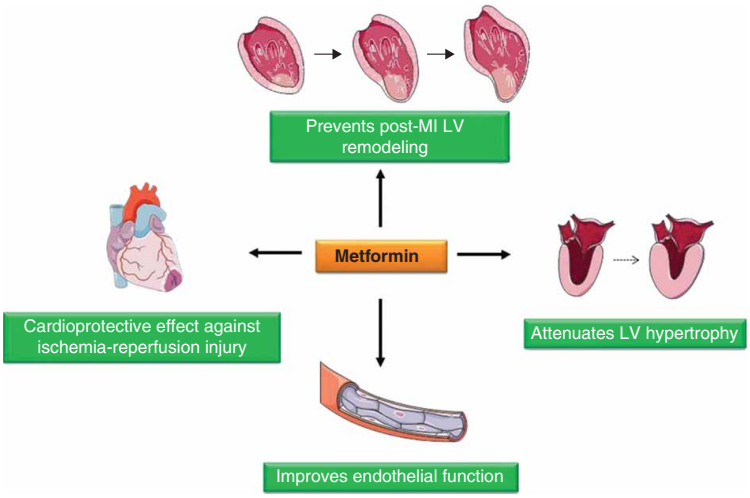

Figure 2. New cardiovascular therapeutic actions of metformin are shown.
This figure was produced using Servier Medical Art [65].
LV: Left ventricle; MI: Myocardial infarction.

7. Conclusion

Metformin started as an insulin-sensitizing drug and is nowadays the first-line drug in the therapy of T2DM. However, the body of evidence accumulated during the past 20 years has shown that metformin has several other beneficial effects on the metabolic profile and cardiovascular system (**Figure 2**, **Table 1**). Indeed, it may not only prevent the progression of a pre-diabetic state to full blown T2DM but it can also prevent the progressive cardiac anatomic and functional changes that accompany the diabetic continuum, contributing to improved systolic and diastolic functions. Furthermore, metformin seems to play a promising role in the prevention of I/R injury associated with acute coronary syndromes – an effect that may be extended to diabetic and non-diabetic individuals. The cellular and subcellular mechanisms responsible for these effects are still being explored, including both AMPK-dependent and -independent signaling pathways. Future research may help unravel novel mechanisms that may represent therapeutic targets of metformin pleiotropic action, further refining its potential to prevent and manage CVD.

8. Expert opinion

Targeting insulin resistance with metformin paved the way for new emerging therapeutic targets for this drug. Basic science has been unraveling the mechanisms that are responsible for its favorable effect in vascular and cardiac functions, improving endothelial function, preventing ischemic heart failure and even reversing cardiac fibrosis. The inhibition of mitochondria as the main effector of metformin's action has recently been challenged by other intracellular mechanisms, including AMPK-dependent and -independent responses. Furthermore, new intracellular actions that may account for its long-term effects such as interference with the protein kinase A and TGF-β signaling cascades are promising findings. In this way, the intracellular effectors of metformin's action represent an interesting field of research that may allow a better understanding of its use as a therapeutic weapon.

Metformin is nowadays viewed as a well tolerated, widely available and inexpensive drug to manage diseases such as MetS and CVD that represent global health problems with epidemic proportions.

However, the scarcity of randomized clinical trials aimed at CVD prevention or treatment and including non-diabetic individuals, as well as the neutral results of some trials (e.g., GIPS-III), have hindered the widening of the therapeutic targets of metformin. Basic science will help us to mechanistically understand the subcellular effect of metformin, possibly discovering new signaling pathways or mediators. Furthermore, robust results in animal models will definitely bring forward the opportunity to translate those findings to humans.

In the following years, one should look carefully at basic science results to successfully design and conduct clinical trials, emphasizing patients without full-blown T2DM, but who otherwise might have increased insulin resistance. Topics such as the prevention of cardiac fibrosis and heart failure with preserved ejection fraction, the attenuation of I/R injury

Table 1. Novel cardiovascular effects of metformin from basic and clinical studies.

Novel cardiovascular effects of metformin	Possible mechanism	Ref.
Animal studies		
Increased endothelial NO production	AMPK activation of eNOS	[13]
Decreased reactive oxygen species production by endothelial cells	Inhibition of mitochondrial complex	[14]
Inhibition of collagen synthesis	Inhibition of TGF-β1-Smad3 pathway	[28]
Attenuation of ventricular hypertrophy in pressure overload	AMPK activation of eNOS	[29]
Protective effect against ischemia-reperfusion injury	AMPK activation of eNOS; phosphoinositide-3-kinase and Akt activation (reperfusion injury salvage kinase pathway); adenosine receptor stimulation	[48,51,53]
Attenuated cardiac remodeling and heart failure after myocardial infraction	Activation of AMPK	[49]

Novel cardiovascular effects of metformin	Ref.
Clinical studies	
Smaller infarct sizes in diabetic patients already on metformin	[50]
Reduced arterial stiffness and improved endothelial function	[58]
Failed to improve short-term left ventricular function after STEMI	[57]
Possible neutral effect on blood pressure	[64]
Improved inflammatory and atherothrombotic blood profile	[59]

AMPK: AMP kinase; eNOS: Endothelial nitric oxide synthase; NO: Nitric oxide; STEMI: ST-elevation myocardial infarction.

on an acute coronary syndrome, and the post-MI left ventricle remodeling surely deserve a special interest and should be faced as potential therapeutic targets for metformin. Clinical investigators may face several challenges in trial design: dose of metformin (low and better-tolerated daily doses such as 500 mg twice daily versus higher doses that may be more effective but also elicit side effects and lead to drug discontinuation), timing of treatment administration (especially in trials addressing the attenuation of I/R injury or the prevention of cardiac fibrosis and heart failure in insulin-resistant patients), need for robust surrogate end points because of the low number of clinical events in the target population and many others. Thoughtful trial design will be challenging but may bring forward positive studies that have potential to change clinical practice.

The interplay among insulin resistance, MetS and cardiac fibrosis and the potential therapeutic role of metformin in this continuum are the active fields of research in the topic. Insulin resistance and MetS are associated with cardiac fibrosis and subclinical diastolic dysfunction. More interestingly, changes in diastolic function are not only typical of diabetic cardiomyopathy but also already present before the onset of diabetes, which reinforces the hypothesis that diastolic dysfunction is mainly associated with the state of insulin resistance and not only to sustained hyperglycemia. An ongoing clinical trial from our center will determine if insulin sensitizers, such as metformin, can improve diastolic function and provide cardioprotection.

In conclusion, the MetS and CVDs are novel therapeutic targets of metformin. Refining our knowledge of metformin's pleiotropic action, including its short- and long-term effects, will definitely need basic and clinical research that may extend the therapeutic indications of this widely available drug, in a global effort to prevent and manage CVD.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents, received or pending, or royalties.

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Affiliation

Ricardo Ladeiras-Lopes[†1,2,3] MD,
Ricardo Fontes-Carvalho[1,2] MD,
Nuno Bettencourt[1,2] MD PhD,
Francisco Sampaio[1,2] MD PhD,
Vasco Gama[1,2] MD &
Adelino Leite-Moreira[2] MD PhD
[†]Author for correspondence
[1]Gaia Hospital Centre, Department of Cardiology, Vila Nova de Gaia 4400-129, Portugal
Tel: +00351227865100;
E-mail: ricardoladeiraslopes@gmail.com
[2]University of Porto, Cardiovascular Research Centre, Faculty of Medicine, Porto, Portugal
[3]Cardiovascular Research and Development Centre, Faculty of Medicine of the University of Porto, Porto, Portugal

2. METABOLIC SYNDROME AND CARDIOVASCULAR DISEASE

2.1 Metabolic syndrome and major adverse cardiovascular events

CVD is the leading cause of death globally[33]. In 2017, high blood pressure, increased blood glucose levels and obesity were among the top five risk factors for premature death[34]. Therefore, it is not surprising that MetS is associated with a two to three fold increased risk for the development of CVD[35] and is also associated with subclinical coronary atherosclerosis, even in non-diabetic patients[36]. Overall, patients with MetS should be systematically identified and a comprehensive management plan addressing this cardiometabolic-based chronic disease continuum (Figure 1) should be established, especially targeting stages 1 (screening for risk factors), 2 (primary prevention of disease in patients at risk and with pre-disease) and 3 (secondary prevention of disease progression in patients with early disease), in order to avoid complications of stage 4 (tertiary prevention of worsening symptom burden in patients with late disease, the prevailing care plan today)[11].

The primary metabolic drivers of MetS, adiposity and dysglicaemia, are associated with deleterious changes in the cardiovascular system. The most important pathophysiological mechanisms involved in the association between MetS and CVD include: 1) atherogenic dyslipidaemia, characterized by reduced HDL-C and increased triglycerides[37]; 2) insulin resistance, a central player in the noxious impact of MetS, promoting abnormalities in myocardial contractile proteins and impaired relaxation, change in substrate utilization, cellular injury, microvascular dysfunction and neurohormonal activation[38]; 3) proinflammatory and prothrombotic state, including increased plasma levels of plasminogen activator inhibitor-1, fibrinogen and C-reactive protein[39, 40]; 4) microvascular dysfunction, as demonstrated by impaired peripheral and endothelial-mediated coronary flow reserve[41, 42]; and 5) activation of renin-angiotensin-aldosterone and symphatetic nerve systems[43, 44].

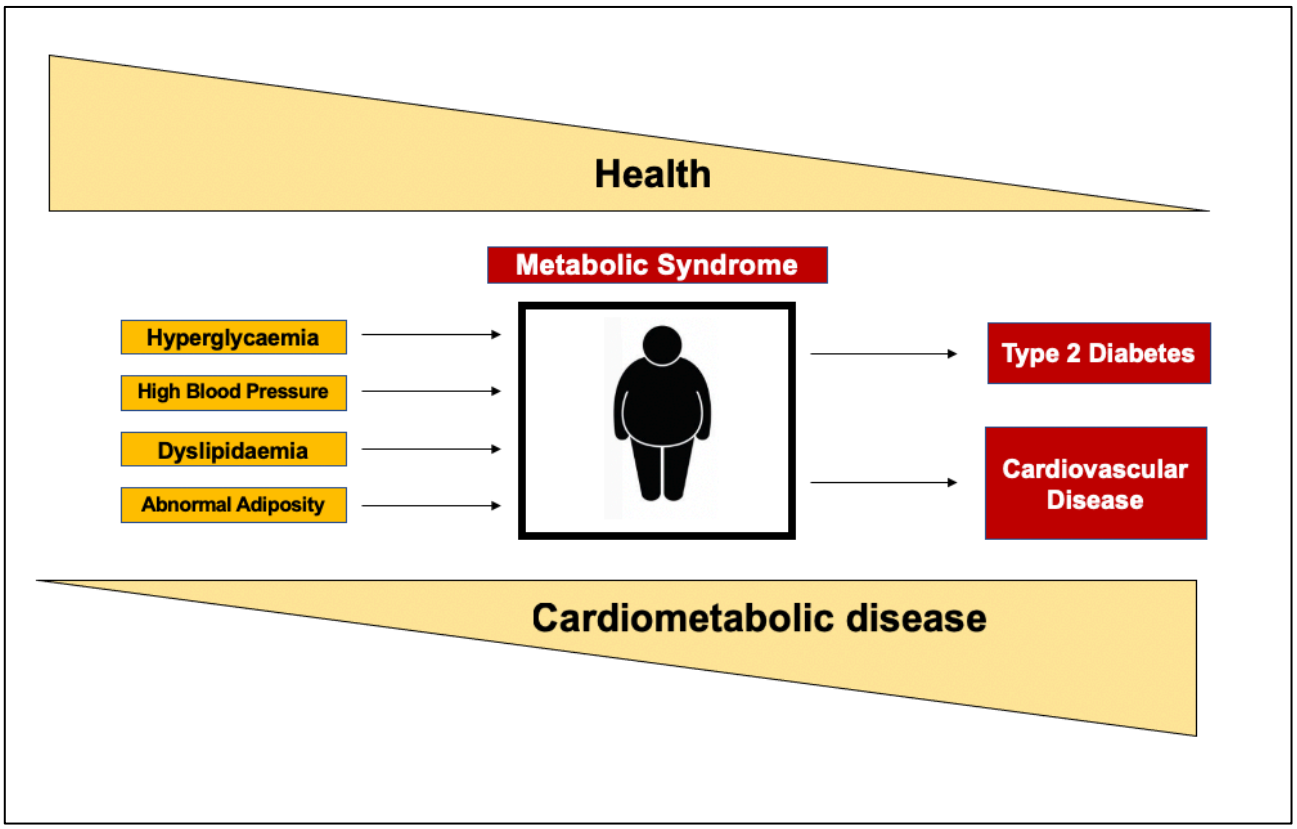

Figure 1. The cardiometabolic continuum

MetS denotes a patient-centric viewpoint of a cluster of cardiovascular risk factors that are associated with increased risk of T2DM and CVD.

2.2. Metabolic syndrome, diastolic dysfunction and heart failure with preserved ejection fraction

The prevalence of heart failure is approximately 1-2% of the adult population in developed countries, exceeding more than 10% among individuals aged 70 or older[45, 46]. MetS is a predictor of incident heart failure[47] and is also highly prevalent in heart failure patients[48].

Interestingly, obesity and cardiometabolic traits including insulin resistance are more strongly associated with risk of future heart failure with preserved ejection fraction (HFpEF) than heart failure with reduced ejection fraction (HFrEF)[47]. When putting together every component contributing to the cardiovascular impact of MetS, several bridges to the pathophysiological paradigm of HFpEF are found[49], leading to impaired myocardial relaxation, stiffer cardiomyocytes and increased myocardial fibrosis, nicely summarized by the complex term "diastolic dysfunction". HFpEF affects approximately 5% of individuals over 60 years old[50], a growing number that seems to track the epidemic of obesity and diabetes. There are several deleterious cardiac changes in HFpEF besides diastolic dysfunction, such as myocyte hypertrophy, systolic dysfunction, energetic abnormalities, interstitial fibrosis, increased inflammation

and oxidative stress and endothelial and microvascular dysfunction[49, 51, 52]. However, diastolic dysfunction plays a key role in the pathophysiology of HFpEF and will be further explored in the next topic.

3. FROM RISK FACTORS TO SUBCLINICAL CARDIAC DISEASE – THE CASE OF DIASTOLIC DYSFUNCTION

Left ventricular diastolic dysfunction (LVDD) is characterized by abnormal myocardial relaxation or altered passive properties, with associated impairment of left ventricular (LV) suction capacity and elevated LV filling pressures[53]. As previously stated, it could be regarded as one component of the subclinical, pre-symptomatic phase of heart failure, especially HFpEF, spanning phases 2 and 3 of the cardiometabolic-based chronic disease continuum[54]. LVDD affects approximately 27% of the general population[55] and 36% of the population older than 60 years[50], and its prevalence is increasing and is higher than that of systolic dysfunction[56].

LVDD is closely associated with several components of MetS, including hypertension, obesity and diabetes[57]. Indeed, there seems to be an association between the grade of LVDD and the number of coexisting MetS criteria[58], but LVDD is often subclinical and therefore hard to detect and follow-up adequately[59]. Diastolic function is often assessed by echocardiography, but no single echocardiographic parameter is considered sufficiently accurate and reproducible to establish the diagnosis of LVDD and several parameters must be combined for the diagnosis[60, 61]. The identification of patients with LVDD is critical because it is considered a risk factor for symptomatic heart failure, with dyspnoea, oedema and fatigue[62]. Furthermore, LVDD seems to be associated with an increased risk of death[46], although this was not systematically quantified yet.

The paradigm shift proposed by Paulus and Tschope for the pathophysiology of HFpEF[49], as a process of comorbid conditions (emphasizing impaired glucose homeostasis, obesity and arterial hypertension) leading to a systemic inflammatory state and microvascular inflammation, converge to LVDD, the major cardiac functional deficit in HFpEF. Therefore, LVDD has definite clinical significance and a better understanding of its association to cardiometabolic risk factors and exploring potential therapeutic interventions to improve it are warranted to reduce patients' morbidity and mortality.

PURPOSE

PURPOSE

This research project aimed to explore the cardiometabolic continuum and its association with LVDD as a key driver of morbidity and mortality, putting into perspective the components of MetS, its pathophysiology, and assessing different ways of gauging its cardiometabolic impact.

The following specific aims were established:

1. To evaluate the relationship between MetS (and insulin resistance) and the risk of LVDD;
2. To assess if visceral and subcutaneous abdominal fat is associated with all-cause mortality and cardiovascular events;
3. To detail if different categories of blood pressure, especially pre-hypertension, are already associated with subclinical diastolic dysfunction ;
4. To evaluate if the continuous MetS severity score is associated with LVDD and inflammatory and insulin-resistance biomarkers;
5. To assess if metformin can improve diastolic function in non-diabetic patients with MetS;
6. To evaluate the association between LVDD and the incidence of major adverse cardiovascular events and death, and quantify the strength of this association.

RESULTS / PUBLICATIONS

RESULTS/PUBLICATIONS

The results and publications of this research project will be presented in three sub-chapters:

A. New perspectives on the impact of cardiometabolic risk factors in CVD

1. Ricardo Ladeiras-Lopes, Francisco Sampaio, Nuno Bettencourt, Ricardo Fontes-Carvalho, Nuno Ferreira, Adelino Leite-Moreira, Vasco Gama. The ratio between visceral and subcutaneous abdominal fat assessed by computed tomography is an independent predictor of mortality and cardiac events. Rev Esp Cardiol (Engl Ed) 2017;70(5):331-337 (doi: 10.1016/j.rec.2016.09.010).

2. Ricardo Ladeiras-Lopes, Ricardo Fontes-Carvalho, Eduardo M. Vilela, Paulo Bettencourt, Adelino Leite-Moreira, Ana Azevedo. Diastolic function is impaired in patients with prehypertension: data from the EPIPorto Study. Rev Esp Cardiol (Engl Ed) 2018;71(11):926-934 (doi: 10.1016/j.rec.2017.11.015).

3. Ricardo Ladeiras-Lopes, Henrique T. Moreira, Nuno Bettencourt, Ricardo Fontes-Carvalho, Francisco Sampaio, Bharath Ambale-Venkatesh, Colin Wu, Kiang Liu, Alain G. Bertoni, Pamela Ouyang, David A. Bluemke, Joao A. Lima, for the MESA Investigators. Metabolic syndrome is associated with impaired diastolic function independently of MRI-derived myocardial extracellular volume: The MESA Study. Diabetes 2018;67(5):1007-1012 (doi: 10.2337/db17-1496).

4. Ricardo Ladeiras-Lopes, Pedro Teixeira, Ana Azevedo, Adelino Leite-Moreira, Nuno Bettencourt, Ricardo Fontes-Carvalho. Metabolic syndrome severity score is associated with diastolic dysfunction and low-grade inflammation in a community-based cohort. Eur J Prev Cardiol 2019; in press (doi: 10.1177/2047487319895400).

B. The contribution of left ventricular diastolic dysfunction to cardiovascular morbidity and mortality

5. Ricardo Ladeiras-Lopes, Margarida Araújo, Francisco Sampaio, Adelino Leite-Moreira, Ricardo Fontes-Carvalho. The impact of diastolic dysfunction as a predictor of cardiovascular events: a systematic review and meta-analysis. Rev Port Cardiol 2019;38(11):789-804 (doi: 10.1016/j.repc.2019.03.007).

C. Improving subclinical LV diastolic dysfunction in non-diabetic metabolic syndrome: a role for metformin?

6. Ricardo Ladeiras-Lopes, Ricardo Fontes-Carvalho, Nuno Bettencourt, Francisco Sampaio, Vasco Gama, Adelino Leite-Moreira. METformin in DIastolic Dysfunction of MEtabolic Syndrome (MET-DIME) trial: rationale and study design. Cardiovasc Drug Ther 2014;28(2):191-196 (doi: 10.1007/s10557-014-6512-2).

7. Ricardo Ladeiras-Lopes, Francisco Sampaio, Sara Leite, Diogo Santos-Ferreira, Eduardo Vilela, Adelino Leite-Moreira, Nuno Bettencourt, Vasco Gama, Pedro Braga, Ricardo Fontes-Carvalho. Metformin in non-diabetic patients with metabolic syndrome and diastolic dysfunction: the MET-DIME randomized trial. (Submitted)

A. New perspectives on the impact of cardiometabolic risk factors in CVD

Rev Esp Cardiol. 2017;**70(5)**:331–337

Original article

The Ratio Between Visceral and Subcutaneous Abdominal Fat Assessed by Computed Tomography Is an Independent Predictor of Mortality and Cardiac Events

Ricardo Ladeiras-Lopes,[a,b,*] Francisco Sampaio,[a,b] Nuno Bettencourt,[b] Ricardo Fontes-Carvalho,[a,b] Nuno Ferreira,[a,b] Adelino Leite-Moreira,[b] and Vasco Gama[a,b]

[a] *Department of Cardiology, Gaia/Espinho Hospital Centre, Vila Nova de Gaia, Portugal*
[b] *Cardiovascular Research and Development Centre, Faculty of Medicine of the University of Porto, Porto, Portugal*

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ABSTRACT

Introduction and objectives: Obesity is an important cardiovascular risk factor and the location of fat deposits seems to be an important determinant of its metabolic impact. Visceral adipose tissue (VAT) exerts a harmful effect on metabolic homeostasis, but few longitudinal studies have evaluated the prognostic impact of the ratio of VAT to subcutaneous adipose tissue (SAT). This study aimed to evaluate whether the VAT/SAT ratio was associated with all-cause mortality and cardiac events.
Methods: Registry-based retrospective cohort study. Eligible patients consisted of those without known heart disease referred to cardiac computed tomography (CT) angiography to evaluate suspected coronary artery disease (CAD). We included all patients with available information on VAT and SAT areas and coronary artery calcium (CAC) score. We assessed the combined endpoint of all-cause mortality, myocardial infarction or revascularization procedure at least 1 month after cardiac CT.
Results: The final population consisted of 713 participants (61% male; mean age, 57.7 ± 10.2 years) followed up for a median of 1.3 years. The combined endpoint occurred in 66 patients; these patients showed a higher VAT/SAT ratio (1.06 ± 0.74 vs 0.80 ± 0.52, *P* = .0001). The VAT/SAT ratio was an independent predictor of death and cardiac events (HR = 1.43; 95%CI, 1.03-1.99), irrespective of cardiovascular risk factors, CAC, and the presence of CAD.
Conclusions: The ratio between abdominal VAT/SAT was an independent predictor of death and coronary events, irrespective of cardiovascular risk factors, CAC, and the presence of CAD. This ratio is a CT-derived metric that may help to better identify patients with increased risk of death or cardiac events.

El cociente entre la grasa abdominal visceral y la subcutánea evaluado por tomografía computarizada es un predictor independiente de mortalidad y eventos cardiacos

Palabras clave:
Coronariografía por tomografía computarizada
Tejido adiposo visceral
Obesidad abdominal
Enfermedad cardiovascular
Enfermedad arterial coronaria

RESUMEN

Introducción y objetivos: La obesidad es un importante factor de riesgo cardiovascular, y parece ser que la localización de los depósitos de grasa determina de manera importante su impacto metabólico. El tejido adiposo visceral (TAV) ejerce un efecto perjudicial en la homeostasis metabólica, pero pocos estudios longitudinales han evaluado el impacto pronóstico de la relación entre el TAV y el tejido adiposo subcutáneo (TAS). Este estudio tiene por objetivo evaluar si el cociente TAV/TAS se asocia con la mortalidad por todas las causas y los eventos cardiacos.
Métodos: Registro basado en estudio de cohortes retrospectivo. Se eligió para el estudio a pacientes sin enfermedad cardiaca conocida remitidos a coronariografía por tomografía computarizada (TC) por sospecha de enfermedad arterial coronaria (EAC). Se incluyó a todos los pacientes con información disponible sobre el TAV, las áreas del TAS y el *score* de calcio de las arterias coronarias (CAC). Se evaluó el criterio de valoración combinado de mortalidad por todas las causas, infarto de miocardio o revascularización al menos 1 mes después de la TC cardiaca.
Resultados: La población final incluida fue de 713 participantes (el 61% varones; media de edad, 57,7 ± 10,2 años), seguidos una media de 1,3 años. Sufrieron el criterio de valoración combinado 66 pacientes, que mostraron una mayor relación TAV/TAS (1,06 ± 0,74 frente a 0,80 ± 0,52; p = 0,0001). El

* Corresponding author: Department of Cardiology, Gaia/Espinho Hospital Centre, Rua Conceição Fernandes, 4434-502 Vila Nova de Gaia, Portugal.
E-mail address: ricardoladeiraslopes@gmail.com (R. Ladeiras-Lopes).

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1885-5857/

cociente TAV/TAS fue un predictor independiente de muerte y eventos cardiacos (HR = 1,43; IC 95%, 1,03-1,99), independientemente de los factores de riesgo cardiovascular, la CAC y la presencia de EAC.
Conclusiones: El cociente TAV/TAS a nivel abdominal fue un predictor independiente de eventos coronarios y muerte, independientemente de los factores de riesgo cardiovascular, la CAC o la presencia de EAC. Esta relación es una medida derivada de la TC y puede ser útil para identificar mejor a los pacientes con mayor riesgo de muerte o eventos cardiacos.

Abbreviations

CAC: coronary artery calcium
CAD: coronary artery disease
CT: computed tomography
MACE: major acute cardiovascular events
SAT: subcutaneous adipose tissue
VAT: visceral adipose tissue

INTRODUCTION

Obesity is a challenging global epidemic with at least one third of the adult population being obese.[1] It is associated with most cardiovascular diseases, including hypertension, coronary heart disease, and heart failure.[2,3]

The harmful impact of obesity is not only related to fat quantity but also to fat "quality" and distribution.[4] Increased accumulation of fat in the abdomen, especially in the visceral compartment, is associated with metabolic risk factors and atherosclerosis.[5,6] Visceral adipose tissue (VAT) is metabolically active by secreting adipokines, causing vascular inflammation and insulin resistance.[7,8] VAT is associated with cardiovascular disease and represents a cardiometabolic risk marker.[9] Prospective data from the Framingham Heart Study support the role of VAT as a predictor of mortality and cardiovascular disease,[10,11] but longitudinal studies exploring this association are scarce and have limited external validity.[12–15] In contrast, fat accumulation in the subcutaneous compartment is associated with a neutral or even beneficial metabolic impact.[16] Therefore, the ratio of visceral to subcutaneous fat (VAT/subcutaneous adipose tissue [SAT] ratio) may provide a better assessment of the true cardiometabolic impact of body fat distribution, due to the differing systemic contributions of these anatomically close but functionally different fat depots.

This study aimed to evaluate whether the abdominal VAT/SAT ratio is associated with all-cause mortality and cardiovascular morbidity.

METHODS

Study Participants

This was a registry-based retrospective cohort study using data from our Cardiovascular Diagnosis and Intervention Unit from a tertiary care hospital. The study sample was drawn from all patients who were referred for coronary computed tomography (CT) angiography for evaluation of coronary artery disease (CAD) from January 2008 to December 2013. Most patients (n = 584) were referred without a previous ischemia test, and the remainder (n = 129) had a previous inconclusive treadmill exercise test or single-photon emission CT scan. Only patients from our primary catchment area were considered for this study. We excluded patients with known cardiovascular disease (previous myocardial infarction, stroke or revascularization procedure, valvular heart disease, previous myocarditis or cardiomyopathy) or with serious life-threatening illness (life expectancy less than 1 year). The final population included patients with full available data on abdominal adipose tissue areas and coronary artery calcium (CAC) score who were followed up for a maximum of 3 years (Figure 1).

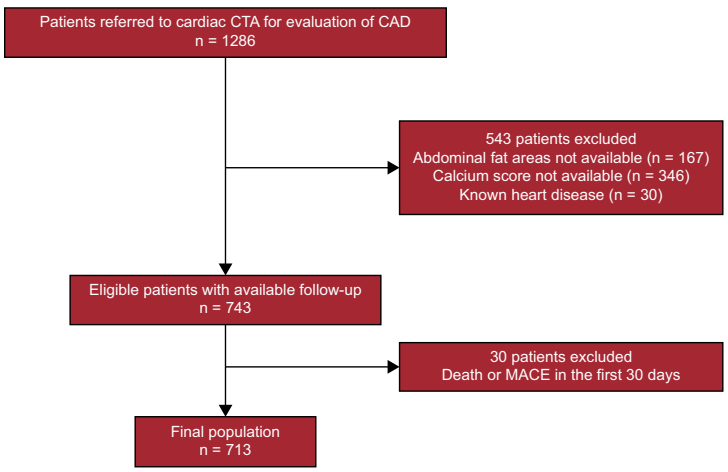

Figure 1. Patient selection flowchart. From the initial 1286 patients referred for cardiac CT due to suspected CAD, the final population included 713 patients. CAD, coronary artery disease; CT, computed tomography; CTA, computed tomography angiography; MACE, major acute cardiovascular events.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institution's ethics committee. All participants gave their written informed consent to participate in the study.

Risk Factor Assessment

Arterial hypertension was defined as history of hypertension (according to the European Society of Cardiology Guidelines[17]) or being on medical treatment with antihypertensive drugs. Smoking history was classified as never, former (participants who smoked for a minimum of 6 months during their lifetime but did not smoke at study entry) and current smoker. Type 2 diabetes mellitus was defined as a history of diabetes (according to current worldwide consensus criteria[18]) or being on medical treatment for diabetes. Family history of premature coronary heart disease was defined as having a first-degree relative who experienced a fatal or nonfatal myocardial infarction and/or coronary revascularization procedure before the age of 55 years in male relatives and 65 years in female relatives. Dyslipidemia was considered if the patient had a past history of dyslipidemia or was taking lipid-lowering drugs (statins, ezetimibe or fibrates).

Cardiac Computed Tomography Scan Protocol

All patients underwent a cardiac multidetector CT scan in a 64-slice CT scanner (SOMATOM, Sensation 64, Siemens Medical Solutions, Forchheim, Germany) with 3 different acquisitions: the first for abdominal fat quantification, the second for CAC quantification and the third for coronary angiography. To assess abdominal fat, a single-slice abdominal CT scan was performed between L4 and L5, according to the method described by Borkan et al.[19] The scan parameters were 120 kV and 216 mA with 5-mm thickness. This resulted in an estimated radiation exposure of 0.06 mSv. A blinded expert used the obtained slice to measure abdominal fat distribution: a cursor pointer was used to trace the VAT area by delineating the abdominal wall muscular layer[20] and adipose tissue was identified in the areas with attenuation values ranging from − 150 to − 50 Hounsfield units (HU).[21] Total abdominal fat area was measured, and the SAT area was obtained by subtracting VAT from the total abdominal fat area. As an estimate of the relative contribution of the VAT to the total abdominal fat, the VAT to SAT area was calculated (VAT/SAT ratio).

The following scan parameters were used to quantify the CAC: collimation, 24 × 1.2 mm; gantry rotation time, 330 ms; pitch, 0.2; tube voltage, 120 kV; and tube current, 190 mAs. Image reconstruction of the calcium score acquisition was performed using an effective slice thickness of 3 mm. CAC score was reported as the mean Agatston score and was calculated using a detection threshold of 130 HU using semiautomated software (Syngo Calcium Scoring, Siemens Medical Solutions) as described previously.[22]

Following CAC acquisition, CT angiography was performed (collimation, 64 x 0.6 mm; tube current, 850 mAs; all other parameters similar to CAC acquisition scan). Tube current modulation with electrocardiographic pulsing for decreasing radiation dose was used, with full tube current applied at 60% to 65% of the RR interval. In patients with body weight lower than 70 kg, tube voltage was reduced to 100 kV. A bolus of 80 to 100 mL of contrast (Ultravist, iopromide 370 mg/mL, Bayer Schering Pharma AG, Berlin, Germany) was injected at 5 mL/s via a power injector (Stellant D, Medrad Inc, Warrendale, Pennsylvania, United States) followed by a 40 mL saline "chaser", using a dedicated antecubital vein 18-gauge access catheter. A bolus-tracking technique was used, with a region of interest placed within the ascending aorta, set to detect a predefined threshold of 150 HU. For assessment of CAD, multiphasic sets of the CT reconstructed images were processed on a dedicated workstation (Aquarius Tera Recon Inc, San Mateo, California, United States) and analyzed for detection of at least 1 luminal diameter narrowing higher than 50% in any coronary artery segment by an experienced cardiologist. Severely calcified segments precluding lumen assessment were classified as positive for CAD.

Patient Follow-up and Combined Endpoint

According to our department's policy, only patients from our primary catchment area were followed up in order to collect information on major acute cardiovascular events (MACE). Patient follow-up data were collected by telephone interviews and electronic health record review at 12 and 36 months after cardiac CT. The combined endpoint included death from all causes, myocardial infarction or a revascularization procedure (percutaneous coronary intervention or coronary artery bypass graft surgery) at least 1 month after cardiac CT. Myocardial infarction was defined according to the most recent consensus definition.[23] The decision to proceed to myocardial revascularization was made by the attending cardiologist/cardiac surgeon.

Statistical Analysis

Continuous variables are reported as mean ± standard deviation or median (interquartile range). Discrete variables are expressed as frequencies and percentages.

An independent *t* test was used to assess differences in abdominal adipose tissue areas and the VAT/SAT ratio between participants according to the presence or absence of the combined endpoint.

Cox proportional hazards regression was used to perform the multivariate analysis using the following models: model 1 included age and sex; model 2 included age, sex, current smoking status, history of hypertension, type 2 diabetes mellitus, dyslipidemia, and familial history of premature CAD. Model 3 included all variables from model 2 plus coronary calcium score. A final model (model 4) was built including all variables from model 3 plus a dichotomic variable for presence/absence of CAD. The proportional hazards assumption of the Cox models was evaluated with Schoenfeld residuals.[24] There was no evidence of departure from the assumption of proportionality. VAT, SAT, VAT/SAT ratio and CAC + 1 were included in the models as a base-2 logarithm due to their skewed distribution (proportionality was assessed using the Kolmogorov-Smirnov test). One unit variation of the base-2 logarithmic transformation would be equivalent to a doubling of the variable of interest.

All statistical analyses were conducted using Stata 13.1 for Mac (StataCorp, College Station, Texas, United States).

RESULTS

Participants' Characteristics and Follow-up Results

The final population included 713 participants, 437 (61% men), with a mean age of 57.7 ± 10.2 years. The participants' characteristics according to the incidence of the combined endpoint during the follow-up period are depicted in Table 1.

All-cause Mortality and Major Acute Cardiovascular Events

During a median follow-up of 1.3 years (interquartile range, 1.1-1.9 years) there were 18 deaths, 2 myocardial infarctions, and

Table 1
Participants' Characteristics

Variable	Combined endpoint			
	Total (n = 713)	Yes (n = 66)	No (n = 647)	*P*
Age, y, mean ± standard deviation	57.7 ± 10.2	60.5 ± 9.7	57.4 ± 10.3	.014
Male sex, n (%)	437 (61)	56 (76)	381 (60)	.007
Hypertension, n (%)	406 (57)	47 (64)	359 (56)	.228
DM2, n (%)	95 (13)	10 (14)	85 (13)	.960
Hyperlipidemia, n (%)	336 (47)	39 (53)	297 (46)	.310
Current smoking, n (%)	113 (16)	16 (22)	97 (15)	.151
Obesity (BMI ≥ 30 kg/m^2)	250 (35)	27 (36)	223 (35)	.786
Total abdominal fat area, cm^2	365.4 ± 156.4	355.5 ± 134.3	366.6 ± 158.8	.566
VAT area, cm^2	151.8 ± 75.9	166.9 ± 71.2	150.0 ± 76.2	.070
SAT area, cm^2	213.6 ± 120.2	188.6 ± 94.7	216.5 ± 122.5	.058
VAT/SAT ratio	0.83 ± 0.55	1.06 ± 0.74	0.80 ± 0.52	< .001
CAC score	11 (0-147)	196 (42-561)	6 (0-110)	< .001
CAD n (%)	208 (29)	62 (84)	146 (23)	< .001

BMI, body mass index; CAC, coronary artery calcium; CAD, coronary artery disease; DM2, type 2 diabetes mellitus; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.
Values are expressed as n (%), mean ± standard deviation or median (interquartile range).

54 revascularization procedures, for a total of 66 events of the combined endpoint. These participants showed a significantly increased VAT/SAT ratio (1.06 ± 0.74 vs 0.80 ± 0.52, *P* = .0001) and a trend to having a higher VAT area (166.9 ± 71.2 vs 150.0 ± 76.2 cm^2) and lower SAT area (188.6 ± 94.7 vs 216.5 ± 122.5 cm^2), which was statistically significant (Table 1).

The total event rate was 6.0 (95% confidence interval [95%CI], 4.7-7.6) per 100 person-years of follow-up (1.2% in patients without obstructive CAD; 19.1% in patients with obstructive CAD in cardiac CT angiography). There was a significant increase in the event rate across the tertiles of the VAT/SAT ratio (3.0 events in the first tertile, 6.5 events in the second tertile and 8.5 events per 100 person-years for the last tertile, *P* = .0076). Figure 2 depicts the cumulative hazard curves of the combined endpoint over time according to the tertile of the VAT/SAT ratio.

In the age- and sex-adjusted analysis, doubling of the VAT/SAT ratio was associated with a 1.47 (95%CI, 1.05-2.07) increased hazard of the combined endpoint (Table 2). This association remained significant after adjustment for traditional cardiovascular risk factors (model 2, adjusted hazard ratio, 1.50; 95%CI, 1.06-2.13).

Visceral adipose tissue was not associated with the combined endpoint in the multivariate analyses (Table 3). However, higher SAT was independently associated with a lower risk of death or MACE irrespective of age, sex, cardiovascular risk factors, and CAC (HR, 0.70, 95%CI 0.51-.97, *P* = .033).

Secondary Analysis

In the model including CAC score and traditional risk factors for CAD, a doubling of the VAT/SAT ratio was associated with a 1.43-fold increased hazard of the combined endpoint (95% CI, 1.01-2.01) (Table 2). The CAC and VAT/SAT ratio seemed to have an additive

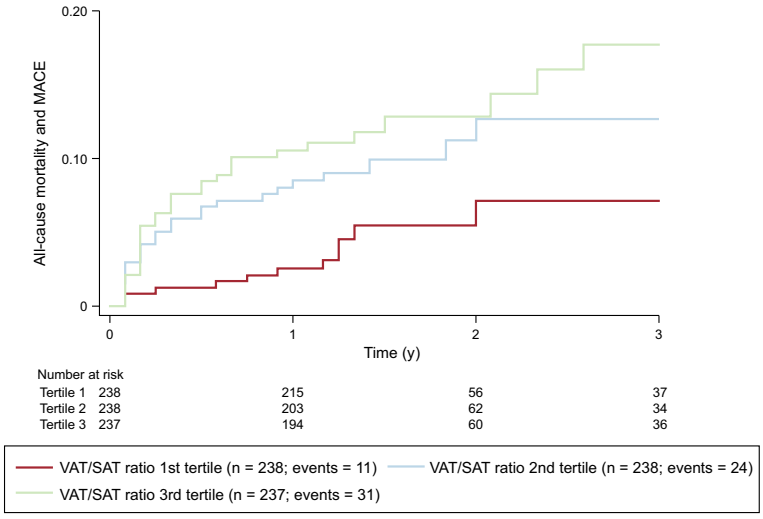

Figure 2. Cumulative incidence of the combined endpoint over time according to the tertile of the VAT/SAT ratio. There was an increase in the event rate across the tertiles of the VAT/SAT ratio. MACE, major acute cardiovascular events; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Table 2
Multivariate Cox Regression Analyses for Evaluation of Log_2 (VAT/SAT Ratio) as a Predictor of the Combined Endpoint (All-cause Mortality or Major Acute Cardiovascular Events)

Variable	Model 1			Model 2			Model 3			Model 4		
	Adjusted HR	95%CI	*P*	Adjusted HR	95%CI	*P*	Adjusted HR	95%CI	*P*	Adjusted HR	95%CI	*P* value
Age (per y)	1.02	0.99-1.05	.151	1.02	0.99-1.05	.113	0.99	0.95-1.02	.447	1.00	0.96-1.03	.889
Male sex	1.94	1.00-3.77	.050	1.93	0.98-3.80	.059	1.24	0.62-2.47	.546	1.55	0.76-3.17	.224
log_2 (VAT/SAT ratio)	1.47	1.05-2.07	.025	1.50	1.06-2.13	.021	1.43	1.01-2.01	.041	1.43	1.03-1.99	.035
Hypertension	-	-	-	1.14	0.66-1.96	.648	1.03	0.59-1.78	.930	1.03	0.59-1.79	.917
DM2	-	-	-	0.95	0.47-1.92	.895	0.80	0.39-1.62	.531	0.92	0.45-1.89	.826
Current smoking	-	-	-	1.34	0.72-2.48	.356	1.16	0.62-2.18	.640	0.98	0.51-1.87	.955
Hyperlipidemia	-	-	-	1.14	0.68-1.90	.613	1.11	0.66-1.86	.698	1.12	0.67-1.89	.670
Obesity	-	-	-	1.02	0.61-1.71	.929	0.95	0.57-1.60	.857	1.10	0.65-1.85	.727
Familial history of premature CAD	-	-	-	1.90	1.04-3.46	.036	1.67	0.91-3.05	.097	1.52	0.83-2.81	.179
log_2(CAC)	-	-	-	-	-	-	1.24	1.14-1.36	<.001	0.97	0.88-1.07	.547
Presence of obstructive CAD	-	-	-	-	-	-	-	-	-	15.40	6.80-34.72	<.001

95%CI, 95% confidence interval; CAC, coronary artery calcium; CAD, coronary artery disease; DM2, type 2 diabetes mellitus; HR, hazard ratio; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Table 3
Multivariate Cox Regression Analyses for Evaluation of the Association Between Abdominal Visceral and Subcutaneous Adipose Tissues and the Combined Endpoint of All-cause Mortality or Major Acute Cardiovascular Events

	Visceral adipose tissue		Subcutaneous adipose tissue	
	Adjusted HR (95%CI)	*P*	Adjusted HR (95%CI)	*P*
Model 1: age + sex	1.07 (0.75-1.54)	.708	0.71 (0.52-0.98)	.040
Model 2: model 1 + CVRF[a]	1.07 (0.71-1.60)	.758	0.66 (0.48-0.93)	.016
Model 3: model 2 + log_2 (CAC)	1.05 (0.72-1.54)	.796	0.70 (0.51-0.97)	.033
Model 4: model 3 + presence of CAD	1.16 (0.81-1.68)	.415	0.80 (0.59-1.09)	.154

95%CI, 95% confidence interval; BMI, body mass index; CAC, coronary artery calcium; CAD, coronary artery disease; CVRF, cardiovascular risk factors; HR, hazard ratio.
[a] The following covariates were included in model 2: age, sex, hypertension, type 2 diabetes mellitus, current smoking, hyperlipidemia, obesity (BMI ≥ 30 kg/m^2), and familial history of premature coronary artery disease.

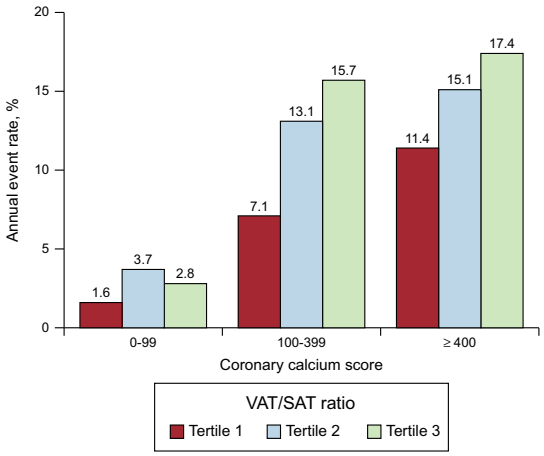

Figure 3. Annual combined endpoint event rates stratified by coronary artery calcium score and tertiles of the VAT/SAT ratio. CAC and the VAT/SAT ratio seemed to have an additive effect in the prediction of the combined endpoint of death or MACE. CAC, coronary artery calcium; MACE, major acute cardiovascular events; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

effect in the prediction of the combined endpoint of death or MACE (Figure 3).

The prevalence of CAD was 29% (208 patients). The VAT/SAT ratio was a significant predictor of the combined endpoint (1.43-fold increased hazard of death or MACE per doubling of the VAT/SAT ratio) after adjustment for cardiovascular risk factors, CAC, and the presence of CAD (model 4, Table 2).

DISCUSSION

In this study, including an adult population referred for cardiac CT angiography to evaluate CAD without known previous cardiovascular disease, we found that an increased abdominal VAT/SAT ratio was associated with a higher total mortality and incidence of MACE, independently of traditional vascular risk factors, CAC, and the presence of obstructive CAD. To our knowledge, this is the first longitudinal study to explore the influence of the relative distribution of abdominal fat between visceral and subcutaneous compartments on total mortality and incidence of cardiovascular events.

During the median follow-up of 1.3 years, there were 66 events of the combined endpoint (6.0 per 100 person-years of follow-up). Our results are in agreement with a meta-analysis on the

association between CAD detected by CT angiography and cardiac events, including 32 studies and 41 960 patients over a mean follow-up of 1.96 years, reporting a composite MACE rate of 5.8% over the follow-up period.[25] As expected, patients with coronary stenosis of at least 50% on cardiac CT angiography had a higher rate of MACE during the follow-up (19.1% vs 1.2% in patients without obstructive CAD).

Adipose tissue has been increasingly recognized as an important modulator of cardiovascular homeostasis. For example, during recent decades it was considered an abundant source of mesenchymal stem cells that produce several factors with angiogenic and immunomodulatory properties that might play a role in regenerative medicine targeting the heart.[26] Furthermore, the effect of VAT and SAT upon cardiovascular health is still a matter of debate, with contradictory evidence, suggesting the need for a better biomarker for the impact of fat distribution on cardiovascular risk.[27] According to the "portal vein hypothesis",[28] VAT is associated with increased delivery of free fatty acids to the liver and production of key inflammatory mediators, such as tumor necrosis factor-α and interleukin-6, leading to insulin resistance and systemic low-grade inflammation.[29] On the other hand, the role of SAT in cardiometabolic risk is still controversial. Previous studies have shown that SAT may be associated with metabolic risk factors and increased insulin resistance, despite having a weaker association when compared with VAT.[5,30] However, in overweight and obese patients, higher SAT is associated with insulin sensitivity,[16] suggesting that in patients with increased body fat, the relative distribution in the abdominal compartment may be important to determine its global metabolic impact. In this study, VAT was not an independent predictor of the combined endpoint. However, our results showed that the SAT area was lower in participants who died or had a MACE during the follow-up, although this result was not statistically significant ($P = .0584$), and that higher SAT was associated with a lower risk of death or cardiac events after adjustment for age, sex, the presence of cardiovascular risk factors, and CAC. Our results support a protective role for SAT, which, according to previously published data, may be associated with improved insulin sensitivity and, therefore, a better metabolic profile.[16]

Visceral adipose tissue affects not only metabolic homeostasis but also cardiac function, especially diastole. Increased VAT is associated with impaired diastolic function both in asymptomatic persons[31] and in those after a myocardial infarction.[32] Its potential harmful impact upon cardiovascular health seems to be related to higher metabolic activity than SAT, with production of inflammatory mediators that generate a systemic proinflammatory state that represents a key mediator in the pathophysiology of heart failure with preserved ejection fraction.[33] However, in our population, VAT was not a significant predictor of death or cardiac events. Nevertheless, even after adjustment for traditional risk factors, coronary calcium score and the presence of coronary stenosis of at least 50%, a higher VAT/SAT ratio predicted death and MACE during a median follow-up of 1.3 years. These findings support the recent "ectopic fat storage model" as the emerging paradigm of body fat contribution to metabolic risk.[34] A dysfunctional SAT favors ectopic fat deposition in other compartments associated with insulin resistance and inflammation.[35] In this way, the VAT/SAT ratio may provide a better index of the cardiometabolic impact of body fat composition than absolute quantification of each deposit independently.

The VAT/SAT ratio was a significant predictor of MACE and all-cause mortality irrespective of CAC score, a powerful and well-validated predictor of coronary events and mortality.[36,37] Considering that CAC is an estimate of overall coronary plaque burden, we hypothesize that a higher VAT/SAT ratio may be associated with increased chronic low-grade systemic inflammation, predisposing patients to have vulnerable plaques, plaque rupture, and thrombosis,[38] and therefore CAC and the VAT/SAT ratio seem to have an additive effect on the risk of death and cardiac events, as depicted in Figure 3.

Limitations

The limitations of this study include the observational design and caution should be exercised when drawing conclusions about causality. All decisions to proceed to myocardial revascularization were made by the attending cardiologist and cardiac surgeon. We excluded revascularization procedures performed during the first month after coronary CT to avoid procedures that were prompted in the short-term by the result of the coronary CT angiography. Therefore, we can assume that most revascularization procedures were performed due to persistent angina despite anti-ischemic therapy or a positive ischemia test. Our cohort included a white population, so its external validity, especially to other ethnic groups, remains to be shown. Only patients from our primary catchment area were followed up to accurately collect information on MACE. Those patients would necessarily come to our center if they had a MACE, and therefore we tried to reduce to a minimum the potential loss of information on MACEs that would be managed in other centers. Only a small proportion of the initial sample underwent the 3-year follow-up (n = 107). A sensitivity analysis (Table of the supplementary material) showed no significant differences in age, sex, cardiovascular risk factors, calcium score, areas of abdominal fat, and prevalence of CAD between groups according to the presence or absence of 3-year follow-up information. In addition, we did not systematically collect detailed information about the medical drugs patients were prescribed.

CONCLUSIONS

This study showed that the VAT/SAT ratio is an independent predictor of death and MACE. In addition to CAC quantification, this marker may become clinically relevant by providing a tool to better identify patients with an increased risk of death and cardiovascular disease.

WHAT IS KNOWN ABOUT THE TOPIC?

- Obesity is a well-established cardiovascular risk factor and recent evidence has shown that its cardiovascular impact depends not only on its quantity but also on its location.
- In the abdominal compartment, VAT seems to have a deleterious impact on cardiovascular health, but the role of SAT is less well established. Furthermore, the association between the VAT/SAT ratio and cardiovascular disease has not been adequately explored.

WHAT DOES THIS STUDY ADD?

- This retrospective study provides longitudinal data indicating that the ratio between VAT and SAT in the abdominal compartment is independently associated with all-cause death and MACE.
- This ratio may become a clinically relevant tool to better identify patients with an increased risk of death and cardiovascular disease.

CONFLICTS OF INTEREST

None declared.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rec.2016.09.010.

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Original article

Diastolic Function Is Impaired in Patients With Prehypertension: Data From the EPIPorto Study

Ricardo Ladeiras-Lopes,[a,b,c,◇,*] Ricardo Fontes-Carvalho,[a,b,c,◇] Eduardo M. Vilela,[b] Paulo Bettencourt,[c,d] Adelino Leite-Moreira,[a,c,e] and Ana Azevedo[a,f,g]

[a] *Departamento de Cirurgia e Fisiologia, Faculdade de Medicina, Universidade do Porto, Porto, Portugal*
[b] *Departamento de Cardiologia, Centro Hospitalar de Gaia/Espinho, Vila Nova de Gaia, Portugal*
[c] *Unidade de Investigação Cardiovascular, Universidade do Porto, Porto, Portugal*
[d] *Departamento de Medicina, Faculdade de Medicina, Universidade do Porto, Porto, Portugal*
[e] *Departamento de Cirurgia Cardiotorácica, Centro Hospitalar São João, Porto, Portugal*
[f] *Departamento de Epidemiologia Clínica, Medicina Preditiva e Saúde Publica, Faculdade de Medicina, Universidade do Porto, Porto, Portugal*
[g] *Epidemiology Research Unit (EPIUnit) – Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal*

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ABSTRACT

Introduction and objectives: Hypertension causes subclinical changes in left ventricular structure and function, namely diastolic dysfunction. Diastolic dysfunction is a predictor of heart failure, being involved in the association between hypertension and heart failure with preserved ejection fraction. We aimed to determine whether patients with prehypertension have early changes in diastolic function in a large community-based cohort of asymptomatic adults.

Methods: A cross-sectional evaluation was performed of a community-based cohort consisting of 925 adults, aged 45 years or older, without known cardiovascular disease. All participants underwent detailed clinical and echocardiographic examination. The participants were categorized according to the European guidelines for the classification of office blood pressure (BP) levels as optimal, prehypertensive (normal and high-normal categories), and hypertensive. Diastolic function was evaluated by echocardiography using e' velocities and E/e' ratio. Diastolic dysfunction was defined using the 2016 ASE/EACVI Joint Recommendations and a 2017 clinically-oriented algorithm.

Results: In this cohort (61.5 ± 10.5 years; 37% men), prehypertension was present in 30.4% and hypertension in 51.0%. Using optimal BP as the reference, there was a progressive decrease of e' velocity in prehypertensive and hypertensive individuals (12.2 ± 3.5 vs 11.3 ± 3.1 vs 9.6 ± 2.9 cm/s, respectively; *P* for trend < .001). After multivariable adjustment, both BP categories were independent predictors of a lower e' velocity (β = -0.56, *P* = .035 for prehypertension and β = −1.08, P < .001 for hypertension).

Conclusions: In this large community-based cohort, adults with prehypertension already showed impaired cardiac relaxation before the onset of hypertension.

Palabras clave:
Prehipertensión
Hipertensión
Función diastólica

La función diastólica se altera en pacientes con prehipertensión: datos del estudio EPIPorto

RESUMEN

Introducción y objetivos: La hipertensión causa cambios subclínicos en la estructura y la función del ventrículo izquierdo, es decir, disfunción diastólica. La disfunción diastólica es un predictor de insuficiencia cardiaca, pues participa en la asociación entre hipertensión e insuficiencia cardiaca con fracción de eyección conservada. El objetivo es evaluar en una gran cohorte poblacional de adultos asintomáticos si los pacientes con prehipertensión tienen cambios precoces en la función diastólica.

Métodos: Se evaluó de manera transversal una cohorte poblacional consistente en 925 adultos de 45 años o más sin enfermedad cardiovascular conocida. Todos los participantes se sometieron a un examen clínico y ecocardiográfico detallado. Se clasificó a los participantes, según las guías europeas para la clasificación de la presión arterial (PA) en la consulta, como óptima, prehipertensión (normal y normal-alta) e hipertensión. La función diastólica se evaluó mediante ecocardiografía usando las velocidades de e' y la razón E/e'. La disfunción diastólica se definió utilizando las recomendaciones conjuntas de ASE/EACVI de 2016 y un algoritmo de orientación clínica de 2017.

* Corresponding author: Departamento de Cardiologia, Centro Hospitalar de Gaia/Espinho, Rua Conceição Fernandes, 4434-502 Vila Nova de Gaia, Portugal.
E-mail address: ricardoladeiraslopes@gmail.com (R. Ladeiras-Lopes).
◇ Both authors equally contributed to this work.

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Resultados: En esta cohorte (61,5 ± 10,5 años; el 37% varones), tenía prehipertensión el 30,4% e hipertensión el 51,0%. Se utilizó la PA óptima como referencia, y se observó una disminución progresiva de la velocidad e' en los individuos prehipertensos e hipertensos (12,2 ± 3,5 frente a 11,3 ± 3,1 frente a 9,6 ± 2,9 cm/s respectivamente; p de tendencia < 0,001). Después del ajuste multivariable, ambas categorías de PA fueron predictoras independientes de una menor velocidad e' (prehipertensión, β = −0,56; p = 0,035; hipertensión, β = −1,08; p < 0,001).

Conclusiones: En esta cohorte poblacional, los adultos con prehipertensión mostraron una relajación cardiaca alterada antes del inicio de la hipertensión.

Abbreviations

BP: blood pressure
DBP: diastolic blood pressure
DD: diastolic dysfunction
PP: pulse pressure
SBP: systolic blood pressure

INTRODUCTION

Arterial hypertension is an important independent risk factor for cardiovascular disease and the single largest contributor to global mortality.[1] In hypertension, subclinical organ damage represents an intermediate stage in the cardiovascular continuum, being associated with cardiovascular events.[2] Therefore, current European guidelines recommend a holistic approach to the hypertensive patient, which includes the assessment of organ damage (cardiac, vascular, renal, and ophthalmic) as part of the diagnostic workup of these patients.[3] Regarding cardiac involvement, hypertension can cause changes in both structure and function,[4] particularly left ventricular hypertrophy and impaired diastolic function.[5] Both have been shown to be independently associated with mortality and cardiovascular events.[6]

Diastolic dysfunction (DD) is prevalent among the general population, affecting 20% to 30% of individuals[7,8] and is strongly associated with aging,[9] obesity,[8] insulin resistance,[10] and hypertension.[11] Although usually subclinical,[11] DD is an important predictor of heart failure, especially of heart failure with preserved ejection fraction,[12] and of long-term mortality.[13] These findings support the role of DD as an intermediate step between hypertension and blood pressure (BP).[11] The importance of assessing diastolic function in hypertension is acknowledged in recent recommendations, which state that evaluation of diastolic parameters should be an integral part of the echocardiogram of the hypertensive patient.[14] However, the recently updated 2016 Joint Guideline of the American Society of Echocardiography and the European Association of Cardiovascular Imaging ("50% rule")[15] has been criticized due to the absence of validation data to support its use and the potential for underdiagnosing DD, and new diagnostic algorithms have been proposed.[16]

Observational studies have shown that the risk of cardiovascular events is directly related to systolic BP (SBP) and diastolic BP (DBP) values, with a progressively higher risk for BP levels over 115 mmHg of SBP and 75 mmHg of DBP.[17] However, although hypertension is clearly associated with DD, few studies have assessed whether "prehypertensive patients" (BP 120-139/80-89 mmHg) already have subclinical impaired diastolic function.[18] Moreover, few studies have assessed the impact of different BP parameters on diastolic function.[19]

In this study we aimed to assess: *a)* whether individuals in the prehypertensive range already have changes in diastolic function; and *b)* the association between diastolic function and different BP parameters, such as SBP, DBP, and pulse pressure (PP).

METHODS

Study Sample

This was a cross-sectional study including participants selected within the first follow-up of a cohort representative at baseline of the adult population of Porto, Portugal—the EPIPorto cohort study. From 1999 to 2003 the cohort assembly was made by random-digit dialing using households as the sampling frame, followed by random selection of 1 person aged 18 years or older in each household. Refusals were not substituted within the same household. The proportion of participation was 70%. At baseline, 2485 participants were recruited. Between October 2006 and July 2008, participants aged 45 years or over were eligible for a systematic evaluation of parameters of cardiac structure and function, which included a cardiovascular clinical history, physical examination, detailed anthropometric evaluation, collection of a fasting blood sample, and a transthoracic echocardiogram (Figure 1). Among 2048 cohort members in the eligible age range at this time, 134 (6.5%) had died, 198 (9.7%) refused to be re-evaluated, and 580 (28.3%) were lost to follow-up (unreachable by telephone or post). Of the 1136 participants who underwent cardiac evaluation, we excluded from this study those with previous myocardial infarction, percutaneous or surgical revascularization, prior cardiac surgery or moderate to severe valvular heart disease, atrial fibrillation, abnormal left ventricle ejection fraction and symptoms of angina, or heart failure (Figure 1). Written informed consent was obtained from all the individuals and the study was approved by the local ethics committee.

Clinical Variable Definitions

Participants were instructed to take their usual medication and abstain from alcohol, tea, coffee, smoking, and exercise in the 30 minutes before the medical evaluation and BP measurement. Systolic BP was identified by phase I Korotkoff sound and DBP by phase V. Two measurements of BP separated by at least 5 minutes were taken, on a single occasion, in the sitting position, with an ERKA 300 sphygmomanometer after a 10-minute rest, with no tight clothes, on the right upper arm, and at heart level. The mean was considered and when the difference was larger than 5 mmHg for systolic or DBP a third measurement was taken and the mean of the 2 closest values was registered. According to the current guidelines,[3] participants were divided into groups according to their BP levels: "optimal BP" (SBP < 120 mmHg and DBP < 80 mmHg); "prehypertension" (SBP 120-139 mmHg or DBP 80-89 mmHg); "hypertension" (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or use of antihypertensive medication).

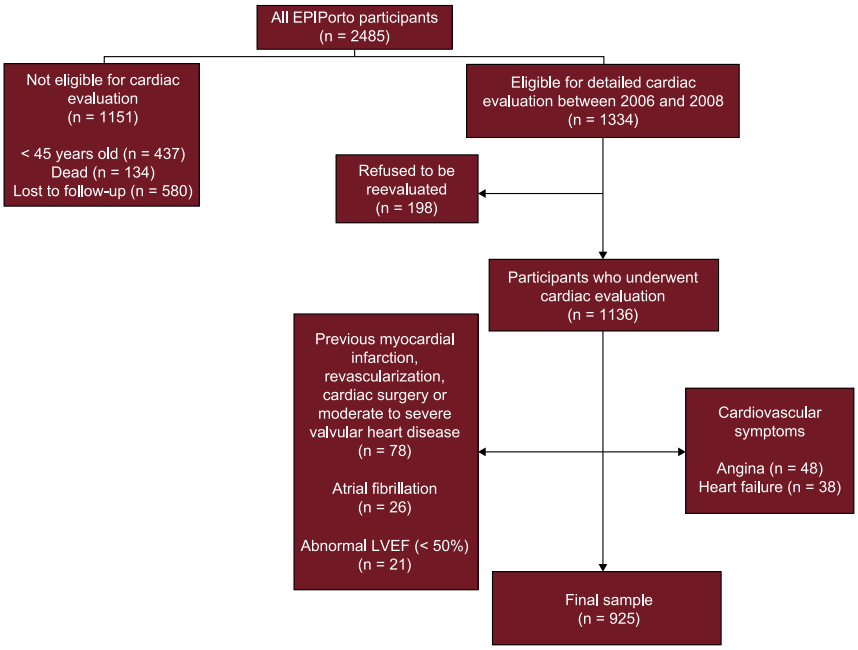

Figure 1. Flowchart of EPIPorto cohort study participants who were included in this study. LVEF, left ventricular ejection fraction.

Diabetes was defined as fasting blood glucose ≥ 126 mg/dL or the patient's self-reported history of diabetes or use of diabetes medications.

Analytical and Anthropometric Evaluation

A fasting venous blood sample was obtained in the morning for measurement of glucose, total cholesterol, LDL, HDL, and triglycerides.

Anthropometric measurements were performed after an overnight fast, with the participant wearing light clothing and no footwear. Body weight was measured to the nearest 0.1 kg using a digital scale, and height was measured to the nearest centimeter in the standing position using a wall stadiometer. Body mass index was calculated as weight (kg) divided by squared height (m^2). Overweight was defined as body mass index ≥ 25 and below 30 kg/m^2, and obesity as body mass index ≥ 30 kg/m^2.

Echocardiography Data

All echocardiographic studies were acquired using the same equipment (Hewlett-Packard Sonos 5500) and immediately after assessment of BP. Images were stored on videotape for subsequent offline analysis by 2 experienced cardiologists, blinded to clinical data. Cardiac chamber dimensions, volumes and left ventricular mass were measured following the standard recommendations[20] and indexed to body surface area. Systolic function was evaluated by ejection fraction calculation using the modified biplane Simpson's rule. Diastolic function was assessed according to the 2016 Joint Guidelines on Diastolic Function Evaluation[15] with measurement of mitral inflow velocities (E-wave, A-wave, E/A ratio) and E-wave deceleration time and isovolumetric relaxation time using pulsed-wave Doppler in the apical 4-chamber view. Velocities were recorded at end-expiration and averaged over 3 consecutive cardiac cycles. Pulsed-wave tissue Doppler velocities were acquired at end-expiration, in the apical 4-chamber view, at the lateral side of the mitral annulus, measuring early diastolic (e′) and late diastolic (a′) velocities and estimating the E/e′ ratio accordingly.

The main definition of DD used in the study followed the recommendations in the 2016 consensus document,[15] where DD was defined if more than 2 of the following were present: lateral E/e' ratio > 13, lateral e' velocity < 10 cm/s, left atrial maximum volume index > 34 mL/m^2, and peak tricuspid regurgitation velocity > 2.8 m/s. Diastolic function was classified as normal if less than 2 were present and indeterminate if 2 of the 4 conditions were present (Figure 2). In addition, considering the limitations of the 2016 recommendations, we also included data using 2 additional DD definitions: the previous European Association of Cardiovascular Imaging/American Society of Echocardiography (EACVI/ASE) consensus from 2009[21] and a recently published clinically-oriented algorithm to assess DD and left ventricle filling pressure (see Figure 2 for a detailed description of the algorithm).[16]

Statistical Analysis

Continuous variables are reported as mean ± standard deviation or median [interquartile range]. Discrete variables are given in frequency and percentage. ANOVA or the chi-squared test were used to test for significant differences between demographic and clinical variables across BP groups (optimal, prehypertension, hypertension).

The Spearman rank correlation was used to assess the relationship between SBP, DBP, and PP and diastolic indices. The "nptrend" command in Stata was used to perform a nonparametric test of trend for the ranks across ordered groups.

To assess whether the categories of BP were associated with lateral E' and E/E' ratio, we used multivariable linear regression

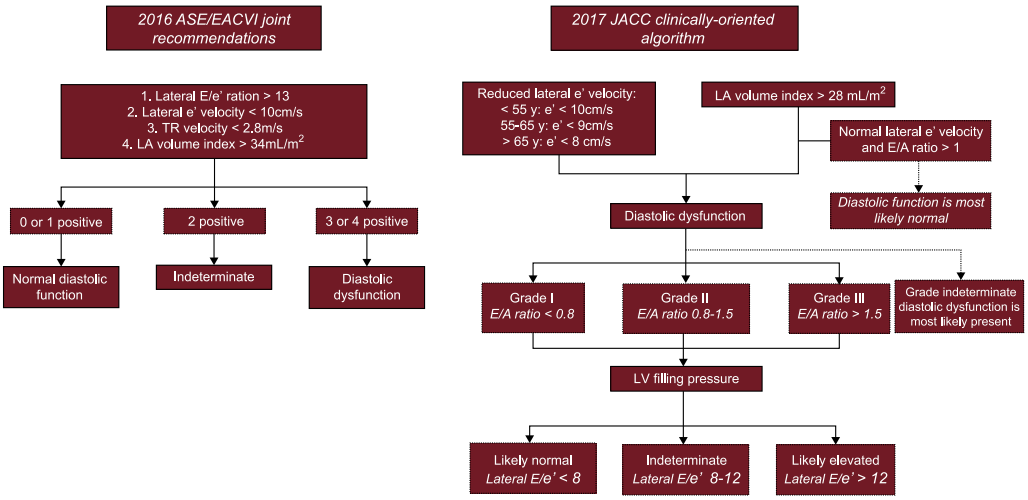

Figure 2. A classification scheme for characterization of diastolic function according to the 2016 ASE/EACVI Joint Recommendations and 2017 clinically-oriented algorithm. ASE/EACVI, American Society of Echocardiography/European Association of Cardiovascular Imaging; LA, left atrial; LV, left ventricle; TR, tricuspid regurgitation.

analysis including age, sex, body mass index, presence of diabetes, and BP category in the model, the latter as a categorical variable with "optimal BP" as the reference category. For evaluation of the association between DD and BP variables, we used Firth-type penalized likelihood logistic regression analysis, to correct for small-sample bias in beta coefficient estimation. McFadden's R-squared was used to assess the goodness-of-fit of the final regression models. Cases of indeterminate diastolic function (n = 134) were excluded from this analysis.

Considering the mean e' velocities in the groups of interest (optimal BP, prehypertension, and hypertension), assuming a within-group variance of 12 and the number of individuals in each group of interest, we had a power of approximately 100% to detect a significant difference in e' velocities with a type I error probability below 5%.

All statistical analyses were conducted using Stata 14.0 for Mac (StataCorp, College Station, Texas, United States).

RESULTS

The final analysis included 925 participants with a mean age of 61.5 ± 10.5 years (37% men). The prevalence of hypertension was 51.0% (472 individuals) and 30.4% (281 individuals) were prehypertensive (normal or high-normal BP categories). The clinical, anthropometric, analytical and echocardiographic characteristics of the study sample are shown in Table 1. Individuals with prehypertension and hypertension showed an increased left ventricular mass index and the latter was an independent predictor of e' velocity and E/e' ratio. Regarding antihypertensive therapy, 22.2% were taking a renin-angiotensin axis modifier, 5.5% a calcium channel antagonist and 10.4% were on diuretics.

According to the 2016 ASE/EACVI Joint Recommendations, diastolic function was considered normal in 783 individuals (84.7%), abnormal in 8 (0.9%) and indeterminate in 134 (14.5%). However, when the 2017 clinically-oriented algorithm was used, the prevalence of DD was 49.2%: 16.2% had grade I DD, 5.2% had grade II, and 0.3% grade III DD. In 254 individuals, DD was graded as indeterminate.

Association Between Different Blood Pressure Parameters and Diastolic Function

Systolic BP values correlated with diastolic function parameters, showing a negative correlation with e' velocity (Spearman's ρ = −0.3; $P < .001$) and a positive correlation with E/e' ratio (Spearman's ρ = 0.2; $P < .001$), as detailed in Figure 3. After adjusting for age, sex, body mass index, and diabetes, we observed that for each 10 mmHg increase in SBP there was a 0.2 cm/s decrease in e' velocity and a 0.1 increase in the E/e' ratio, as detailed in Table 2. Systolic BP was not associated with DD using the more stringent 2016 criteria. However, each 10 mmHg increase in SBP was associated with a 20% increase in the adjusted odds for DD according to the 2017 clinically-oriented algorithm.

As shown in Figure 3, there was also an inverse correlation between DBP and e' velocity (Spearman's ρ = −0.1; $P = .01$), but not with E/e' ratio (Spearman's ρ = −0.1; $P = .23$). In the multivariable analysis, for each 10 mmHg increase in DBP we observed a 0.3 cm/s decrease in e' velocity, and a 30% increase in the adjusted odds for DD according to the 2017 algorithm (no significant association was found when using the 2016 joint criteria).

PP was inversely correlated with the e' velocity (Spearman's ρ = −0.4; $P < .001$) and positively correlated with the E/E' ratio (Spearman's ρ = 0.3; $P < .001$), as detailed in Figure 3. In the multivariable regression analyses, PP was significantly associated with E/e' ratio (each 10 mmHg increase in PP was associated with a 0.2 increase in the E/e' ratio) but not with e' velocity. PP was associated with an increased odds of DD when we used both the 2016 criteria (odds ratio [OR], 1.07; $P < .001$) and 2017 algorithm (OR, 1.01; $P = .013$).

Higher SBP and PP were associated with an increase in left atrial volume index in the univariate analyses, but not DBP. However, after adjustment for age, sex, body mass index and diabetes, only PP remained a significant predictor of left atrial volume index.

Diastolic Function Parameters in Different Categories of Blood Pressure

When compared with individuals with optimal BP, prehypertensive and hypertensive participants showed a progressive

Table 1
Study Participant Characteristics, According to Blood Pressure Levels

	Total (n = 925)	Optimal BP (n = 172)	Prehypertension (n = 281)	Hypertension (n = 472)	*P*
Age, y	61.5 ± 10.5	56.5 ± 10.0	58.7 ± 9.8	65.0 ± 9.8	< .001
Male sex	346 (37)	41 (24)	132 (47)	173 (37)	< .001
Cardiovascular risk factors					
Diabetes	99 (11)	6 (3)	17 (6)	76 (16)	< .001
Total cholesterol, mg/dL	220.6 ± 53.1	222.3 ± 72.2	220.7 ± 58.9	220.0 ± 39.7	.881
LDL-C, mg/dL	134.8 ± 53.3	139.2 ± 74.7	136.3 ± 61.2	132.3 ± 35.9	.305
HDL-C, mg/dL	61.9 ± 45.9	67.7 ± 72.7	62.2 ± 57.9	59.6 ± 13.7	.141
Triglycerides, mg/dL	152.8 ± 468.1	164.9 ± 757.9	166.0 ± 601.0	140.6 ± 78.6	.724
BMI, kg/m^2	27.3 ± 4.6	25.6 ± 4.3	26.9 ± 4.6	28.2 ± 4.5	< .001
SBP, mmHg	132.5 ± 19.4	109.3 ± 6.5	127.4 ± 7.5	144.1 ± 18.5	< .001
DBP, mmHg	78.4 ± 11.2	67.1 ± 6.2	78.3 ± 8.0	82.6 ± 11.4	< .001
Pulse pressure, mmHg	52.1 ± 16.7	41.7 ± 10.0	47.2 ± 12.8	58.8 ± 17.8	< .001
Echocardiographic data					
Septum, mm	8.6 ± 1.4	7.9 ± 1.1	8.4 ± 1.2	9.0 ± 1.5	< .001
Posterior wall, mm	7.9 ± 1.2	7.2 ± 1.0	7.7 ± 1.1	8.2 ± 1.3	< .001
LV mass index, g/m^2	78.3 ± 18.8	70.3 ± 13.8	75.2 ± 17.1	83.2 ± 19.9	< .001
LA volume index, mL/m^2	28.2 ± 9.5	26.9 ± 9.2	27.7 ± 8.6	29.0 ± 10.0	.023
LVED volume index, mL/m^2	65.6 ± 15.9	64.9 ± 16.0	65.8 ± 14.8	65.8 ± 16.5	.800
LVES volume index, mL/m^2	26.4 ± 8.8	26.1 ± 8.5	26.5 ± 8.8	26.5 ± 8.8	.835
Ejection fraction, %	60.7 ± 6.1	60.9 ± 5.7	60.9 ± 6.3	60.5 ± 6.0	.594
E-wave, cm/s	71.6 ± 15.3	73.3 ± 15.6	71.1 ± 14.9	71.2 ± 15.4	.274
A-wave, cm/s	78.2 ± 19.9	68.9 ± 19.5	72.5 ± 16.8	85.0 ± 19.3	< .001
E/A ratio	0.96 ± 0.30	1.12 ± 0.33	1.03 ± 0.31	0.87 ± 0.26	< .001
Deceleration time, ms	236.3 ± 54.1	226.6 ± 51.1	228.7 ± 47.7	244.4 ± 57.6	< .001
IVRT, ms	91.3 ± 15.8	87.9 ± 12.3	91.1 ± 15.2	92.7 ± 17.0	.003

BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; IVRT, isovolumic relaxation time; LDL-C, low-density lipoprotein cholesterol; LA, left atria; LV, left ventricle; LVED, left ventricle end-diastolic; LVES, left ventricle end-systolic; SBP, systolic blood pressure.
Data are presented as mean ± standard deviation for continuous variables and No. (%) for categorical variables; *P* value for ANOVA or chi-squared test for significant differences between BP groups.

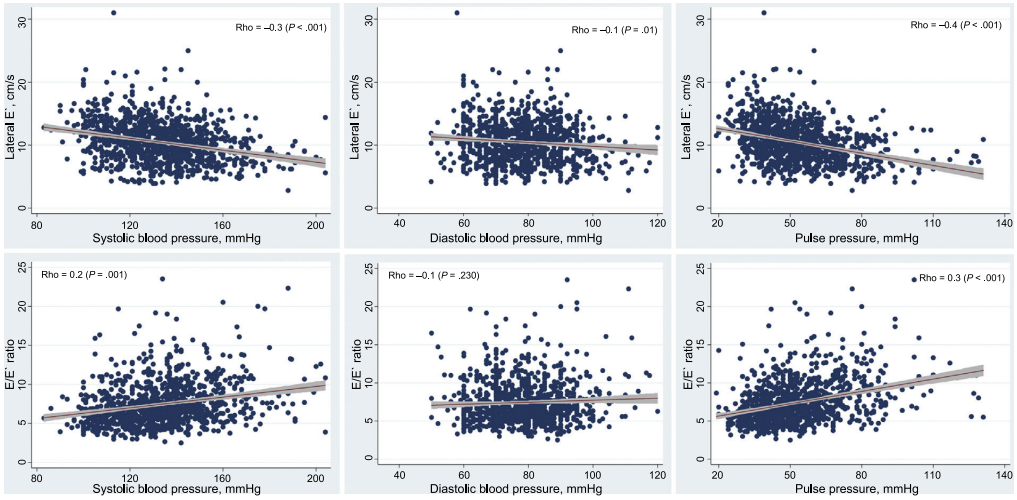

Figure 3. Correlations between blood pressure variables (systolic blood pressure, diastolic blood pressure, and pulse pressure) and diastolic function parameters.

deterioration in diastolic function parameters (*P* for trend < .001). Prehypertension was associated with lower E' velocity (11.3 ± 3.1 cm/s vs 12.2 ± 3.5 cm/s in individuals with optimal BP; *P* = .003), which was even lower in those with hypertension (9.6 ± 2.9 cm/s vs 12.2 ± 3.5 cm/s; *P* < .001), as depicted in Figure 4. In the multivariable analysis, both prehypertension (β = –0.56; *P* = .035) and hypertension (β = –1.08; *P* < .001) were associated with a significant decrease in the E' velocity, as detailed in Table 3.

Table 2
Regression Analyses Evaluating the Association Between SBP, DBP and PP and Echocardiographic Diastolic Indexes (E' Velocity, E/E' Ratio, and Presence of Diastolic Dysfunction)

	Univariable analysis			Multivariable analysis*		
	β coefficient	SE	*P*	β coefficient	SE	*P*
E' velocity						
SBP (per mmHg)	−0.049	0.005	< .001	−0.015	0.005	.003
DBP (per mmHg)	−0.030	0.010	.001	−0.031	0.008	< .001
PP (per mmHg)	−0.065	0.006	< .001	−0.010	0.006	.126
E/E' ratio						
SBP (per mmHg)	0.032	0.004	< .001	0.013	0.004	.004
DBP (per mmHg)	0.009	0.008	.238	0.009	0.007	.225
PP (per mmHg)	0.051	0.005	< .001	0.020	0.005	< .001
LA volume index						
SBP (per mmHg)	0.061	0.016	< .001	0.032	0.018	.065
DBP (per mmHg)	−0.016	0.028	.567	−0.017	0.029	.559
PP (per mmHg)	0.124	0.018	< .001	0.094	0.022	< .001
	Odds ratio	SE	*P*	Odds ratio	SE	*P*
Diastolic dysfunction						
2016 joint ASE/EACVI recommendations						
SBP (per mmHg)	1.05	0.016	.002	1.03	0.017	.108
DBP (per mmHg)	0.98	0.032	.447	0.99	0.032	.658
PP (per mmHg)	1.08	0.017	< .001	1.07	0.018	< .001
2017 clinically-oriented algorithm						
SBP (per mmHg)	1.03	0.004	< .001	1.02	0.004	< .001
DBP (per mmHg)	1.02	0.006	< .001	1.03	0.007	< .001
PP (per mmHg)	1.04	0.005	< .001	1.01	0.005	.013

ASE/EACVI, American Society of Echocardiography/European Association of Cardiovascular Imaging; DBP, diastolic blood pressure; PP, pulse pressure; SBP, systolic blood pressure; SE, standard error.
For the univariable analyses, each blood pressure variable (SBP, DBP and PP) is included individually in the model for prediction of each of the diastolic indexes (linear regression) and diastolic dysfunction (logistic regression).
* In the multivariable analyses, age, sex, body mass index, and the presence of diabetes were included in the model, in addition to the blood pressure variable assessed in each regression equation. All multivariable models showed McFadden's R-squared between 0.2 and 0.4, therefore providing good-fit models.

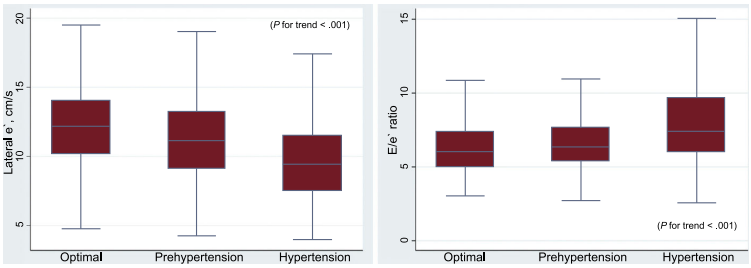

Figure 4. Diastolic function (e' velocity and E/e' ratio) according to blood pressure categories.

There was a significant trend toward a progressive increase in the E/e' ratio as BP levels increased (6.4 ± 2.1 for optimal BP, 6.7 ± 2.1 for prehypertension and 8.0 ± 2.9 for hypertensive individuals; *P* for trend < .001). However, after adjustment for age, sex, body mass index, and diabetes, only hypertension was significantly associated with the lateral E/e' ratio (Table 3).

According to the 2016 Joint Recommendations, DD was present in 0.8% of prehypertensive and 1.3% of hypertensive patients (Table 4). Using the less stringent 2017 algorithm, there was a progressive and significant increase in the prevalence of DD across BP categories, from 28% in individuals with optimal BP, 39% in prehypertension and 63% in hypertension (Table 4). Furthermore, we found an increasing prevalence of likely elevated left ventricle filling pressures (1%, 5%, and 11% in the optimal, prehypertensive and hypertensive groups, respectively).

We found no significant association between BP categories and DD using the 2016 criteria (8 cases of DD). Prehypertension was significantly associated with increased odds of DD in the univariate analysis, using the 2017 algorithm (OR, 1.68; *P* = .013), although the *P* value was slightly higher than .05 in the multivariable analysis (Table 3).

DISCUSSION

In this cross-sectional study using a community-based cohort of asymptomatic individuals without known cardiovascular disease,

Table 3
Association Between Blood Pressure Categories and Diastolic Function Parameters

	Univariable analysis			Multivariable analysis*		
	β coefficient	SE	*P*	β coefficient	SE	*P*
E' velocity						
Optimal	Reference			Reference		
Prehypertension	−0.94	0.30	.002	−0.56	0.27	.035
Hypertension	−2.58	0.27	< .001	−1.08	0.26	< .001
E/E' ratio						
Optimal	Reference	Reference				
Prehypertension	0.31	0.24	.206	0.24	0.23	.289
Hypertension	1.61	0.22	< .001	0.78	0.23	.001
	Odds ratio	SE	*P*	Odds ratio	SE	*P*
Diastolic dysfunction						
2016 joint ASE/EACVI recommendations						
Optimal	Reference			Reference		
Prehypertension	3.25	5.05	.448	2.47	3.88	.566
Hypertension	5.90	8.68	.227	2.42	3.68	.561
2017 clinically-oriented algorithm						
Optimal	Reference			Reference		
Prehypertension	1.68	0.35	.013	1.48	0.33	.080
Hypertension	4.31	0.84	< .001	2.47	0.53	< .001

ASE/EACVI, American Society of Echocardiography/European Association of Cardiovascular Imaging; SE, standard error.
* In the multivariable analyses, age, sex, body mass index, and the presence of diabetes were included in the model, in addition to the blood pressure categories assessed in each regression equation. All multivariable models showed McFadden's R-squared between 0.2-0.4, therefore providing good-fit models.

Table 4
Prevalence and Grade of Diastolic Dysfunction Across Blood Pressure Categories Using the 2016 Joint Recommendations and the 2017 Clinically-oriented Algorithm

	2016 ASE/EACVI Joint Recommendations			2017 Clinically-Oriented Algorithm				
				Diastolic function		LV filling pressures		
	Normal	DD	Indeterminate	Normal	DD	Likely normal	Indeterminate	Likely elevated
BP category								
Optimal	165 (95.9)	0 (0.0)	7 (4.1)	124 (72.1)	48 (27.9)	57 (71.2)	22 (27.5)	1 (1.2)
Prehypertension	254 (90.4)	2 (0.8)	25 (8.9)	170 (60.5)	111 (39.5)	118 (70.2)	42 (25.0)	8 (4.8)
Hypertension	364 (77.1)	6 (1.3)	102 (21.6)	176 (37.3)	296 (62.7)	169 (49.6)	132 (38.7)	40 (11.7)

ASE/EACVI, American Society of Echocardiography/European Association of Cardiovascular Imaging; BP, blood pressure; DD, diastolic dysfunction; LV, left ventricle.
Data are expressed as No. (%).

we found a continuous association between the deterioration of diastolic function and BP levels, including SBP, DBP, and PP. More importantly, we observed that although diastolic function impairment is more pronounced in hypertensive individuals, these changes were already present in prehypertensive individuals, reflecting subclinical organ damage in this population.

Impairment of Diastolic Function in Prehypertensive Individuals

There is currently strong evidence supporting a continuum of cardiovascular risk in function of BP values, not exclusive to the hypertensive range.[11,12,17] This relationship was highlighted in a meta-analysis of 61 prospective studies, which showed a strong relationship between cardiovascular mortality and BP values, down to BP values of 115/75 mmHg.[17] Also, previous studies have shown that prehypertensive individuals have more target-organ damage than normotensive individuals, namely vascular damage.[22,23]

In our study we showed a continuous relationship between increasing degrees of BP (especially SBP and DBP) and a deterioration in E' velocity, suggesting impaired cardiac relaxation. This observation supports the notion that these changes may reflect the cumulative effect of hypertension on the myocardium.[6] Moreover, we observed that prehypertensive individuals had significantly lower E' velocities compared with patients with "optimal BP", showing that changes in diastolic function are already present in the prehypertensive stage. These results are in agreement with a previously published study based on an analysis from the ARIC cohort[18] comprising a sample of 4871 older individuals (mean age 75 years), showing a progressive impairment of diastolic function parameters throughout different BP thresholds (from optimal BP to hypertension). Although there were significant differences between the groups in terms of diastolic function parameters (e' lateral, E/e' lateral) and prevalence of DD, there were no differences in systolic function parameters.

In our study, PP was associated with a higher E/e' ratio and left atrial volume index (denoting increased left ventricle filling pressure), and with increased odds of DD. PP is an indirect index of arterial stiffness and an independent predictor of cardiovascular mortality.[24] Data from the Framingham Heart Study provide support that in middle-aged and elderly individuals, PP has more prognostic power for cardiovascular events than SBP or DBP,[25] and identifies the highest-risk patients for developing heart failure.[26] Increased arterial stiffness might increase left ventricle hypertrophy due to cardiac pressure overload, therefore contributing to the morphological and functional changes involved in the pathophysiology of DD and elicit subclinical cardiac damage.

Our data corroborate the sensitivity of diastolic function parameters as markers of myocardial subclinical organ damage in this clinical setting. From a pathophysiological standpoint, several mechanisms might account for the progressive deterioration of cardiac relaxation and increased myocardial stiffness[27]: increased fibrosis, hypophosphorylation of titin, altered myocardial metabolism, decreased nitric oxide availability and a proinflammatory milieu.

The Role of Diastolic Function as a Target Organ in Cardiovascular Risk Assessment

The presence of DD, even when subclinical, is considered an independent predictor of cardiovascular events and mortality.[7] For example, in hypertensive individuals from the ASCOT substudy, the E/e' ratio was an independent predictor of cardiovascular events.[6] Curiously, most E/e' ratio values were within the normal range, reflecting that this is indeed a sensitive parameter. Even in hypertensive patients with left ventricular hypertrophy, which is an established marker of target organ damage,[3] impairment of diastolic function parameters adds prognostic information, allowing a better assessment of risk in this population.[28]

In our study, we decided to include 2 different criteria to define DD: the 2016 ASE/EACVI Joint Recommendations[15] and a recently published clinically-oriented algorithm.[16] The former replaced the previous recommendations for echocardiographic assessment of diastolic function.[21] However, it has been strongly criticized because it was not validated and because its "50% rule" is very stringent, resulting in a large proportion of patients being included as "indeterminate" group (14.5% in our study). Indeed, according to the 2016 Joint Recommendations, we found a prevalence of DD of 0.9%, markedly different from the 49.2% using the 2017 algorithm and 22.0% using the previous recommendations from 2009 (Table of the supplementary material). This prevalence of DD is in line with a recently published study using data from 1485 participants of the community-based STANISLAS cohort (1.3%).[29] The small number of cases of DD using the 2016 criteria might account for the lack of significant association in the multivariable analysis both with prehypertension and hypertension. On the other hand, using the 2017 clinically-oriented algorithm, hypertensive patients had a 2.47 increased odds of DD and prehypertension was associated with a 1.48 increased odds of DD ($P = .080$). Given the importance of DD in the interface between hypertension and the development of BP,[4,30] our findings offer a possible explanation for the increased cardiovascular risk in prehypertensive individuals.[31]

Strengths and Limitations

The strengths of this study include the assessment of a relatively large sample of the general population, without other cardiac diseases, using contemporary echocardiographic techniques for the assessment of diastolic function.[21] In this study, which comprised individuals 45 years or older, the prevalence of hypertension was 53.6%, which is similar to that reported in European individuals aged between 35 and 64 years (44.2%),[32] and for Portuguese individuals between 35 and 64 years (46.9%).[33] Diastolic function was evaluated according to the recommendations of the consensus document of the European Association of Echocardiography and the American Society of Echocardiography,[21] which recommend the evaluation of E' velocities and E/E' ratio from tissue Doppler. In this study, we observed a stronger association between BP parameters and E' velocity, which is considered an early and preload-independent index of left ventricular relaxation,[28] whereas the E/E' ratio is used to estimate increased left ventricle filling pressures.[34]

In this study, most patients were female (63%) and we adopted a cross-sectional design, which partially limits comments on causality. Furthermore, among the 2048 cohort members within the eligible age range, 580 individuals (28%) were unreachable by telephone or post. Although we excluded patients with clinical signs of coronary artery disease, we did not perform any stress tests to exclude myocardial ischemia, which is also a determinant of DD. In the assessment of diastolic function, we did not evaluate intraobserver or interobserver variability. However, all 4 cardiologists had extensive experience in echocardiography and worked in the same institution and a detailed procedure protocol was discussed between the team, prior to the start of the study, to harmonize the methodology and the measurements. Regarding the logistic regression models, due to the small number of positive cases of DD using some of the definitions, maximum likelihood estimation of conventional logistic model may suffer from small-sample bias. To address this problem, as well as the risk of over-adjustment after forcing 4 variables in the multivariable model (age, sex, body mass index, and diabetes), we used Firth-type penalized likelihood logistic regression analysis. Finally, in this study, all BP measurements were obtained using office BP, and not with ambulatory BP monitoring, which is currently the gold-standard method recommended in hypertension guidelines and provides a more accurate approach to the relationship between cardiovascular morbidity and mortality than office BP.[3] The use of ambulatory BP monitoring would potentially result in the reclassification of some individuals in a lower BP category.[35]

CONCLUSIONS

In this large sample of the general population, there was a continuous relationship between BP levels and deterioration of diastolic function parameters. Changes in diastolic function were already present in prehypertensive individuals, reflecting myocardial subclinical organ damage in this population. Given the prognostic impact of DD in hypertensive patients, these observations reinforce the importance of assessing diastolic function in the workup of both hypertensive and prehypertensive patients.

CONFLICTS OF INTEREST

None declared.

WHAT IS KNOWN ABOUT THE TOPIC?

- Hypertension is a major cardiovascular risk factor that is associated with structural and functional deleterious cardiac changes, contributing to impaired diastolic function and heart failure with preserved ejection fraction. Few studies have specifically evaluated the association between prehypertension and DD, especially considering the recent 2016 updated recommendations for the evaluation of diastole.

WHAT DOES THIS STUDY ADD?

- Using a community-based cohort free of known cardiovascular disease, we found a continuous relationship between BP levels and deterioration of diastolic function parameters. Furthermore, prehypertensive individuals seemed to have an increased odds of DD using both the 2016 ASE/EACVI Joint Recommendations and a 2017 clinically-oriented algorithm. Our findings emphasize the importance of assessing diastolic function in the workup of both hypertensive and prehypertensive patients

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found in the online version available at: http://dx.doi.org/10.1016/j.rec.2017.11.015.

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Metabolic Syndrome Is Associated With Impaired Diastolic Function Independently of MRI-Derived Myocardial Extracellular Volume: The MESA Study

Ricardo Ladeiras-Lopes,[1,2] Henrique T. Moreira,[3,4] Nuno Bettencourt,[1] Ricardo Fontes-Carvalho,[1,2] Francisco Sampaio,[1,2] Bharath Ambale-Venkatesh,[3] Colin Wu,[5] Kiang Liu,[6] Alain G. Bertoni,[7] Pamela Ouyang,[3] David A. Bluemke,[5] and João A. Lima[3]

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The relationship of metabolic syndrome (MetS) and insulin resistance (one of its key pathophysiological mediators) with diastolic dysfunction and myocardial fibrosis is not well understood. This study aimed to evaluate the association of MetS with diastolic function and myocardial extracellular matrix (ECM) using cardiac MRI (CMRI) in a large community-based population. This cross-sectional analysis included 1,582 participants from the Multi-Ethnic Study of Atherosclerosis (MESA) with left ventricular ejection fraction ≥50% and no history of cardiac events. Diastolic function was assessed using tagged CMRI parameters including end-diastolic strain rate (EDSR) and strain relaxation index (SRI). ECM was evaluated using extracellular volume (ECV) quantification. Participants' mean age was 67.4 ± 8.6 years, and 48.1% were males. MetS was present in 533 individuals (33.7%), and type 2 diabetes in 250 (15.8%). In the multivariable analyses, MetS (irrespective of the presence of type 2 diabetes) and higher insulin resistance were associated with impaired diastolic function (higher SRI and lower EDSR), independent of ECV. In conclusion, MetS, irrespective of the presence of type 2 diabetes, was independently associated with impaired diastole. These functional myocardial changes seem to result from intrinsic cardiomyocyte alterations, irrespective of the myocardial interstitium (including fibrosis).

Metabolic syndrome (MetS) is a constellation of cardiovascular risk factors that reached epidemic proportions during the last two decades. Approximately 20–40% of the adult populations of U.S. and Europe have MetS (1).

Insulin resistance and inflammation play a key role in the pathophysiology of MetS, contributing to a prothrombotic and oxidative state that increases the risk of cardiovascular disease due to microvascular and macrovascular damage (2). This metabolic dysfunctional status is associated with the deterioration of cardiac structure and function, also known as "insulin-resistant cardiomyopathy" (3). Furthermore, myocardial fibrosis plays a pivotal role in cardiac remodeling in hypertensive and advanced diabetic heart disease (4), being associated with diastolic dysfunction (5). However, the relationships of MetS and insulin resistance with left ventricular (LV) myocardial fibrosis and diastolic dysfunction have not been well characterized in population studies (5).

New cardiac MRI (CMRI) techniques allow an accurate evaluation of both cardiac structure and function in populations (6,7). First, extracellular volume (ECV) quantification offers noninvasive assessment of changes in the myocardial extracellular matrix (ECM), including fibrosis and steatosis (8). Indeed, ECV quantification is derived from data obtained from magnetic resonance parameter T1 (the longitudinal relaxation time) without a contrast agent and postcontrast, both from myocardium and blood, as well as from hematocrit data. ECV is a marker of myocardial tissue remodeling and a physiologically intuitive unit of measurement. Recently, "synthetic" ECV calculation was described and shown to be associated with cardiovascular

[1]Cardiovascular Research and Development Center, Faculty of Medicine of the University of Porto, Porto, Portugal
[2]Cardiology Department, Gaia Hospital Centre, Vila Nova de Gaia, Portugal
[3]The Johns Hopkins University, Baltimore, MD
[4]University of São Paulo, Ribeirão Preto, Brazil
[5]National Institutes of Health, Bethesda, MD
[6]Northwestern University, Chicago, IL
[7]Department of Epidemiology & Prevention, Wake Forest School of Medicine, Winston-Salem, NC

Corresponding author: Ricardo Ladeiras-Lopes, ricardoladeiraslopes@gmail.com.

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outcomes, overcoming the need for blood sampling in order to have the hematocrit data (9). Moreover, tagged CMRI measurements of diastolic function, such as end-diastolic strain rate (EDSR) and strain relaxation index (SRI), were recently described as predictors of heart failure in a population free of cardiovascular disease (7).

This study aimed to: *1*) evaluate the relationship between MetS and insulin resistance with LV diastolic function in a large community-based cohort using CMRI and *2*) assess whether this association is dependent on myocardial ECM.

RESEARCH DESIGN AND METHODS

Study Population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective, population-based, epidemiological study started in 2000 and aiming to investigate the prevalence and progression of subclinical cardiovascular disease in a multiethnic cohort (Caucasian, African American, Hispanic, Chinese American) of 6,814 individuals. The study protocol was approved by the institutional review boards of participating institutions. The characteristics of subjects enrolled in the MESA have been described previously (10).

From all individuals who underwent evaluation as part of the MESA "Exam 5" (the fifth round of examinations of the MESA study), which happened from 2010 to 2012, participants with prior clinical cardiac events (myocardial infarction, resuscitated cardiac arrest, definite angina, probable angina if followed by revascularization, and cardiac death) (n = 459), LV ejection fraction <50% (n = 133), unknown MetS status (n = 2,024), and positive or unknown late gadolinium enhancement during CMRI (n = 2,660) were excluded from the analysis. The final sample size was composed of 1,582 individuals, with data available on myocardial tagging and synthetic ECV quantification. The characteristics of the participants are described in Table 1.

Definition of MetS and Insulin Resistance

MetS was defined according to the 2005 definition of the American Heart Association/National Cholesterol Education Panel (i.e., if three or more of the following were present): *1*) abdominal obesity based on waist circumference ≥88 cm (35 inches) for women and ≥102 cm (40 inches) for men (≥80 cm and ≥90 cm for Asian American females and males, respectively); *2*) HDL cholesterol (HDL-C) <1.0 mmol/L (40 mg/dL) for men or <1.3 mmol/L (50 mg/dL) for women or receiving treatment to increase HDL-C levels; *3*) fasting triglyceride measurements ≥1.7 mmol/L (150 mg/dL) or receiving treatment to reduce triglyceride levels; *4*) blood pressure of ≥130 mmHg systolic or ≥85 mmHg diastolic, or receiving antihypertensive treatment; or *5*) impaired fasting glucose (IFG) defined as a fasting glucose level of 5.55–6.99 mmol/L (100–125 mg/dL) or type 2 diabetes (fasting plasma glucose of ≥7.0 mmol/L [≥126 mg/dL]) (1).

BMI was calculated as weight divided by the square of height (in kilograms per square meter). Resting blood pressure was measured three times in the seated position using a Dinamap PRO-100 Sphygmomanometer (Critikon; Wipro GE Healthcare, Waukesha, WI). Fasting blood glucose was assessed using Vitros analyzer (Johnson & Johnson Ortho-Clinical Diagnostics, Rochester, NY), and fasting insulin with the Elecsys assay (electrochemiluminesce

Table 1—Participants' characteristics

	Total (N = 1,582)
Age, years	67.4 ± 8.6
Male sex, *n* (%)	761 (48.1)
Ethnicity, *n* (%)	
Caucasian	706 (44.6)
Chinese	163 (10.3)
African American	375 (23.7)
Hispanic	338 (21.4)
eGFR (MDRD, mL/min/1.73 m^2)	84.9 ± 18.7
BMI, kg/m^2	28.4 ± 5.2
Waist circumference, cm	98.5 ± 13.4
Body weight, kg	78.4 ± 16.5
Height, cm	166.1 ± 9.7
Heart rate, bpm	64.2 ± 10.0
Cigarette smoking, *n* (%)	
Never	693 (44.0)
Former	770 (48.8)
Current	113 (7.2)
SBP, mmHg	121.9 ± 19.1
DBP, mmHg	68.5 ± 9.6
HDL-C, mg/dL	55.1 ± 16.3
Triglycerides, mg/dL	110.3 ± 61.8
Total cholesterol, mg/dL	184.5 ± 36.2
Fasting glucose, mg/dL	100.1 ± 24.3
log_2(HOMA-IR)	3.4 ± 1.0
MetS, *n* (%)	533 (33.7)
Increased waist circumference, *n* (% of MetS)	472 (88.6)
Triglycerides ≥150 mg/dL or receiving fibrates, *n* (% of MetS)	233 (43.7)
Decreased HDL-C or taking niacin, *n* (% of MetS)	290 (54.4)
SBP ≥130 or DBP ≥85 mmHg, *n* (% of MetS)	464 (87.1)
IFG or type 2 diabetes, *n* (% of MetS)	390 (73.2)
Presence of MetS but no type 2 diabetes, *n* (%)	350 (22.1)
Type 2 diabetes, *n* (%)	250 (15.8)
LV ejection fraction, %	62.5 ± 6.1
LVEDVi, mL/m^2	65.0 ± 12.7
LVMi, g/m^2	65.4 ± 12.1
LV mass-to-volume ratio, mL/g	1.0 ± 0.2
Antihypertensive medication, *n* (%)	758 (47.9)

Values are reported as the mean ± SD, unless otherwise indicated. DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; LVEDVi, indexed LV end-diastolic volume; LVMi, indexed LV mass; MDRD, Modification of Diet in Renal Disease; SBP, systolic blood pressure.

immunoassay; Roche Diagnostics, Mannheim, Germany). Estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease equation (11).

Insulin resistance was calculated for patients not taking insulin or hypoglycemic agents (N = 1,069) using the HOMA of insulin resistance (HOMA-IR) (fasting glucose [in milligrams per deciliter] × fasting insulin [in milliunits per liter]/405) (12).

CMRI

Images were acquired using 1.5-T MRI scanners using electrocardiogram-triggered segmented k-space fast spoiled gradient-echo pulse sequences during breath-holds. Torso phase array coils were used for signal reception. CMRI myocardial horizontal and vertical tagging were performed on three LV short-axis slices (base to apex) by nonselective radiofrequency pulses separated by a spatial modulation of magnetization-encoding gradients. Parameters for imaging and analysis methods have been previously described (13). LV volume and mass were indexed according to body surface area.

Additional information on the analyses of ECV using T1 mapping and diastolic function using myocardial tagging is included as Supplementary Data.

Statistical Analysis

Summary statistics were presented as the mean ± SD for continuous variables and as percentages for categorical variables.

Subgroups according to the presence of MetS and type 2 diabetes were defined as follows: 1) absence of both MetS and type 2 diabetes; 2) presence of MetS and the absence of type 2 diabetes; and 3) presence of type 2 diabetes, irrespective of the presence or absence of MetS. To verify a progressive increase in insulin resistance from subgroup 1 toward subgroup 3, a comparison of HOMA-IR (base-2 log transformed) between the subgroups was performed.

A t test and one-way ANOVA (with Bonferroni post hoc analysis) were used to test continuous variables in two or more than two subgroups. A χ^2 test was used for comparisons with categorical variables.

Multivariable linear regression analysis was used to assess the association of MetS and insulin resistance with diastolic function and ECV using the following two models: model 1, adjusting for age, sex, ethnicity, smoking status, and antihypertensive medication; and model 2, adjusting for all variables included in model 1 plus ECV.

All analyzes were performed using Stata version 14.0 (StataCorp, College Station, TX). Statistical significance was defined as $P < 0.05$. All reported P values are two tailed.

RESULTS

Participants' Characteristics

The characteristics of the participants are presented in Table 1. The mean age of the final sample was 67.4 ± 8.6 years, and 48.1% were males. MetS was present in 533 individuals

Table 2—CMRI-derived diastolic variables and ECV according to the presence of MetS and type 2 diabetes

	No MetS	MetS+	*P* value	MetS−/type 2 diabetes−	MetS+/type 2 diabetes−	Type 2 diabetes+	*P* value
Diastolic parameters							
EDSR, 1/ms	0.115 ± 0.051	0.110 ± 0.054	0.049	0.116 ± 0.051	0.111 ± 0.057	0.106 ± 0.051	0.020
LV TRR, °/cm per ms	−22.231 ± 8.769	−22.099 ± 9.123	0.792	−22.299 ± 8.801	−22.187 ± 9.349	−21.749 ± 8.584	0.709
SRI, ms/%	2.602 ± 1.731	3.103 ± 2.167	<0.001	2.568 ± 1.695	3.139 ± 2.298	3.048 ± 1.960	<0.001
ECV, %	27.024 ± 2.718	26.599 ± 2.749	0.013	27.046 ± 2.731	26.506 ± 2.901	26.763 ± 2.425	0.020

Values are reported as the mean ± SD. An independent t test was used to compare diastolic variables and ECV according to the presence or absence of MetS. One-way ANOVA (with Bonferroni post hoc analysis) was used to compare the three subgroups according to the presence or absence of MetS and type 2 diabetes.

(33.7%), and type 2 diabetes was present in 250 (15.8%). Glycated hemoglobin levels in patients with IFG and type 2 diabetes were 5.9 ± 0.4 and 7.1 ± 1.4, respectively. The mean LV ejection fraction, LV end-diastolic volume index, and LV mass index were 62.5 ± 6.1%, 65.0 ± 12.7 mL/m^2, and 65.4 ± 12.1 g/m^2.

Influence of MetS and Type 2 Diabetes on Diastolic Function

Table 2 presents a comparison of the diastolic variables (EDSR, SRI, and torsion recoil rate [TRR]) and ECV according to MetS and type 2 diabetes status. Individuals with MetS and type 2 diabetes showed a lower EDSR and higher SRI (i.e., worse diastolic function). In the multivariable analyses, MetS was an independent predictor of higher SRI (adjusted β = 0.503; SE = 0.114; $P < 0.001$) and lower EDSR (adjusted β = −0.008; SE = 0.003; $P = 0.012$) irrespective of age, sex, ethnicity, smoking status, and antihypertensive medication (Table 3, model 1). In addition, the presence of MetS (with or without type 2 diabetes) was associated with deteriorated diastolic function (higher SRI and lower EDSR) after adjusting for the previous variables and also ECV (Table 3, model 2), a surrogate for myocardial interstitium changes, including fibrosis. There was no association of MetS and type 2 diabetes with TRR.

Influence of Insulin Resistance on Diastolic Function

When considering the association of insulin resistance with diastolic function variables, the multivariable linear regression analyzes performed showed that $\log_2$(HOMA-IR) was an independent predictor of worse diastolic function, as reflected by lower EDSR (adjusted β = −0.005; SE = 0.002; $P = 0.002$) and higher SRI (adjusted β = 0.254; SE = 0.059; $P < 0.001$), irrespective of age, sex, ethnicity, smoking status and antihypertensive medication. Furthermore, increased insulin resistance was associated with a deteriorated diastolic function even after adjustment for ECV (model 2). There was no association of $\log_2$(HOMA-IR) with TRR (adjusted β = 0.069; SE = 0.198; $P = 0.726$).

DISCUSSION

In the current study using a large community-based cohort, adults with MetS without type 2 diabetes, as well as adults with type 2 diabetes, had higher SRI and lower EDSR than individuals without MetS, meaning impaired diastolic function. MetS and type 2 diabetes were not associated with increased myocardial ECM, as assessed by ECV quantification using CMRI. To our knowledge, this is the first study to simultaneously assess myocardial extracellular space (ECV by CMRI) and diastolic dysfunction (EDSR, TRR, and SRI) in relation to presence or absence of MetS and type 2 diabetes.

MetS is reaching epidemic proportions with ~34% of American adults fulfilling MetS criteria (14). A recently published consensus article (15) emphasized that MetS is a complex pathophysiological state, clinically underrecognized, and associated with serious and extensive comorbidity. In this study, we report the first detailed analysis of

Table 3—Linear regression analyses for the association of MetS and type 2 diabetes with LV diastolic function

	SRI						EDSR						LV TRR					
	Univariate		Model 1		Model 2		Univariate		Model 1		Model 2		Univariate		Model 1		Model 2	
	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
Presence of MetS (vs. no MetS)	0.501 (0.110)	<0.001	0.503 (0.114)	<0.001	0.516 (0.137)	<0.001	−0.006 (0.003)	0.049	−0.008 (0.003)	0.012	−0.008 (0.003)	0.004	0.132 (0.499)	0.792	0.131 (0.509)	0.797	0.287 (0.597)	0.631
Comparison between subgroups																		
1) No MetS/no type 2 diabetes	Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref		Ref	
2) MetS with no type 2 diabetes	0.571 (0.129)	<0.001	0.523 (0.139)	<0.001	0.531 (0.155)	0.001	−0.005 (0.003)	0.128	−0.006 (0.003)	0.097	−0.007 (0.003)	0.021	0.112 (0.586)	0.849	0.114 (0.582)	0.845	0.267 (0.676)	0.693
3) Type 2 diabetes	0.480 (0.146)	0.001	0.461 (0.146)	0.002	0.421 (0.177)	0.018	−0.010 (0.004)	0.008	−0.011 (0.004)	0.005	−0.010 (0.003)	0.004	0.550 (0.662)	0.407	0.140 (0.655)	0.831	0.220 (0.781)	0.778

When comparing subgroups according to the presence of MetS and type 2 diabetes, the group with no MetS or type 2 diabetes (subgroup 1) was the reference group. Variables included model 1: age, sex, ethnicity, smoking status, and antihypertensive medication. Variables included in model 2: all from model 1 plus ECV. Ref, reference value.

CMRI-derived diastolic deformation parameters in a large community-based cohort with a prevalence of MetS of ~35%. Our results add value to previously published literature by unraveling subclinical impairment of cardiac diastolic function in patients with MetS, even without the presence of type 2 diabetes (16). Specifically, patients with MetS showed higher SRI, a recently described CMRI-derived diastolic index that reflects the combined influence of impaired cardiac relaxation and abnormal tissue properties and represents an independent predictor of atrial fibrillation and heart failure (7).

Furthermore, HOMA-IR was an independent predictor of EDSR and SRI, showing that increasing insulin resistance is associated with impaired diastolic function. Insulin resistance is central to the pathophysiology of MetS (15) and the concept of "insulin-resistant" cardiomyopathy is emerging and its pathophysiology includes myocardial metabolic deregulation, oxidative stress, and inflammation (3). Indeed, insulin resistance is associated with post-translational modifications of contractile proteins and calcium overload (17), activation of the sympathetic nervous system (18), and cellular injury (19). For example, titin hypophosphorylation might contribute to higher myocardial stiffness without an extracellular increase in myocardial fibrosis. Interestingly, increased myocyte stiffness rather than increased fibrosis has been proposed as the main contributor to diastolic dysfunction in patients with diabetes who have heart failure and preserved ejection fraction (20). Overall, these mechanisms might also be key mediators and triggers for impaired relaxation, myocardial stiffening, and diastolic dysfunction in patients with MetS (21).

The limitations of this study include its cross-sectional design, which prohibits inference about causality. Higher ECV might also be ascribed to other factors than increased myocardial fibrosis, such as increased myocardial inflammation and neovascularization (22). The reference standard for the evaluation of diffuse fibrosis is endomyocardial biopsy, which was not performed in this cohort. Moreover, T1 times and myocardial tagging–derived variables were acquired at the midventricular level and might not represent overall LV mechanics.

In conclusion, using CMRI ECV quantification and myocardial tagging we showed that adults without diabetes with MetS, as well as patients with diabetes, have impaired diastolic function irrespective of myocardial interstitium. Subclinical deleterious changes in cardiac function might help to better stratify patients with MetS and lead to earlier and more aggressive decisions in the management of these patients.

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Metabolic syndrome severity score is associated with diastolic dysfunction and low-grade inflammation in a community-based cohort

Ricardo Ladeiras-Lopes[1,2], Pedro Teixeira[1,2], Ana Azevedo[3,4], Adelino Leite-Moreira[2], Nuno Bettencourt[2] and Ricardo Fontes-Carvalho[1,2]

Metabolic syndrome (MetS) affects approximately one out of three adults in western countries and it combines a cluster of cardiovascular risk factors.[1] A continuous gender and race/ethnicity-specific MetS severity score was recently described and validated,[2] and is an independent predictor of cardiovascular events, beyond individual MetS components.[3] We aimed to assess if this score was associated with subclinical diastolic dysfunction. Additionally, we searched for a potential relationship between MetS severity score and inflammatory/insulin-resistance markers.

This was a cross-sectional study of a community-based cohort consisting of 925 adults, aged 45 years or older, without any known cardiovascular disease. A detailed description of the cohort assembly is provided elsewhere.[4] All participants underwent clinical, analytical (insulin, adiponectin, leptin and high-sensitivity C-reactive protein (hs-CRP)) and echocardiographic examination (including e' velocities and E/e' ratio). Insulin resistance was estimated according to the homeostatic model assessment (HOMA), as the product of fasting glucose (in milligrams per decilitre) and insulin (in milliunits per litre) divided by a constant of 405.

MetS was defined using the 2005 American Heart Association/National Heart, Lung, and Blood Institute criteria (MetS criteria showing the strongest association with cardiovascular disease in the Portuguese population[5]). A continuous MetS severity z-score was applied to all patients, in theory normally distributed and ranging from theoretical negative to positive infinity with mean = 0 and standard deviation = 1. We used two of the six equations, as described by Gurka et al.[2]: a) non-Hispanic white males: score = $-5.4559 + 0.0125 \times$ waist circumference $- 0.0251 \times$ high-density lipoprotein cholesterol (HDL-C) $+ 0.0047 \times$ systolic blood pressure (SBP) $+ 0.8244 \times \ln$(triglycerides) $+ 0.0106 \times$ glucose; b) non-Hispanic white females: score = $-7.2591 + 0.0254 \times$ waist circumference $- 0.0120 \times$ HDL-C $+ 0.0075 \times$ SBP $+ 0.5800 \times \ln$(triglycerides) $+ 0.0203 \times$ glucose.

Diastolic dysfunction was defined using three criteria: 2009[6] and 2016[7] European Association of Cardiovascular Imaging/American Association of Echocardiography (EACVI/ASE) recommendations and a 2017 clinically oriented algorithm.[8]

Regarding the statistical analyses, continuous variables are reported as mean ± standard deviation or median (interquartile range), according to normality of distribution. Discrete variables are described using frequency and percentage. The HOMA index, hs-CRP, adiponectin and leptin were included in the analyses as a base-2 logarithm due to their skewed distribution (normality was assessed using the Kolmogorov–Smirnov test). One unit variation of the base-2 logarithmic transformation would be equivalent to a doubling of the variable of interest. Bivariate correlations were assessed by Pearson's (r) correlation coefficient (non-normally distributed variables were previously logarithmically transformed). Independent t-test was used to compare MetS severity score in individuals with normal diastolic function and individuals with diastolic dysfunction. Multivariable linear regression analysis

[1]Serviço de Cardiologia, Centro Hospitalar de Vila Nova de Gaia/Espinho, Vila Nova de Gaia, Portugal
[2]Unidade de Investigação e Desenvolvimento Cardiovascular, Faculdade de Medicina, Universidade do Porto, Porto, Portugal
[3]EPIUnit – Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal
[4]Departamento de Ciências da Saúde Pública e Forenses, e Educação Médica, Faculdade de Medicina, Universidade do Porto, Porto, Portugal

Corresponding author:
Ricardo Ladeiras-Lopes, Cardiology Department, Gaia Hospital Centre and Cardiovascular Research Centre, Faculty of Medicine, University of Porto, Rua Conceicao Fernandes, 4434-502 Vila Nova de Gaia, Portugal.
Email: ricardoladeiraslopes@gmail.com
Twitter: @rladeiraslopes

was used to assess the association between diastolic echocardiographic indices and MetS severity score using two models: a) including age; b) including age and each individual MetS component (dichotomized). In addition, multivariable logistic regression was used to assess if MetS severity score was a predictor of diastolic dysfunction, independently of each individual's MetS component. All statistical analyses were conducted using Stata 14.0 for Mac (StataCorp, College Station, TX, USA).

The final sample included 925 participants with a mean age of 61.5 ± 10.5 years (37% men). The clinical, anthropometric, analytical and echocardiographic characteristics of the study sample are shown in Table 1. The prevalence of MetS was 39.3% (358 individuals), and 10.7% (99 individuals) had type 2 diabetes. The prevalence of diastolic dysfunction was 22% according to the 2009 joint guideline, 1% according to the 2016 joint guideline (with 14.5% categorized as "indeterminate") and 49.2% according to the 2017 algorithm proposed by Mitter et al.[8]

The mean MetS severity score was 0.05 ± 0.83. This score was positively correlated with the HOMA insulin resistance index ($r = 0.57$, $p < 0.001$), hs-CRP ($r = 0.28$, $p > 0.001$) and leptin ($r = 0.36$, $p < 0.001$), and inversely correlated with adiponectin ($r = -0.27$, $p < 0.001$). Higher MetS severity score was associated with a decrease in e' velocity and an increase in E/e' ratio, irrespective of age (Figure 1).

In multiple linear regression analyses, MetS score was a negative predictor of e' velocity (beta-coefficient: -0.62; p-value < 0.001) and a positive predictor of E/e' ratio (beta-coefficient: 0.30; p-value $= 0.042$), even when age and all individual MetS components were included in the model. There was a higher MetS severity score in patients with criteria for diastolic dysfunction according to the 2009 criteria (0.36 ± 0.83 vs -0.03 ± 0.81 in normal diastolic function) and 2017 algorithm (0.25 ± 0.81 vs -0.14 ± 0.80 in normal diastolic function), as well as in patients with positive or indeterminate criteria using the 2016 guideline (0.31 ± 0.79 vs 0.01 ± 0.83 in normal diastolic function). MetS score was an independent predictor of diastolic dysfunction according to the 2017 clinically oriented algorithm, irrespective of age and individual MetS components (odds ratio 1.38, $p = 0.027$), but not using the 2009 (odds ratio 1.26, $p = 0.136$) and 2016 (odds ratio 1.66, $p = 0.410$) EACVI/ASE recommendations.

In this study, we observed that an increasing MetS severity score is associated with higher insulin resistance, increased inflammatory biomarkers and metabolically dysfunctional adipokines profile (high leptin and low adiponectin). These biomarkers might be viewed as surrogates for "metabolic dysfunction",

Table 1. Study participant characteristics.

	Total ($n = 925$)
Age (years)	61.5 ± 10.5
Male sex, *n* (%)	346 (37)
Cardiovascular risk factors	
Total cholesterol, mg/dL	219.9 ± 38.7
LDL-C, mg/dL	132.9 ± 34.4
HDL-C, mg/dL	59.9 ± 13.2
Triglycerides, mg/dL	131.2 ± 76.4
Waist circumference, cm	92.7 ± 11.4
BMI, kg/m^2	27.3 ± 4.6
SBP, mmHg	132.5 ± 19.4
DBP, mmHg	78.4 ± 11.2
Fasting glucose, mg/dL	101.5 ± 24.8
Log2(HOMA-IR)	0.1 ± 1.4
MetS, *n* (%)	358 (39.3)
Increased waist circumference, *n* (% of MetS)	279 (77.9)
Triglycerides $\geq$ 150 mg/dL or receiving fibrates, *n* (% of MetS)	206 (57.5)
Decreased HDL-C or taking niacin, *n* (% of MetS)	210 (58.9)
SBP $\geq$ 130 or DBP $\geq$ 85 mmHg, *n* (%of MetS)	332 (92.7)
IFG or type 2 diabetes, *n* (%of MetS)	254 (70.9)
Type 2 diabetes, *n* (%)	99 (10.7)
Oral antidiabetic medication and/or insulin, *n* (%)	65 (7.0)
Echocardiographic data	
Septum, mm	8.6 ± 1.4
Posterior wall, mm	7.9 ± 1.2
LV mass index, g/m^2	78.3 ± 18.8
LA volume index, mL/m^2	28.2 ± 9.5
LVED volume index, mL/m^2	65.6 ± 15.9
LVES volume index, mL/m^2	26.4 ± 8.8
Ejection fraction, %	60.7 ± 6.1
E wave, cm/s	71.6 ± 15.3
A wave, cm/s	78.2 ± 19.9
E/A ratio	0.96 ± 0.30
Deceleration time, ms	236.3 ± 54.1
IVRT, ms	91.3 ± 15.8

Data are presented as mean $\pm$ standard deviation for continuous variables and count (percentage) for categorical variables.

BMI: body mass index; DBP: diastolic blood pressure; HOMA-IR: homeostatic model assessment of insulin resistance; LV: left ventricle; LA: left atria; LVED: left ventricle end-diastolic; LVES: left ventricle end-systolic; IVRT: isovolumic relaxation time; MetS: metabolic syndrome; SBP: systolic blood pressure; LDL: low-density lipoprotein; HDL: high-density lipoprotein; HDL-C: high-density lipoprotein cholesterol; IFG: impaired fasting glucose.

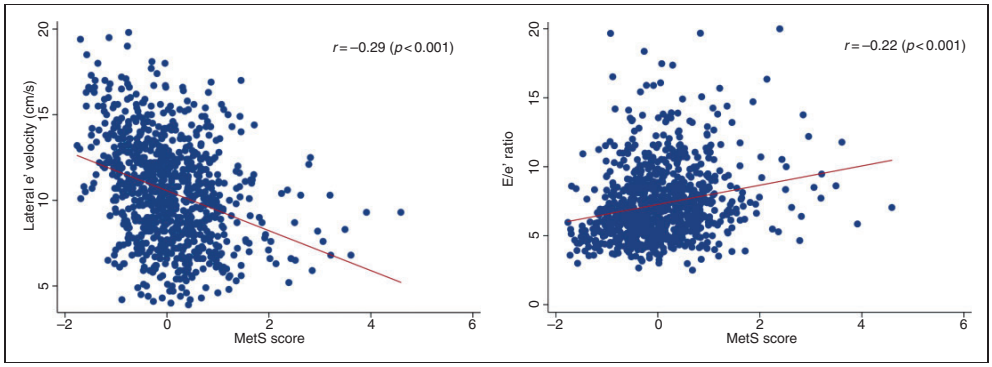

Figure 1. Scatter plots with regression line showing inverse correlation between MetS severity score and lateral e' velocity (surrogate for LV relaxation), and positive correlation with E/e' ratio (surrogate for LV filling pressures).

and all are key players in MetS pathophysiology and important contributors to diastolic dysfunction and heart failure with preserved ejection fraction,[9] as was previously shown for other biomarkers such as amino acids.[10] In addition, higher MetS score was associated with decreased e' velocity (impaired relaxation) and increased E/e' ratio (higher left ventricle (LV) filling pressures). Lastly, patients with diastolic dysfunction showed higher MetS score and the latter was an independent predictor of diastolic dysfunction (defined according to a 2017 clinically oriented algorithm[8]), irrespective of age and individual MetS components, although this was not the case for the definition of diastolic dysfunction according to 2009 and 2016 EACVI/ASE recommendations. There was substantial criticism regarding the updated 2016 diastolic dysfunction definition, due to its higher specificity and the potential for identifying as positive only the most advanced cases of diastolic dysfunction.[11] This might account for the low prevalence of diastolic dysfunction of 1% in our cohort according to this criterion, whereas its prevalence in community-based cohorts according to older criteria is usually around 35%.[12] Further research is needed to assess how using a continuous MetS severity score can improve diastolic dysfunction prediction beyond the dichotomous MetS categorization and its individual components, and even be integrated with other screening models for diastolic dysfunction in the community.[13]

Interestingly, metformin treatment of non-diabetic patients with insulin resistance or pre-diabetes significantly reduced LV mass and oxidative stress.[14] The MET-DIME trial will further explore this topic, by evaluating if the initiation of metformin therapy in non-diabetic patients with MetS and diastolic dysfunction is associated with improvement in diastolic function.[15]

Overall, we showed that a MetS severity score provides an integrated index of metabolic dysfunction, combining insulin resistance, low-grade inflammation and metabolically unfavourable adipokine profile. MetS score is associated with LV relaxation and filling pressures, and is an independent predictor of diastolic dysfunction, beyond MetS individual components.

Declaration of conflicting interests

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B. The contribution of left ventricular diastolic dysfunction to cardiovascular morbidity and mortality

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ORIGINAL ARTICLE

The impact of diastolic dysfunction as a predictor of cardiovascular events: A systematic review and meta-analysis

Ricardo Ladeiras-Lopes[a,b,c,*,◊], **Margarida Araújo**[a,◊], **Francisco Sampaio**[b,c], **Adelino Leite-Moreira**[a,c], **Ricardo Fontes-Carvalho**[a,b,c]

[a] *Department of Surgery and Physiology, Faculty of Medicine of the University of Porto, Porto, Portugal*
[b] *Cardiology Department, Gaia/Espinho Hospital Centre, Vila Nova de Gaia, Portugal*
[c] *Cardiovascular Research Centre, Faculty of Medicine, University of Porto, Porto, Portugal*

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Abstract
Introduction: Diastolic dysfunction is highly prevalent and a key pathophysiological contributor to several cardiovascular diseases, especially heart failure with preserved ejection fraction. In addition, some evidence suggests diastolic dysfunction is a risk factor for major adverse cardiovascular events. This study aimed to systematically review the evidence and to quantify the association between diastolic dysfunction and risk of cardiovascular events and death.
Methods: MEDLINE was systematically searched from 1974 up to October 2017. We included cohort studies that assessed diastolic function in adults in the community, providing a definition of diastolic dysfunction regarding the occurrence of any cardiovascular event or mortality. For the quantitative analysis, relative risk estimates comparing individuals with versus without diastolic dysfunction were combined using a random effects model.
Results: Nineteen studies were identified for inclusion in the systematic review, assessing a total of 63 802 participants. Nine studies were included in the meta-analysis. Diagnostic criteria and classification of diastolic dysfunction differed substantially between studies. The median prevalence of diastolic dysfunction in studies including individuals with and without diastolic dysfunction was 35.1% (range 5.3-65.2%). Comparing diastolic dysfunction with normal diastolic function, the summary relative risk estimate for cardiovascular events or mortality was 3.53 (95% CI: 2.75-4.53; I^2=85.5%; nine studies).

* Corresponding author.
E-mail address: ricardoladeiraslopes@gmail.com (R. Ladeiras-Lopes).
◊ Equally contributing authors.

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Conclusions: Although the definitions found in the literature differ, the diagnosis of diastolic dysfunction is associated with a 3.53-fold increased risk of cardiovascular events or death. This finding highlights the importance of developing easily applicable and consensual diagnostic criteria, as well as fostering research on effective treatment strategies when diastolic dysfunction is identified in the subclinical stage.

PALAVRAS-CHAVE
Diástole;
Relaxamento miocárdico;
Disfunção diastólica;
Prognóstico;
Eventos cardiovasculares;
Insuficiência cardíaca

O impacto da disfunção diastólica como preditor de eventos cardiovasculares: uma revisão sistemática e meta-análise

Resumo
Introdução: A disfunção diastólica (DD) é muito prevalente e representa um mecanismo fisiopatológico central para várias doenças cardiovasculares, especialmente para a insuficiência cardíaca com fração de ejeção preservada. Além disso, alguns estudos sugerem que a DD se associa a um aumento do risco de eventos cardiovasculares. Este estudo pretende determinar se a DD é um preditor de eventos cardiovasculares e mortalidade através de uma revisão sistemática e meta-análise.
Métodos: Foi realizada uma pesquisa na Medline, desde 1974 até outubro de 2017. Foram incluídos estudos de coorte que avaliassem a função diastólica em adultos da comunidade, comparando participantes com e sem DD, no que diz respeito ao desenvolvimento de eventos cardiovasculares ou morte. Na meta-análise, os riscos relativos foram combinados usando um modelo de *random-effects analysis*.
Resultados: Foram identificados dezanove estudos para a revisão sistemática, avaliando um total de 63 802 participantes, dos quais nove foram incluídos na meta-análise. Observámos que os critérios de diagnóstico e classificação de DD foram bastante diferentes entre os estudos. A prevalência mediana de DD foi 35,1% (variabilidade 5,3%-65,2%). A presença de DD associou-se a um aumento significativo do risco relativo combinado de evento cardiovascular ou mortalidade (3,53; IC 95%: 2,75-4,53; I2=85,5%; 9 estudos).
Conclusões: Apesar da heterogeneidade na definição de DD, a sua presença está associada a um aumento marcado do risco de eventos cardiovasculares ou morte. Este resultado realça a importância de desenvolver critérios objetivos e consensuais para o diagnóstico de DD, de modo a promover a sua identificação numa fase subclínica e eventualmente estimular uma investigação dirigida à abordagem terapêutica precoce.

Introduction

Diastolic dysfunction (DD) is a commonly used term that denotes the presence of pathophysiological changes in cardiac function which include abnormal relaxation, increased myocardial stiffness and increased end-diastolic pressure.[1]

The prevalence of DD is increasing and is now higher than that of systolic dysfunction.[2] According to a recent systematic review, DD affects approximately 36% of the population older than 60 years.[3] Echocardiography is often used to assess diastolic function. However, no single echocardiographic parameter is considered sufficiently accurate and reproducible to establish the diagnosis of DD and several parameters must be combined for the diagnosis. Recently, a joint effort involving the European Association of Cardiovascular Imaging and the American Society of Echocardiography set out to harmonize the assessment of diastolic function and to develop a new definition of DD.[4]

DD is closely associated with several cardiovascular risk factors, including hypertension, obesity and diabetes.[5–8] Furthermore, DD is mechanistically involved in the cardiovascular changes that accompany common cardiac diseases, such as stable coronary artery disease, myocardial infarction and cardiomyopathies, with a very strong link to heart failure with preserved ejection fraction (HFpEF).[9] DD is one of the best predictors of exercise capacity in patients with heart failure and after myocardial infarction.[10] More interestingly, it has also been suggested that increasing severity of DD increases the risk of heart failure[1] and is predictive of all-cause mortality.[11] However, to the best of our knowledge, the available evidence has not yet been systematically reviewed.

This study aimed to systematically review cohort studies assessing the association between DD and the incidence of major adverse cardiovascular events (MACE) and death, and to quantify the strength of this association using meta-analytical methods.

Methods

Data sources and query

This study was performed according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement.[12] Studies were identified by searching the PubMed electronic database (MEDLINE) and scanning reference lists of articles. The following search terms were used: (''Diastole''[MeSH] OR diastolic OR ''myocardial relaxation'' OR ''cardiac relaxation'' OR ''myocardial stiffness'' OR ''cardiac stiffness'') AND (''Prognosis''[MeSH] OR ''Heart Failure''[MeSH] OR ''Mortality''[MeSH]) AND (community OR ''general population''). A language filter was used to restrict the search to English, Portuguese and Spanish papers. There were no date restrictions applied to the electronic searches – all reports from 1974 until October 26 2017 (when the last search was conducted) were eligible. There were no other methodological filters.

Eligibility criteria

Cohort studies that included adults from the community assessing the impact of DD on cardiovascular events (including heart failure, myocardial infarction, and hospitalization from cardiovascular cause) and/or mortality (both cardiac and all-cause) over time were eligible. Studies in specific population groups, such as end-stage renal disease on dialysis, were excluded. Only studies clearly describing how DD was defined and providing data on incident MACE and/or mortality were selected for inclusion. Studies comparing outcomes between individuals with and without DD were eligible for meta-analysis. Studies that only assessed diastolic function parameters as continuous variables, without defining a cut-off to distinguish DD from normal diastolic function, were excluded.

Study selection and data collection

After studies were identified using the search query, they were screened by one investigator (M.A.) based on the title and abstract. Eligibility was assessed and article data were extracted by two reviewers independently (M.A., R.L.) using a standardized form. Any disagreement was subsequently resolved by the two authors. Information was extracted from each included report on the following: characteristics of participants (number of individuals, age, gender, ethnicity, risk factors such as hypertension, diabetes, smoking, body mass index, systolic blood pressure, diastolic blood pressure, medication, left ventricular [LV] hypertrophy, systolic dysfunction, heart failure, coronary artery disease) and diastolic echocardiographic measures (e' velocity, E/e' ratio, left atrial size, tricuspid regurgitation velocity, E/A ratio); imaging method used for the diagnosis of DD and diagnostic criteria; and type of outcome measure (MACE and/or mortality).

To avoid double counting of a cohort, one set of results was selected when multiple publications were available for the same cohort. Priority was given to the study with the longest follow-up.

Regarding the studies eligible for meta-analysis and not providing the required data in the full-text and supplementary material publications, the first and/or corresponding authors were contacted by email in order to provide the required information.

The methodological quality of all studies was assessed using the revised and validated version of the Methodological Index for Non-Randomized Studies (MINORS),[13] as detailed in Supplementary Table 1.

Statistical analysis

Study-specific measures were pooled using random-effects model meta-analysis to provide a single summary estimate. Random-effects model meta-analysis makes allowance for between-study heterogeneity. Pooled estimates along with their 95% confidence intervals (CI) were provided. Heterogeneity between studies was assessed using Q and I^2 statistics (I^2 values of $\leq$25%, 50%, and $\geq$75% represent low, moderate, and high levels of heterogeneity, respectively (www.cochrane-handbook.org).

A forest plot was constructed showing the individual studies with the pooled estimates. Publication bias was assessed using the Egger test and the funnel plot analysis. All statistical analyses were performed using STATA software (version 13.1, StataCorp LP, College Station, TX, USA).

Results

General characteristics of the included studies

Of a total of 604 initially identified studies, only 12 matched our eligibility criteria and were included (Figure 1). Seven additional studies were included after checking the reference list of the articles, yielding 19 studies for the qualitative analysis. Ten studies were excluded from quantitative analysis (meta-analysis) because they provided data only for patients with DD without a clearly defined group with normal diastolic function (n=4), or provided insufficient data for the required calculations (as full-text and supplementary material publications) and did not respond to multiple contact attempts (n=6).

Nine studies were eventually included in the meta-analysis. For the 19 studies included in the qualitative analysis, the mean follow-up period ranged from one year[14] to 11 years.[15] Eleven studies were conducted in the USA,[11,15–24] six in Europe,[14,25–29] one in Israel[30] and one in Japan.[31] Six of the included studies[14,17,21–23,31] were retrospective. Included studies were assessed as high-quality publications (median MINORS score of 17; 25th and 75th percentile of 16 and 18, respectively).

The included studies provided data on 63 802 participants from community-based cohorts, with mean ages ranging from 50.929 to 82 years,[14] with some studies focusing on the elderly population.[16,20,21,30] Most of the studies had a balanced gender distribution, except for Ren et al.,[19] which

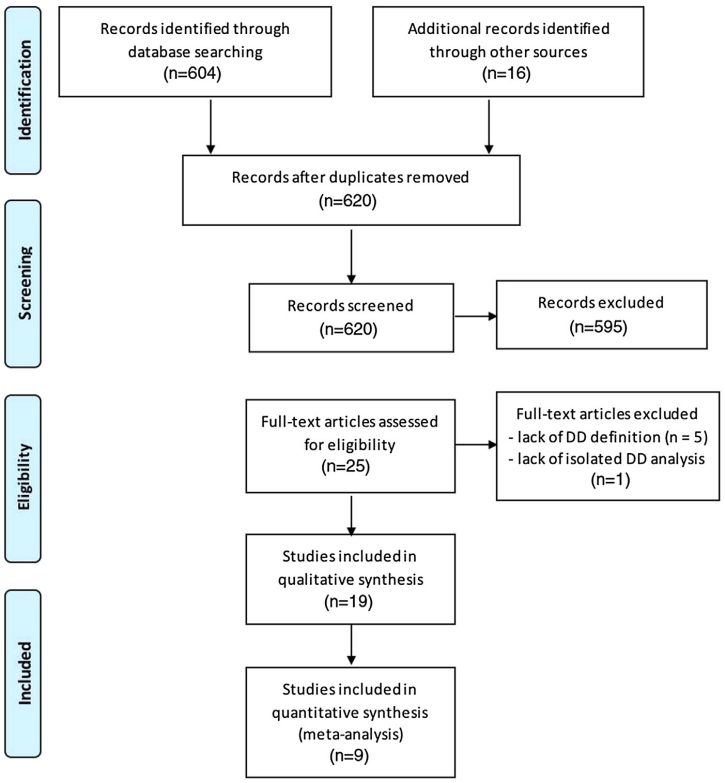

Figure 1 Flowchart showing search strategy for published data and selection process for inclusion in the systematic review and meta-analysis (according to the PRISMA flow Diagram[12]).

included 81% males. Kuznetsova et al.[29] included only white participants and Brady et al.[22] had 57% black participants. As depicted in Table 1, the prevalence of major cardiovascular risk factors was in agreement with what would be expected in cohorts coming from the community. However, Blomstrand et al.[25] and From et al.[17] focused only on subjects with diabetes. Most of the studies included individuals with systolic dysfunction and only six studies excluded this population.[14,19,22–24,31] Kardys et al.[28] reported as much as 39% of systolic dysfunction in their baseline population. Symptomatic heart failure was present in eight studies,[11,14,18,22,24,25,30,31] and most included individuals with known coronary artery disease (only Aurigemma et al.,[16] Tsang et al.,[21] Brady et al.[22] and Kardys et al.[28] used this as an exclusion criterion).

Assessment and definition of diastolic dysfunction

All studies used echocardiography (pulsed-wave Doppler and/or tissue Doppler imaging) to assess diastole and to define DD, except Brady et al.,[22] who used cardiac catheterization and measurement of LV pressure. The prevalence of DD in the studies ranged from 5.3%[20] to 65.2%,[24] with a median prevalence of 35.1%.

Significant heterogeneity was observed regarding the definition and grading of DD. Ten studies[11,15,16,18,19,21,23,24,28,29] used a one-level classification tree (criteria were presented for each grade and DD was defined as fulfillment of the criteria for any of these grades), two studies[26,27] used a two-level classification tree (criteria for DD were defined and, if fulfilled, subsequent grading took place with additional variables), and seven studies[14,17,20,22,25,30,31] only defined the criteria for DD, without grading. As shown in Table 2, there was also significant variability in the core echocardiographic parameters for diagnosis of DD. The most used variable was E/A ratio (10 studies[11,15,16,18,19,21,23,27–29]), followed by E/e' ratio (eight studies[11,17,20,23,25,27,29,30]), E-wave deceleration time (seven studies[11,15,18,21,23,28,31]), left atrial size (five studies[15,20,21,27,29]) and pulmonary vein flow indices (five studies[11,19,23,29,31]), e' velocity (two studies[20,27]) and LV end-diastolic pressure (one study[22]).

Association between diastolic dysfunction and risk of cardiovascular events and mortality

The median incidence of the primary outcome among individuals with normal diastolic function and participants with

Table 1 General characteristics of cohort studies assessing the association between diastolic dysfunction and risk of cardiovascular events and/or mortality.

Study	Cohort (acronym)	n	Patients with LVSD, Yes/No (criteria), percentage	Patients with HF, Yes/No; percentage according to NYHA	Patients with known CAD, Yes/No; percentage	Patients with LVH, percentage; criteria	Age, years (mean/median, SD/IQR)	Male gender, %	Ethnicity, %	Hypertension, %	Diabetes, %	Smokers, %	Mean BMI, kg/m^2	Mean SBP, mmHg	Mean DBP, mmHg	ACEi/ARB, %	Beta-blockers, %
Johansenet al.[27]	CCHS	1851	Yes (LVEF <50%); 0.8%	No	Yes; 16%	11%; LVMI ≥104 g/m^2 (women); ≥116 g/m^2 (men)	57.9 (SD 16.1)[a]	43	N/A	43[a]	10[a]	NA	25.4 (SD 3.9)[a]	136 (SD 23)[a]	79 (SD 12)[a]	NA	NA
Shah et al.[20]	ARIC	5801	NA	No	NA	NA	76 (SD 5.1)	42	23% black	89	39	6	28.8 (SD 5.6)	131 (SD 18)	67 (SD 11)	NA	NA
Banerjee et al.[14]	N/A	80	No (LVEF ≤45%)	Yes (100%); NYHA I: 22.5%; II: 43.8%; III: 25%; IV: 8.8%	Yes; 9%	N/A	82 (SD 8.1)	34	N/A	83	24	N/A	N/A	N/A	N/A	53	44
Blomstrand et al.[25]	CARDIPP	406	Yes (GLS <-15%); 13%	Yes; 1.5%	Yes; 7.4%	N/A	60.7 (SD 3.1)	68	N/A	65	100	N/A	29.8 (SD 4.5)	136 (SD 15)	81 (SD 10)	48	35
Kuznetsova et al.[29]	FLEMENGHO	793	Yes (LVEF ≤50%); 0.8%	N/A	Yes; 3.2%	N/A	50.9 (SD 15.5)	49	100% white	41	3	21	Women: 26.3 (SD 4.7); men: 26.6 (SD 3.7)	Women: 128 (SD 19); men: 131 (SD 15)	Women: 78 (SD 9); men: 82 (SD 10)	8	15
Di Bello et al.[26]	D.A.VE.S	2142	Yes (LVEF ≤50%); N/A	No	Yes; 14.8%	3.5%; LVMI >49.2 g/m^{27} (men) and >46.2 g/m^{27} women)	63 (IQR 56-68)	54	N/A	57	14	23	25 (IQR 23-29)	140 (IQR 130-150)	80 (SD 80-90)	41	25
Leibowitz et al.[30]	JLCS	502	Yes (LVEF <55%); N/A	Yes (11.2%); NYHA I 90.7%; II 7.7%; III 1.4%; IV 0.2%	Yes; 36.8%	N/A	N/A (all individuals >85)	47	N/A	71	19	3	27.2 (SD 4.4)	N/A	N/A	N/A	N/A
Vogel et al.[23]	RES	388	No (LVEF <50%)	No	Yes; 52%	N/A	67.1 (SD 12.4)	43	N/A	87	30	N/A	29.2 (SD 6.9)	N/A	N/A	N/A	N/A
Halley et al.[24]	N/A	36261	No (LVEF <55%)	Yes; 3.5%	Yes; 0.6%	N/A	58.3 (SD 15.4)	45.6	N/A	14.9	11.6	N/A	N/A	140 (SD 21.6)	82.1 (SD 11.2)	N/A	N/A
Kane et al.[18]	OCHFS	1402	Yes (LVEF <50%); 2.4%;	Yes; 2.2%	Yes; 16.7%	N/A	65.2 (SD 9.5)	49	>95% white	42	10	N/A	28.5 (SD 5.2)	126 (SD 19.1)	69.5 (SD 10.4)	18	22
Lam et al.[15]	FHS	1038	Yes (LVEF ≤45%); 5%	No	Yes; 9%	N/A	76 (SD 5)	39	N/A	77	10	N/A	26.6 (SD 4.5)	147 (SD 22)	N/A	N/A	N/A
From et al.[17]	N/A	1760	N/A	No	Yes; 36%	33%; LVMI ≥104 g/m^2 (women); ≥116 g/m^2 (men)	60 (SD 14)	49	N/A	86	100	N/A	33 (SD 14)	N/A	N/A	N/A	N/A
Kardys et al.[28]	Rotterdam Study	4425	Yes (''qualitative assess-ment''); 39%	No	No	N/A	71.4 (SD 7.3)	39	N/A	N/A	13	16	27.5 (SD 4.1)	150 (SD 21)	80 (SD 11)	11	14
Okura et al.[31]	SHFS	272	No (LVEF <40%)	Yes; NYHA I 41%; II 58%; IV 1%	Yes; 19% (men), 15% (women)	61%; LVMI ≥116 g/m^2 (men); ≥104 g/m^2 (women)	68.5 (SD 8.7) (men); 69.3 (SD 10.6) (women)	58	N/A	46	24	N/A	N/A	N/A	N/A	39	N/A

Table 1 (*Continued*)

Study	Cohort (acronym)	n	Patients with LVSD, Yes/No (criteria), percentage	Patients with HF, Yes/No; percentage according to NYHA	Patients with known CAD, Yes/No; percentage	Patients with LVH, percentage; criteria	Age, years (mean/median, SD/IQR)	Male gender, %	Ethnicity, %	Hypertension, %	Diabetes, %	Smokers, %	Mean BMI, kg/m^2	Mean SBP, mmHg	Mean DBP, mmHg	ACEi/ARB, %	Beta-blockers, %
Ren et al.[19]	HSS	693	No (LVEF <50%)	No	Yes; 100%	49%; LVMI >90 g/m^2	65 (SD 10) normal diastole; 72 (SD 9) impaired relaxation; 70 (SD 12) pseudonormal or restrictive	81	59% white, 32% African-American, 13% Asian, 14% other	71	26	18	28.5 (SD 5.0) normal diastole; 28.2 (SD 4.9) impaired relaxation; 28.6 (SD 5.8) pseudonor-mal or restrictive	132 (SD 21) normal diastole; 138 (SD 22) impaired relaxation; 134 (SD 24) pseudonormal or restrictive	75 (SD 11) normal diastole; 76 (SD 11) impaired relaxation; 71 (SD 11) pseudonormal or restrictive	46	57
Brady et al.[22]	N/A	115	No (LVEF <50%)	Yes; <0.035%	No	N/A	58	37	18% white, 57% black, 22% Hispanic	71	21	N/A	31	N/A	N/A	N/A	N/A
Redfield et al.[11]	Rochester Epidemiol-ogy Project	2042	Yes (LVEF ≤50%); 6%	Yes; 2.2%	Yes; 12.2%	N/A	62.8 (SD 10.6)	41	majority white	25	4.5	8.9	28.4 (5.41)	N/A	N/A	47.5% LVEF≤40%; 14.2% moderate or severe DD	22.5% LVEF≤40%; 40.2% moderate or severe DD
Tsang et al.[21]	N/A	1160	Yes (LVEF <50%); 4%	No	No	3% in group with no events, 6% in group with events; ECG	75 (SD 7)	36	N/A	48% in group with no events, 63% in group with events	7% in group with no events, 11% in group with events	N/A	N/A	142 (SD 23) in group with no events, 146 (SD 23) in group with events	N/A	N/A	N/A
Aurigemma et al.[16]	CHS	2671	Yes (LVEF <45%); 4%	No	No	7.6% in group with no events, 24.3% in group with events; ratio of observed to expected LV mass/height ratio >1.45	72 (SD 5) in group with no events, 74 (SD 6) in group with events	36% in group with no events, 45% in group with events	94% white (in group with no events) 94% white (in group with events)	28% in group with no events, 47% in group with events	5% in group with no events, 12% in group with events	12% in group with no events, 12% in group with events	26 (SD 4) in group with no events, 27 (SD 5) in group with events	134 (SD 21) in group with no events, 145 (SD 22) in group with events	70 (SD 11) in group with no events, 73 (SD 13) in group with events	N/A	N/A

ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; CAD: coronary artery disease; DBP: diastolic blood pressure; DD: diastolic dysfunction; ECG: electrocardiography; GLS: global longitudinal strain; HF: heart failure; IQR: interquartile range; LV: left ventricular; LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; LVMI: left ventricular mass index; LVSD: left ventricular systolic dysfunction; N/A: not available; NYHA: New York Heart Association functional class; SBP: systolic blood pressure; SD: standard deviation.

[a] Unpublished data.

Table 2 Method of assessment of diastolic dysfunction and magnitude of the association with incident cardiovascular events and/or mortality.

Study	DD imaging technique	DD criteria	DD prevalence, %	Mean lateral e', cm/s	Mean septal e'e, cm/s	Mean E/e' ratio (septal or lateral e')	Mean LA size	TR jet velocity (mean)	Mean E/A ratio	Follow-up (mean)	Primary endpoint	Association measure (HR/RR/OR (95% CI)]
Johansenet al.[27]	Echocardiography (including TDI)	e' <7 cm/s (mean of septal and lateral); grading: E/A >2 and/or LAVi ≥34 ml/m^2 and/or E/e' ≥17	52.5	7.1 (SD 2.7) (mean of septal and lateral)[a]		N/A	LAVi 19.1 ml/m^2 (SD 6.5)	N/A	1.11 (SD 0.46)[a]	10.9 years	CV death, MI and hospitalization due to HF	HR 2.54 (95% CI 1.61-4)
Shah et al.[20]	Echocardiography (including TDI)	According to 2016 guidelines[3]	5.3	N/A	N/A	N/A	N/A	N/A	N/A	610 days	All-cause mortality and hospitalization due to HF	Event rate ratio (calculated) 5.36
Banerjee et al.[14]	Echocardiography (including TDI)	According to 2007 diastolic heart failure consensus[33]	100 (only patients with DD)	N/A	N/A	16 (SD 5.74)	N/A	N/A	N/A	1 year	CV death or hospitalization due to CV event	Incidence 25%
Blomstrand et al.[25]	Echocardiography (including TDI)	E/e' ratio >15	34.2	N/A	N/A	14.4 (SD 4.5) (septal)	N/A	N/A	N/A	67 (SD 17) months	CV death, myocardial infarction and stroke	HR 3.05 (95% CI 1.18-7.85)
Kuznetsova et al.[29]	Echocardiography (including TDI)	Impaired relaxation (group 1): ↓ E/A ratio (age-specific) and E/e' ≤8.5; elevated filling pressure (group 2): normal E/A ratio (age-specific), E/e' >8.5 or Adur <ARdur+10 or LAVi ≥28 ml/m^2); combined dysfunction (group 3): ↓ E/A ratio (age-specific) and E/e' >8.5	25.1	Women: 11.3 (SD 3.6); men: 11.5 (SD 3.8) (mean of septum, lateral, inferior, posterior)		Women: 7.5 (SD 2.4); men: 6.7 (SD 1.9) (mean of septum, lateral, inferior, posterior)	LAVi: women: 21.8 ml/m^2 (SD 6.0); men: 24.0 ml/m^2 (SD 6.3)	N/A	Women: 1.2 (SD 0.4); men: 1.3 (SD 0.4)	4.8 years	Cardiac events (MI, coronary revascularization, HF, new-onset angina, cor pulmonale, new-onset AF, life-threatening arrhythmias); CV events (cardiac events plus stroke, TIA, aortic aneurysm, arterial embolism, and peripheral artery revascularization)	HR 1.77 (95% CI: 0.75-4.17) for CV events in group 1 and 2.21 (95% CI: 1.01-3.83) in groups 2+3; HR 2.13 (95%CI: 0.70-6.48) for cardiac events in group 1 and 4.50 (95% CI: 1.73-11.7) in groups 2+3

Table 2 (*Continued*)

Study	DD imaging technique	DD criteria	DD prevalence, %	Mean lateral e', cm/s	Mean septal e'e, cm/s	Mean E/e' ratio (septal or lateral e')	Mean LA size	TR jet velocity (mean)	Mean E/A ratio	Follow-up (mean)	Primary endpoint	Association measure (HR/RR/OR (95% CI)]
Di Bello et al.[26]	Echocardiography (including TDI)	According to 2009 guidelines[30]	N/A	N/A	N/A	N/A	LA diameter 38 mm (IQR 34-45); LA area 15 cm^2 (14-18)	N/A	N/A	26 (SD 11) months	Cardiac death, MI, CABG or PTCA, stroke, TIA, acute pulmonary edema	OR 1.392 (95% CI 1.313-1.712)
Leibowitz et al.[30]	Echocardiography (including TDI)	E/e' >13	N/A	N/A	N/A	12.2 (SD 4.9) (mean of septal and lateral tissue velocities) [survivor]	LAVi 36.6 ml/m^2 (SD 12.5) [survivor]	N/A	0.97 (SD1.1) [survivor]	5 years	All-cause mortality	HR 1.028 (95% CI 0.98-1.084) (E/e' ratio included as continuous variable in the model)
Vogel et al.[23]	Echocardiography (including TDI)	Impaired relaxation: grade 1 - E/A $\leq$0.75, E/e' <10; grade 1a - E/A $\leq$0.75, E/e' >10; pseudonormal pattern: grade 2 - 0.75 <E/A <1.5, DT >140, PV S/D $\geq$1 or E/e' $\geq$10; restrictive (grade 3/4): E/A >1.5 and/or DT <140 ms and/or PV S/D <1 and/or E/e $\geq$10	100 (only patients with DD)	N/A	N/A	15.5 (SD 5.4) (not specified)	LAVi 41.5 ml/m^2 (SD 12.1)	N/A	1.3 (SD 0.7)	3.9 years	HF	Cumulative probability of 2.2%, 5.7% and 11.6% at 1, 2 and 3 years, respectively
Halley et al.[24]	Echocardiography (including TDI)	According to 2002 guidelines[34]	65.2	N/A	N/A	N/A	N/A	N/A	N/A	6.2 (SD 2.3) years	All-cause mortality	Mild DD: HR 1.11 (95% CI 0.85-1.47); moderate DD: HR 1.58 (95% CI 1.20-2.08); severe DD: HR 1.84 (95% CI 1.29-2.62)

Table 2 (*Continued*)

Study	DD imaging technique	DD criteria	DD prevalence, %	Mean lateral e', cm/s	Mean septal e'e, cm/s	Mean E/e' ratio (septal or lateral e')	Mean LA size	TR jet velocity (mean)	Mean E/A ratio	Follow-up (mean)	Primary endpoint	Association measure (HR/RR/OR (95% CI)]
Kane et al.[18]	Echocardiography (including TDI)	Mild DD: E/A ratio <0.75; moderate or pseudonormal DD: E/A 0.75 to 1.5, DT >140 ms, plus 2 other Doppler indices of elevated end-diastolic filling pressure; severe DD: E/A ratio >1.5, DT <140 ms, and Doppler indices of elevated LV end-diastolic filling pressure	39.2	N/A	0.08 (SD 0.05)	10.7 (SD 4.5) (septal)	LAVi 24.7 ml/m^2 (SD 8.5)	N/A	N/A	6.3 (SD 2.3) years	HF	HR 1.81 (95% CI 1.01-3.48)
Lam et al.[15]	Echocardiography (PW Doppler imaging)	Abnormal relaxation: E/A <0.5, DT >280 ms; restrictive filling: mitral E/A >2.0, DT <120 ms; pseudonormal LV filling: distinguished from normal if LA size ≥sex-specific 80th percentile or LV mass ≥sex-specific 80th percentile or any AF	36	N/A	N/A	N/A	N/A	N/A	N/A	11 years	HF	HR 1.32 (95% CI 1.01-1.71)
From et al.[17]	Echocardiography (including TDI)	E/e' ratio >15	23	N/A	N/A	13 (SD 6) (septal)	LAVi 63 ml/m^2 (SD 24)	N/A	N/A	2.9 (SD 1.8) years	HF	HR 1.61 (95% CI 1.17-2.2)
Kardys et al.[28]	Echocardiography (PW Doppler imaging)	Impaired relaxation: E/A <0.75 and DT >240 ms; restrictive pattern: E/A >1.50 and DT <150 ms	11.5	N/A	N/A	N/A	LA diameter 40 mm (SD 5)	N/A	0.83 (IQR 0.71-1.00)	3 years	All-cause mortality	Impaired relaxation: HR 1.55 (95% CI 1.04-2.33); restrictive pattern: HR 7.23 (95% CI 2.16-24.2)

Table 2 (*Continued*)

Study	DD imaging technique	DD criteria	DD prevalence, %	Mean lateral e', cm/s	Mean septal e'e, cm/s	Mean E/e' ratio (septal or lateral e')	Mean LA size	TR jet velocity (mean)	Mean E/A ratio	Follow-up (mean)	Primary endpoint	Association measure (HR/RR/OR (95% CI)]
Okura et al.[31]	Echocardiography (PW Doppler imaging)	LVEF ≥40%+ ≥1 of: (1) DT <140 ms; (2) S/D ratio <1; (3) ARdur-Adur >30 ms	100 (only patients with DD)	N/A	N/A	N/A	LA diameter 42.9 mm (SD 7.5) males; 40.8 (8.0) females	N/A	1.3 (SD 0.5) males; 1.2 (SD 0.4) females	4.4 (SD 1.7) years	All-cause mortality	Incidence rate 6.3%
Ren et al.[19]	Echocardiography (PW Doppler imaging)	Impaired relaxation: E/A ≤0.75 and systolic dominant pulmonary venous flow; Pseudonormal pattern: E/A=0.75-1.5 and LV diastolic dominant pulmonary venous flow; restrictive pattern: E/A >1.5 and LV diastolic dominant pulmonary venous flow	52	N/A	N/A	N/A	N/A	N/A	N/A	3 years	All-cause mortality, heart disease death, non-fatal MI, hospitalization for HF	Impaired relaxation: HR 0.7 (95% CI 0.4-1.3) for all-cause mortality; HR 1.0 (95% CI 0.3-3.8) for heart disease death; HR 1.7 (95% CI 0.7-4.5) for hospitalization for HF; HR 1.7 (95% CI 0.8-3.7) for non-fatal MI; pseudonormal or restrictive: HR 1.2 (95% CI 0.6-2.4) for all-cause mortality; HR 3.9 (95% CI 1.0-14.8) for heart disease death; HR 6.3 (95% CI 2.4-16.1) for hospitalization for HF; HR 1.3 (95% CI 0.5-3.2) for non-fatal MI

Table 2 (*Continued*)

Study	DD imaging technique	DD criteria	DD prevalence, %	Mean lateral e', cm/s	Mean septal e'e, cm/s	Mean E/e' ratio (septal or lateral e')	Mean LA size	TR jet velocity (mean)	Mean E/A ratio	Follow-up (mean)	Primary endpoint	Association measure (HR/RR/OR (95% CI)]
Brady et al.[22]	Cardiac catheterization and LV pressure measurement	LVEDP ≥15 mmHg and LVEF ≥50%	100 (only patients with DD)	N/A	N/A	N/A	N/A	N/A	N/A	63 months	All-cause mortality	Incidence 5%
Redfield et al.[11]	Echocardiography (including TDI)	Mild DD (impaired relaxation): E/A ≤0.75, ΔE/A <0.5, E/e' <10, S >D, ARdur <Adur; moderate DD (pseudonormal): 0.75 <E/A <1.5, DT >140 ms, ΔE/A ≥0.5, E/e' ≥10, S <D or ARdur >Adur+30 ms; severe DD - reversible restrictive: E/A >1.5, DT <140 ms, ΔE/A ≥0.5, E/e' ≥10, S <D or ARdur >Adur+30 ms; fixed restrictive: E/A >1.5, DT <140 ms, ΔE/A <0.5, E/e' ≥10, S <D or ARdur >Adur+30 ms (2 criteria necessary for moderate or severe DD classification)	24.6	N/A	N/A	N/A	N/A	N/A	N/A	5 years (longest)	All-cause mortality	Mild DD: HR 8.31 (95% CI 3.00-23.1); moderate or severe DD: HR 10.17 (95% CI 3.28-31.00)

Table 2 (*Continued*)

Study	DD imaging technique	DD criteria	DD prevalence, %	Mean lateral e', cm/s	Mean septal e'e, cm/s	Mean E/e' ratio (septal or lateral e')	Mean LA size	TR jet velocity (mean)	Mean E/A ratio	Follow-up (mean)	Primary endpoint	Association measure (HR/RR/OR (95% CI)]
Tsang et al.[21]	Echocardiography (PW Doppler imaging)	Abnormal relaxation: mitral E/A <0.75 or DT >240 ms; pseudonormal LV filling: mitral E/A=0.75-1.5 and DT 151-240 ms, but LA volume ≥28 ml/m^2; restrictive filling: E/A >1.5 or DT ≤150 ms	60.1	N/A	N/A	N/A	LAVi 31 ml/m^2 (SD 12) in group with no events; 36 ml/m^2 (SD 12) in group with events	N/A	N/A	3.8 (SD 2.7) years	CV death, MI, coronary revascularization, AF, HF, TIA, stroke	HR 1.64 (95% CI 1.1-2.55)
Aurigemma et al.[16]	Echocardiography (PW Doppler imaging)	E/A <0.7 or >1.5	N/A	N/A	N/A	N/A	LA diameter 3.8 cm (SD 0.6) in group with no events; 4.0 cm (SD 0.7) in group with events	N/A	0.95 (SD 0.3) in group with no events; 0.88 (SD 0.4) in group with events	5.2 years	HF	RR 1.88 (95% CI 1.33-2.68) for E/A ratio <0.7; RR 3.5 (95% CI 1.8-6.8) for E/A >1.5

Adur: mitral A-wave flow duration; ARdur: reverse pulmonary vein flow duration; AF: atrial fibrillation; CABG: coronary artery bypass grafting; CI: confidence interval; CV: cardiovascular; DD: diastolic dysfunction; DT: deceleration time; HF: heart failure; HR: hazard ratio; IQR: interquartile range; LA: left atrial; LAVi: left atrial volume index; LV: left ventricle; LVEF: left ventricular ejection fraction; MI: myocardial infarction; N/A: not available; OR: odds ratio; PW: pulsed-wave; PTCA: percutaneous transluminal coronary angioplasty; PV S/D: pulmonary vein systolic forward flow/diastolic forward flow; RR: risk ratio; SD: standard deviation; TDI: tissue-Doppler imaging; TIA: transient ischemic attack; TR: tricuspid regurgitation.

[a] Unpublished data.

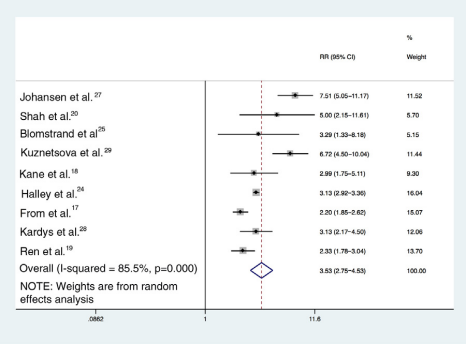

Figure 2 Forest plot showing the overall estimate of the association between diastolic dysfunction and cardiovascular events and/or mortality. CI: confidence interval; RR: relative risk.

DD was 3% (range 2.6-16.8%) and 13.1% (range 5.2-37.4%), respectively. Of the 14 studies providing an association measure, 13 showed a significant association between DD and MACE and/or mortality in the multivariate analysis. The strongest association was reported by Redfield et al.,[11] with an HR of 8.31 (95% CI 3.00-23.1) for all-cause mortality in individuals with mild DD and an HR of 10.17 (95% CI 3.28-31.00) for patients with moderate or severe DD. Only Leibowitz et al.[30] did not find an association (HR 1.028; 95% CI 0.98-1.084) between E/e' ratio (as a continuous variable) and all-cause mortality.

Two studies found an association between diastolic function variables and cardiovascular events and/or mortality: Shah et al.[20] found that abnormal e', E/e' ratio, left atrial dimension and left atrial volume index (LAVi) were significantly associated with incident death or heart failure hospitalization, while Vogel et al.[23] correlated E/A ratio with incident atrial fibrillation.

Banerjee et al.[14] found a significantly worse combined outcome of all-cause mortality and hospitalization in a cohort of patients with DD and increased E/e′ ratio. Okura et al.[31] reported that the cumulative survival rate of DD patients, irrespective of a history of heart failure, was significantly lower than in the general population. However, Brady et al.[22] showed that the mean mortality in the DD group was similar to the general population, and therefore DD with a normal LVEF, in the absence of coronary artery disease and systolic dysfunction, had an excellent prognosis over a long period (5-6 years). Overall, 17 studies showed that DD was a significant predictor of MACE and/or mortality, while two studies did not find this association.

Based on random-effects model meta-analysis, the pooled estimate for relative risk of MACE/mortality for individuals with DD across nine studies was 3.53 (95% CI 2.75-4.53; I^2=85.5%) (Figure 2). Including the studies providing data on hospitalizations and/or mortality (six studies), the pooled estimate using the random-effects model was 3.98 (95% CI 2.91-5.44; I^2=84.2%); in addition, including only the two studies providing all-cause mortality as the primary outcome (Halley et al.[24] and Kardys et al.[28]), DD was associated with a 3.13-fold (95% CI 2.92-3.35; I^2=0%) increased risk of death.

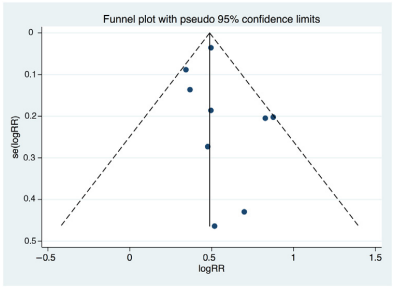

Figure 3 Funnel plot of the studies included in the meta-analysis for assessment of potential asymmetry and risk of publication bias.

Heterogeneity and publication bias

There were significant differences between individual studies in the magnitude of the association between DD and MACE, as indicated by the statistical test for heterogeneity (Q2=55.0, I^2=85.5%, p <0.001). Figure 3 illustrates the funnel plot of studies that excluded small-study effects coming from publication bias (p=0.480 from the Egger test for funnel plot symmetry).

Discussion

This is the most comprehensive systematic review of cohort studies assessing the association between DD and risk of MACE and death. Overall, most studies (17 studies out of 19) showed DD as a significant predictor of cardiovascular events and death. The quantitative analysis using random-effects model meta-analysis yielded a combined risk of MACE and/or death 3.53-fold higher in the presence of DD; in addition, DD was associated with a 3.13-fold increased risk of death.

Diastolic dysfunction as a predictor of increased risk of cardiovascular events and mortality

Research during the last decade has shed some light on the pathophysiological changes leading to DD and its deleterious impact on cardiac function. Common risk factors for cardiovascular disease (such as hypertension, obesity, hypercholesterolemia and diabetes) are associated with systemic inflammation, myocardial oxidative stress and coronary microvascular dysfunction, and are significant contributors to myocardial stiffening and LV DD.[32] In the cardiovascular risk continuum, intermediate stages of risk such as pre-hypertension[6] and non-diabetic metabolic syndrome[33] are already associated with deterioration in indices of diastolic function measured by echocardiography and cardiac MRI, which suggests that there is also a continuum of myocardial structural and functional changes that impact on diastole. Indeed, the complex interplay between a systemic low-grade proinflammatory state, endothelial dysfunction and changes in myocardial extracellular space and intrinsic cardiomyocyte properties are now accepted as the new pathophysiological paradigm for HFpEF.[34] A previous

systematic review and meta-analysis[35] showed that asymptomatic LV DD was associated with an increased risk for incident HF (relative risk 1.7; 95% CI: 1.3-2.2), including data from five studies.[15–19] Therefore, even subclinical DD seems to be strongly involved in the pathophysiology of HF. That being said, both in patients without symptomatic cardiovascular disease and in patients with full-blown cardiovascular disease (such as coronary artery disease), the presence of DD may indicate a more advanced degree of a specific but complex low-grade inflammatory state and structural and functional myocardial changes that appear to be associated with increased risk of CV events and death.

Causes of heterogeneity

In this meta-analysis significant heterogeneity was observed between studies, which may result from three key factors: different study populations, significant differences in the definition of DD, and different definitions of the primary outcome in each of the studies. All of these are potential limitations of this study and are inherent to the methodological approach adopted, especially regarding the quantitative meta-analysis.

Study population

This systematic review included different study populations, coming from different countries and continents and including community-derived individuals, elderly populations, and diabetes cohorts. The mean age differed between the study populations, as did gender distribution and ethnicity. As these were community-derived cohorts, the prevalence of cardiovascular risk factors, such as hypertension, diabetes, smoking and obesity, and cardiac diseases (especially coronary artery disease and heart failure) differed significantly between cohorts. This variability in study populations certainly explains a part of the heterogeneity observed in this meta-analysis. On the other hand, it is interesting to observe that despite the variability in the types of individuals included in this analysis, almost all studies showed a consistent association between DD and increased risk of cardiovascular events.

Diastolic dysfunction criteria

Given the complexity of the pathophysiology of DD, no single echocardiographic parameter can be used to quantify diastole.[36] Therefore, over the last two decades, various parameters and classifications of DD have been used in different studies and in different guidelines, which may also explain some of the heterogeneity observed in this study.

As detailed in Table 2, in our systematic review we critically appraised the DD criteria used in different studies and observed striking differences between studies, even though there are published guidelines on diagnosis and grading. For example, the study by Di Bello et al.[26] cited the 2009 American Society of Echocardiography/European Association of Cardiovascular Imaging (ASE/EACVI) guidelines,[37] but never clarified which variables were used for DD diagnosis. Even Shah et al.,[20] who cited the 2016 ASE/EACVI guidelines,[4] did not proceed exactly as suggested because they used LA diameter >4 cm to define LA dilatation (the guidelines use indexed volumes) and did not include tricuspid regurgitation jet velocity >2.8 m/s, which is one of the four main parameters in the 2016 guidelines. Moreover, in some studies it was difficult to code diastolic function because of the vagueness of definitions and classifications of DD.

Selmeryd et al.[38] examined how the 2009 ASE/EACVI guidelines on the classification of DD were interpreted in the medical community and how variations in the definition of DD affected the reported prevalence. They found that these guidelines have been interpreted differently across studies that cite them with respect to the variables and logical operators used, and that these differences had a substantial impact on the prevalence of DD (range 12-84%).

On the other hand, the 2016 EACVI/ASE consensus on diastolic function[4] was intended to simplify the approach to DD classification. It proposes that four variables with high specificity for myocardial disease should be assessed when determining whether LV diastolic function is normal or abnormal, in order to decrease false positive diagnoses of DD: e' velocity, E/e' ratio, LAVi and peak tricuspid regurgitation (TR) jet velocity. LV DD is present if three or four parameters are abnormal and inconclusive if only two variables are abnormal. A comparison of the impact of the 2016 ASE/EACVI guidelines on the prevalence and grades of DD in comparison with the 2009 ASE/EACVI guidelines showed that the concordance between the classifications is poor and that the former result in a much lower prevalence of DD, apparently only diagnosing the most advanced cases, leaving many patients diagnosed as having indeterminate diastolic function. One possible explanation might be the inclusion of TR jet velocity, which reflects more advanced and severe DD, resulting in lower sensitivity and higher specificity.[39] None of the studies included in this meta-analysis assessed DD using the exact criteria of the 2016 guidelines.

In summary, we strongly believe that it is important to clarify the definition of DD, to correctly assess diastolic function, and to persist in the search for new therapeutic options for DD.

Outcome definitions

In order to assess the prognosis of DD, we included studies that used diverse outcome definitions, such as different combinations of cardiovascular events, HF hospitalization, the combined endpoint of HF hospitalization and mortality, all-cause mortality or cardiac deaths. Since different outcomes were being assessed, some more comprehensive than others, the strength of the associations will inevitably be different.

Clinical relevance

Despite the heterogeneity between the studies included in this work as discussed above, our findings are significant for daily clinical practice and should drive a shift towards a rapid but careful assessment of diastolic function in most patients, as DD was found to be a consistent predictor of cardiovascular events and death. Therefore, we favor the inclusion of a statement concerning diastolic function in all echocardiography reports, when feasible. Notwithstanding, a universal definition of DD is still lacking and therefore most echocardiography laboratories should adopt the one

they feel most confident with and use it for consistency and reproducibility.

Conclusions

In this systematic review we found a consistent association between DD and the risk of cardiovascular events and death in community-based populations with different risk factors and prevalence of cardiac diseases. Individuals with DD showed a 3.53-fold higher risk of cardiac events or death and a 3.13-fold increased risk of mortality. A simple and widely used definition of DD is urgently needed, not only for user-friendly clinical application but also for the development of new therapeutic trials specifically targeting DD in the subclinical phase.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.repc.2019.03.007.

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C. Improving subclinical LV diastolic dysfunction in non-diabetic metabolic syndrome: a role for metformin?

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ORIGINAL ARTICLE

METformin in DIastolic Dysfunction of MEtabolic Syndrome (MET-DIME) Trial: Rationale and Study Design

MET-DIME Trial

Ricardo Ladeiras-Lopes · Ricardo Fontes-Carvalho · Nuno Bettencourt · Francisco Sampaio · Vasco Gama · Adelino F. Leite-Moreira

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Abstract
Purpose Insulin resistance plays a central role in the pathophysiology of metabolic syndrome (MS). Its cardiac deleterious effects are characterized by an increase in fibrous tissue that increases myocardial stiffness and contributes to subclinical left ventricular diastolic dysfunction (LVDD) and heart failure with preserved ejection fraction in patients with MS. In addition to lifestyle counseling (LC), metformin treatment may attenuate or even reverse diastolic dysfunction in these patients. This trial aims to evaluate if treating non-diabetic patients with MS and LVDD with metformin in addition to LC improves diastolic function and assess its impact in functional capacity and health-related quality of life (HRQoL).
Design MET-DIME is a phase II prospective, randomized, open-label, blinded-endpoint trial with a scheduled follow-up of 24 months. Fifty-four patients (adults 40–65 years old with AHA/NHLBI criteria of MS and rest LVDD) will be randomized by minimization to LC only or LC plus metformin (target dose of 1,000 mg twice daily). The primary endpoint will be change in mean of early diastolic mitral annular velocity, an echocardiographic parameter highly correlated with myocardial fibrosis (serial measurements will be performed at 6, 12 and 24 months). The secondary endpoints will include change in diastolic parameters at rest; metabolic, inflammatory and remodeling biomarkers; functional capacity; adipose tissue volumes and HRQoL.
Conclusion MET-DIME is a pragmatic trial designed to evaluate if adding metformin to the standard treatment of patients with MS improves diastolic dysfunction, assessing its impact in metabolic homeostasis, proinflammatory state, functional capacity and HRQoL.

Keywords Metabolic syndrome · Metformin · Diastolic Dysfunction · Insulin resistance

R. Ladeiras-Lopes (✉) · R. Fontes-Carvalho · A. F. Leite-Moreira
Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine of the University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
e-mail: ricardoladeiraslopes@gmail.com

R. Ladeiras-Lopes · R. Fontes-Carvalho · N. Bettencourt · F. Sampaio · V. Gama
Department of Cardiology, Gaia/Espinho Hospital Centre, Vila Nova de Gaia, Portugal

Metabolic Syndrome and Diastolic Dysfunction

The metabolic syndrome (MS) is a constellation of cardiovascular risk factors that reached epidemic proportions during the last two decades. Although there are 6 sets of diagnostic criteria for MS, approximately 20 to 40 % of the adult population of United States and Europe have MS [1]. The prevalence of MS in Portugal also varies with the definition used but approximately 30 % of Portuguese adults fulfill the criteria for MS, with a clear association with cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) [2]. The AHA/NHLBI modification of the NCEP-ATPIII criteria of MS [3] shows the strongest association with CVD in the Portuguese population [4]. Furthermore, although the association with cardiovascular disease and mortality is one of the major concerns on the approach to patients with MS, its harmful impact on global health with functional and psychological repercussions should also be considered [5, 6].

Insulin resistance is central to the pathophysiology of MS, associated with a proinflammatory, prothrombotic and oxidative state that increases the risk of cardiovascular disease, with its micro and macrovascular complications [7]. Moreover, MS is definitely associated with the obesity epidemic [7]. Abdominal obesity is one of the diagnostic criteria for MS, although waist circumference is not capable of distinguish between

visceral fat and subcutaneous adipose tissue, the former with a more ominous effect on cardiovascular function [8]. In the recent years other adipose tissues locations such as pericardial and epicardial fat were also shown to be associated with atherosclerotic burden and coronary disease, representing new cardiometabolic risk markers [9].

The metabolic dysfunctional status previously described is associated with deterioration of cardiac structure and function. Indeed, myocardial fibrosis plays a pivotal role in cardiac dysfunction in hypertensive and diabetic heart disease [10] and is also present in patients with MS [11]. Furthermore, cardiac remodeling with an important fibrotic response is exquisitely associated to left ventricle diastolic dysfunction (LVDD) and is present in older hearts, LV hypertrophy and T2DM [12]. LVDD is also present in patients with MS and there seems to be an association between the grade of LVDD and the number of coexisting MS criteria [13]. However, LVDD in MS patients is often subclinical [5, 14], representing a harder challenge to detect it and follow-up these patients adequately. Not only LVDD is central to the pathophysiology of heart failure with preserved ejection fraction (HF-PEF) [15] but insulin resistance, arterial hypertension, obesity and dyslipidemia are comorbidities of a large proportion of these patients which represent at least half of the heart failure patients [16, 17] The pathophysiological mechanisms of LVDD in HF-PEF are a topic of intense research, and our group has played an active role clarifying some issues regarding not only the modulation of diastolic function but also the mechanisms involved in LVDD [18].

Metformin and Metabolic Syndrome

Considering the dominant role of insulin resistance in the pathophysiology of the MS and its cardiac deleterious effects, it seems reasonable to consider that an increase in insulin sensitivity might be associated with a global improvement in the structure and function of the heart. Metformin is a biguanide approved for the treatment of T2DM known by its insulin-sensitizing effect. Furthermore, in the last years it was demonstrated in animal models of insulin resistance and arterial hypertension that metformin prevents cardiac remodeling and progression to heart failure with an evident benefit in time periods less than a year [19, 20]. The cardioprotection afforded by metformin treatment seems to result from interference with TGF-beta signaling pathway and activation of the AMP-kinase signaling cascade [21, 22].

Since insulin resistance is a dominant player in the MS in non-diabetic patients, improvement of the metabolic profile of these patients with metformin might be associated with favorable remodeling of myocardial structure and an improvement in myocardial function. According to the current recommendations for the management of non-diabetic patients with MS [3], lifestyle changes are mandatory and metformin is an option to these patients, but the demonstration of an unequivocal cardiovascular benefit would provide an inoffensive and widely available pharmacological weapon to improve the quality of life and delay the cardiovascular detrimental effects of MS.

Trial Objectives

1. Assess if treating non-diabetic patients with MS and rest LVDD with metformin, in addition to lifestyle counseling, improves diastolic function and assess its impact in functional capacity and health-related quality of life (HRQoL);
2. Evaluate if biomarkers of cardiac remodeling, inflammation, and glucose homeostasis are predictive factors of response to metformin treatment of non-diabetic patients with MS and LVDD.

Design

The study will include a screening phase and a prospective, randomized, open-label, blinded-endpoint (PROBE) trial to assess the effect of metformin administration in addition to lifestyle counseling to non-diabetic patients with MS during a scheduled duration of 24 months of follow-up.

During the screening phase patients will be recruited from the outpatient clinic of Gaia/Espinho Hospital Centre (tertiary care hospital with a reference population of 700,000 patients) dedicated to non-diabetic hypertensive patients with a total population of approximately 1,000 adult patients, the majority of whom have a clinical diagnosis of MS.

After the screening phase (with a maximum duration of 10 months), eligible subjects will be randomized to lifestyle counseling only or lifestyle counseling plus metformin treatment and followed-up for a 2-year period (Fig. 1). The lifestyle counseling will be provided in the form of written information and individualized during the interview in all clinic visits, emphasizing the importance of a healthy lifestyle, engage on regular moderate-intensity physical activity and healthy diet. Metformin treatment will start with 500 mg once daily (at breakfast) during the first week (Step 1); if well tolerated, the dose will be progressively increased to 500 mg twice daily (at breakfast and dinner) during 1 week (Step 2), to 1,000 mg at breakfast and 500 mg at dinner during 1 week (Step 3), in order to reach the target dose of 1,000 mg twice daily (at breakfast and dinner) during the rest of the follow-up.

Randomization will be performed using the minimization method [23]. Minimization criteria will be age (under 55 years

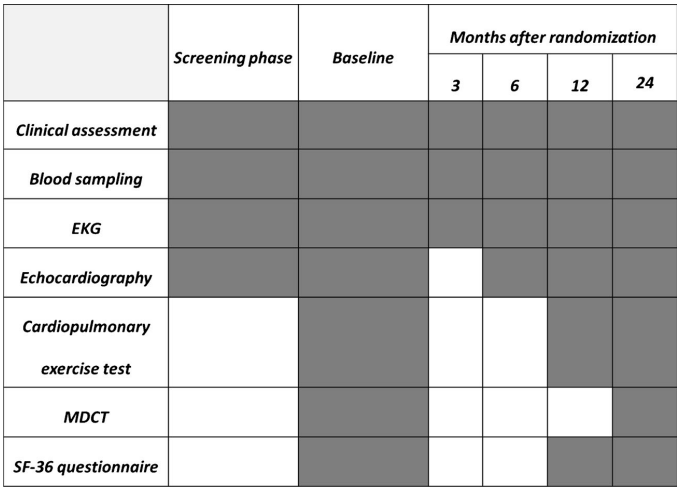

Fig. 1 Planned follow-up for all participants. *EKG* electrocardiography; *MDCT* multidetector computed tomography; *SF-36* Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36)

old or ≥55 years old), gender (male or female), baseline treatment with drugs that affect the renin-angiotensin-aldosterone axis (ACE inhibitors, angiotensin receptor blockers, mineralocorticoids antagonists or renin inhibitors), presence or absence of typical symptoms or most specific signs of heart failure [according to the European Society of Cardiology [24]] and degree of diastolic dysfunction [grades I, II and III, EAE/ASE echocardiographic criteria [25]].

Study Population

Eligible participants will be non-diabetic adults aged between 40 and 65 years fulfilling the AHA/NHLBI criteria for clinical diagnosis of MS [(at least 3 of the following: waist circumference ≥102 cm in males or ≥88 cm in females; fasting plasma triglycerides ≥150 mg/dL or on drug treatment for elevated triglycerides; fasting HDL cholesterol <40 mg/dL in males or <50 mg/dL in females or on drug treatment for reduced HDL; systolic blood pressure≥130 mmHg or diastolic blood pressure≥85 mmHg or on antihypertensive drug treatment in a patient with history of hypertension; fasting plasma glucose≥100 mg/dL) [3]] and with echocardiographic evidence of LVDD at rest, considering the mean of septal and lateral E′ as assessed by Tissue Doppler Imaging echocardiography (E'_{mean}<10.2 cm/s if 40–59 years old or E'_{mean}<7.2 cm/s if aged 60 to 65 years old) [25]. Patients should be in a stable dose of antihypertensive or antidislypidemic medication at least 1 month prior to recruitment, able to perform a cardiorespiratory fitness test and give a written informed consent.

Exclusion criteria are described in Table 1.

Endpoints

The primary endpoint will be the change in E'_{mean} during the 24 month follow-up period. Serial echocardiographic measurements will be performed at baseline, month 6, month 12 and month 24. The E′ is an echocardiographic parameter strongly correlated with myocardial fibrosis (r=−0.7) [25, 26].

The secondary endpoints will include diastolic echocardiographic parameters, metabolic indices, cardiovascular, remodeling and inflammation biomarkers, functional capacity, epicardial, pericardial and abdominal adipose tissue volumes, coronary calcium score and HRQoL (Table 2).

Safety and Adverse Reactions

Although metformin is a widely used drug, its use at the dose of 1,000 mg twice daily is associated with gastrointestinal (GI) side effects at the onset of treatment. These side effects are reduced if the medication is taken with food and the dose titrated from once daily to twice daily over several weeks.

Potential non-gastrointestinal (non-GI) side effects include, but are not limited to: severe headache, moderate edema, disabling leg cramps, arthralgia, myalgia, dizziness, mild rashes, and dysmenorrhea.

If non-GI side effects considered likely to be due to metformin occur and require cessation of treatment during Step 1, metformin will be stopped for 4 weeks. If the non-GI symptoms disappear, a second attempt to introduce metformin will be performed after 4 weeks. If symptoms re-occur, metformin will be discontinued one more time. A last try will be performed after 4 weeks. If metformin continues to be not tolerated, the patient will be excluded from the trial.

Table 1 Study population: inclusion and exclusion criteria

Inclusion criteria
- 40-65 years old
- Metabolic syndrome, AHA/NHLBI criteria [3] (at least 3 of the following):
  - Waist circumference ≥102 cm in males or ≥88 cm in females;
  - Fasting plasma triglycerides ≥150 mg/dL or on drug treatment for elevated triglycerides;
  - Fasting HDL cholesterol <40 mg/dL in males or <50 mg/dL in females or on drug treatment for reduced HDL;
  - Systolic blood pressure≥130 mmHg or diastolic blood pressure≥85 mmHg or on antihypertensive drug treatment in a patient with history of hypertension;
  - Fasting plasma glucose≥100 mg/dL.
- Rest left ventricle diastolic dysfunction, based on the mean of septal and lateral E′ (E'_{mean})
  - E'_{mean}<10.2 cm/s if 40–59 years old;
  - E'_{mean}<7.2 cm/s if aged 60 to 65 years old.
- Stable dose of antihypertensive or antidislypidemic medication at least 1 month prior to recruitment.
- Able to perform a cardiopulmonary exercise test.

Exclusion criteria
- Diabetes mellitus according to the ADA criteria [27] (at least one of the following):
  - fasting plasma glucose ≥126 mg/dL;
  - 2-h plasma glucose ≥200 mg/dL during an oral glucose tolerance test, as described by the WHO;
  - random plasma glucose ≥200 mg/dL in a patient with classical symptoms of hyperglycemia or hyperglycemic crisis;
  - hemoglobin A1C≥6.5 % using a method that is NGSP certified and standardized to the DCCT assay or ≥48 mmol/mol reported in IFCC units.
- Ischemic heart disease (history of angina, acute coronary syndrome, acute myocardial infarction or coronary artery bypass graft surgery);
- Left ventricle ejection fraction less than 50 % (assessed by transthoracic echocardiography);
- Moderate or severe valvular heart disease;
- Pericardial disease;
- Uncontrolled atrial or ventricular tachyarrhythmias;
- History of myocarditis;
- Renal disease or dysfunction (creatinine clearance <60 mL/min, calculated by the Cockcroft-Gault formula)
- Significant liver disease (aspartate aminotransferase or alanine aminotransferase ≥2.5 times upper limit of normal)
- Females who are pregnant, planning to become pregnant or who admit sexual activity without appropriate contraception;
- Lactation.

Table 2 Primary and secondary endpoints

Primary endpoint
- Mean of septal and lateral early diastolic mitral annular velocities (E'_{mean})—baseline, 6, 12, 24 months

Secondary endpoints
- Diastolic echocardiographic parameters:
  - E/E′ratio; Isovolumetric relaxation time (IVRT); E/A ratio, E wave deceleration time, diastolic dysfunction grades according to the ASE/ESE consensus, strain rate during IVRT (SR-IVRT) and E/SR-IVRT ratio
- Metabolic biomarkers:
  - Insulin and glucose plasma levels, insulin resistance (HOMA—Homeostasis Model Assessment) and adiponectin levels;
- Cardiovascular biomarkers:
  - N-terminal pro-BNP and high sensitivity C-reactive protein;
- Remodeling and inflammation biomarkers:
  - TNF-α (tumor necrosis factor α) , TIMP1 (type 1 tissue inhibitor of matrix metalloproteinase) e GDF-15 (growth-differentiation factor 15);
- Functional capacity during cardiopulmonary exercise test:
  - Peak oxygen uptake, anaerobic threshold and ventilatory efficiency
- Epicardial, pericardial and abdominal adipose tissue volumes, and coronary calcium score, assessed by cardiac multidetector CT (MDCT)
- Health-related quality of life, according to Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36).

If non-GI side effects occur that are considered likely due to metformin during Step 2, Step 3 or Target Dose, the participant will drop back to the previous titulation step for 4 weeks. A second attempt to restart the corresponding dose will be made after 4 weeks. If symptoms re-occur during this second attempt, the participant is restarted at the previous titulation step for 4 weeks again. A third attempt to uptitrate metformin is made after 4 weeks. If this third attempt fails, the participant will be maintained with the dosing of the previous titulation step for the remainder of the follow-up.

GI side effects will be managed in the same way as non-GI symptoms but each interruption period (if in Step 1) or dose reduction (if in other titulation steps) will last only 1 week.

Statistical Plan

Statistical Analysis

To specifically address Objective 1, the analysis of the primary endpoint will be performed on an intention to treat basis by repeated-measures analysis of covariance (ANCOVA) including the following variables: baseline mean E′, age and mean arterial pressure, treatment group, gender, treatment with drugs that affect the renin-angiotensin-aldosterone axis at baseline, presence or absence of signs/symptoms of heart failure and baseline degree of diastolic dysfunction. Serial measurements will include baseline, month 6, month 12 and month 24 assessment. An interim analysis of the primary endpoint is planned at the end of the first 12 months of follow-up.

To address trial Objective 2 we will explore the ANCOVA model previously described assessing interaction between treatment group and baseline levels of metabolic indices and inflammatory and remodeling biomarkers.

All statistical tests will be two-sided, with an alpha level of 0.05.

Power and Sample Size

Considering the prespecified analysis of the primary endpoint at 12 months of follow-up, a sample size of 21 patients for each group was estimated to allow the detection of a difference in means of 1.5 cm/s with a power of 80 % and an alpha of 0.05, assuming a standard deviation of 2.3 cm/s in each group [25] and a conservative baseline/follow-up measurement correlation of 0.3. Considering a potential drop-out rate of 20 %, the final estimated sample size needed in each allocation arm was 27 patients.

Ethical and Legal Issues

All eligible participants should be able and willing to provide written informed consent in order to be included in this study.

This randomized trial was approved by the Local Ethics Committee, Portuguese Data Protection Agency, INFARMED (National Pharmacy and Medicines Institute) and CEIC (National Ethics Committee for Clinical Research). It is registered in www.clinicaltrials.gov with the Identifier NCT02017561. The study will be conducted in accordance with the Declaration of Helsinki.

Conclusion

MET-DIME is a pragmatic trial designed to evaluate if adding metformin to the standard treatment of patients with MS improves diastolic dysfunction, assessing its impact in metabolic homeostasis, proinflammatory state, functional capacity and HRQoL.

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

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TITLE

Metformin in non-diabetic patients with metabolic syndrome and diastolic dysfunction: the MET-DIME randomized trial

AUTHORS:

Ricardo Ladeiras-Lopes[1,2], Francisco Sampaio[1,2], Sara Leite[2], Diogo Santos-Ferreira[1,2], Eduardo Vilela[1], Adelino Leite-Moreira[1,2], Nuno Bettencourt[1,2], Vasco Gama[1], Pedro Braga[1], Ricardo Fontes-Carvalho[1,2]

INSTITUTIONS:

1. Cardiology Department, Gaia Hospital Centre, Vila Nova de Gaia, Portugal
2. Cardiovascular Research and Development Centre, Faculty of Medicine of the University of Porto, Porto, Portugal

CORRESPONDING AUTHOR:

Ricardo Ladeiras-Lopes

Cardiology Department, Gaia Hospital Centre & Cardiovascular Research Centre, Faculty of Medicine, University of Porto

Porto, Portugal

Phone: +22 786 51 00

Electronic address: ricardoladeiraslopes@gmail.com

ABSTRACT

Aims: Metabolic syndrome (MetS) affects one out of 3 adults in the western world and is associated with preclinical diastolic dysfunction that impairs functional capacity and quality of life (QoL). This randomized trial was designed to evaluate if the addition of metformin to the standard treatment of non-diabetic patients with MetS improves diastolic dysfunction.

Methods and Results: Prospective, randomized, open-label, blinded-endpoint trial. Fifty-four non-diabetic adults with MetS and diastolic dysfunction were randomized to lifestyle counseling or lifestyle counseling plus metformin (target dose 1000 mg bid). The primary endpoint was the change in mean e' velocity (assessed at baseline, 6, 12 and 24 months). Secondary endpoints were improvements in insulin resistance, functional capacity and QoL. Linear mixed effects modelling was used for longitudinal data analysis using modified intention-to-treat (mITT) and per-protocol (PP) approaches.

Forty-nine patients were included in the mITT analysis (mean age=51.8±6.4; 55% males). Metformin treatment was associated with a significant decrease in HOMA-IR. There was a significantly different mean change in e' velocity during the study period between trial arms, both in the mITT (at 24 months, change of +0.67±1.90cm/s in metformin arm vs. -0.33±1.50cm/s in control arm) and PP populations (+0.80±1.99cm/s in metformin arm vs. -0.37±1.52cm/s in control arm), using a random intercept linear mixed model. There were no significant differences in peak oxygen uptake and SF-36 scores between trial arms.

Conclusion: Treatment with metformin of non-diabetic MetS patients with diastolic dysfunction, on top of lifestyle counseling, is associated with improved diastolic function.

KEYWORDS: metabolic syndrome, diastole, metformin, insulin resistance

INTRODUCTION

Metabolic syndrome (MetS) represents a cluster of cardiovascular risk factors sharing common pathophysiological mechanisms, and affects at least one quarter of the world population(1, 2). It is associated with cardiac remodeling and deterioration of cardiac function, including microvascular dysfunction, coronary atherosclerosis and calcification, and heart failure(3). Myocardial fibrosis and left ventricular (LV) diastolic dysfunction are some of the most frequent manifestations of subclinical cardiac involvement in MetS(4, 5). In particular, diastolic dysfunction is a pivotal player in the pathophysiology of heart failure with preserved ejection fraction (HFpEF), and is associated with an increased risk of cardiovascular events or death(6).

Metformin is the most widely used oral antihyperglycemic agent in patients with type 2 diabetes mellitus (T2DM) and reduces the incidence of T2DM in high-risk individuals (abnormal glucose homeostasis)(7). Recently, new potential therapeutic applications of metformin targeting the cardiovascular system have been described, including the prevention of cardiac fibrosis and HFpEF(8). The cardioprotection afforded by metformin treatment seems to result from interference with TGF-beta signaling pathway and activation of the AMP-kinase signaling cascade(9, 10).

In this randomized trial we aimed to evaluate if metformin can improve diastolic function in non-diabetic patients with MetS and LV diastolic dysfunction. We also aimed to assess the impact of this therapy in functional capacity and health-related quality of life (HRQoL).

METHODS

Study population

The detailed study protocol was previously reported(11). Briefly, this study included non-diabetic adults aged between 40 and 65 years with non-diabetic MetS and diastolic dysfunction. MetS was defined using the AHA/NHLBI criteria, requiring at least 3 of the following: waist circumference ≥94 cm in males or ≥80 cm in females; fasting plasma triglycerides ≥150 mg/dL or on drug treatment for elevated triglycerides; fasting HDL-cholesterol (HDL-C) <40 mg/dL in males or <50 mg/dL in females or on drug treatment for reduced HDL-C; systolic blood pressure≥130 mmHg or diastolic blood pressure≥85 mmHg or on antihypertensive drug treatment in a patient with history of hypertension; fasting

plasma glucose≥100 mg/dL)(12). Echocardiographic evidence of LV diastolic dysfunction at rest was defined as mean e′ velocity <10.2 cm/s if 40–59 years old or mean e' velocity <7.2 cm/s if aged 60 to 65 years, according to previous recommendations(13).

For eligibility, patients had to be in a stable dose of antihypertensive or antidyslipidemic medication at least 1 month prior to recruitment, able to perform a cardiopulmonary exercise test (CPET) and give written informed consent.

Exclusion criteria were: T2DM according to American Diabetes Association criteria(14), known ischemic heart disease, moderate to severe valvular heart disease, pericardial disease, LV ejection fraction less than 50%, uncontrolled atrial or ventricular tachyarrhythmias, history of myocarditis, renal disease or dysfunction (creatinine clearance <60 mL/min, calculated by the Cockcroft-Gault formula), significant liver disease (aspartate aminotransferase or alanine aminotransferase ≥2.5 times upper limit of normal), females who were pregnant, planning to become pregnant or who admitted sexual activity without appropriate contraception; lactation.

All included participants provided written informed consent in order to be included in this study. This randomized trial was approved by the Local Ethics Committee, Portuguese Data Protection Agency, INFARMED (National Pharmacy and Medicines Institute) and CEIC (National Ethics Committee for Clinical Research). It is registered in www.clinicaltrials.gov with the Identifier NCT02017561. The study was conducted in accordance with the Declaration of Helsinki.

Study design

The study was a prospective, randomized, open-label, blinded-endpoint (PROBE) trial to assess the effect of metformin administration in addition to lifestyle counseling to non-diabetic patients with MetS during a scheduled duration of 24 months of follow-up.

Individuals included in the trial were randomized to lifestyle counseling only or lifestyle counseling plus metformin treatment (target dose: 1,000mg twice daily) and followed-up for a 2-year period (Fig. 1). Lifestyle counseling was provided in the form of written information and individualized during the interview in all clinic visits, emphasizing the importance of a healthy lifestyle, regular moderate-intensity physical activity and healthy diet.

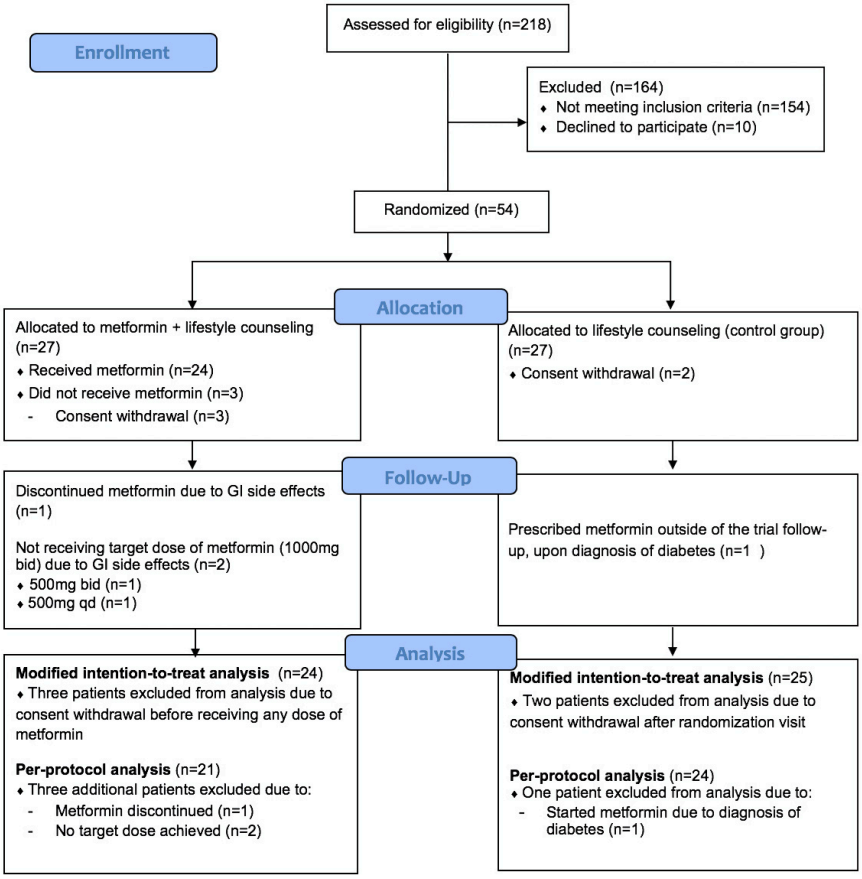

Figure 1. Trial CONSORT diagram.

GI – gastrointestinal.

The intervention consisted in giving metformin starting with 500 mg once daily (at breakfast) during the first week; if well tolerated, the dose was progressively increased to 500 mg twice daily (at breakfast and dinner) during week 2, to 1,000 mg at breakfast and 500 mg at dinner during week 3, in order to reach the target dose of 1,000 mg twice daily (at breakfast and dinner) during the rest of the follow-up.

Randomization was performed using the minimization method(15). The following were criteria used for minimization method: age (under 55 years old or ≥55 years old), gender (male or female), baseline treatment with drugs that affect the renin-angiotensin-aldosterone

axis [angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), mineralocorticoids antagonists or renin inhibitors)], presence or absence of typical symptoms or most specific signs of heart failure (according to the European Society of Cardiology(16)) and degree of diastolic dysfunction (grades I, II and III), EAE/ASE echocardiographic criteria(13).

Endpoints

The primary endpoint was the change in the mean early diastolic mitral annular velocity (mean e'), at 6, 12 and 24 months. In addition, we also assessed the change in the following secondary endpoints: body weight and waist circumference, insulin resistance index (HOMA—Homeostasis Model Assessment), plasma levels of N-terminal pro-BNP (NT-proBNP) and high sensitivity C-reactive protein; functional capacity during cardiopulmonary exercise test (peak oxygen uptake), and health-related quality of life, according to the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36).

Blood tests

Venous blood samples were collected after an overnight fast for measurement of standard analytical parameters and plasma levels of insulin, NT-proBNP, high sensitivity CRP. Insulin resistance was assessed by the HOMA index, calculated as fasting insulin (mIU/L)*fasting glucose (mg/dL)/405.

Echocardiography

All exams were performed using an Acuson SC 2000 heart ultrasound system (Siemens Medical Solutions, Erlangen, Germany) and recorded for offline analysis using specialized software. The operator was blinded for the study treatment. Echocardiographic studies included standard 2D, spectral Doppler, color-Doppler and tissue-Doppler imaging. Cardiac chamber dimensions, volumes and left ventricular mass were measured following the standard recommendations(17) and indexed to body surface area. LV systolic function was evaluated by ejection fraction using the modified biplane Simpson's rule. Diastolic function was assessed according to the 2009 Guidelines on Diastolic Function Evaluation(13) with measurement of mitral inflow velocities (E-wave, A-wave, E/A ratio) and E-wave deceleration time and isovolumetric relaxation time using pulsed-wave Doppler in the apical 4-chamber

view. Velocities were recorded at end-expiration and averaged over 3 consecutive cardiac cycles. Pulsed-wave tissue Doppler velocities were acquired at end-expiration, in the apical 4-chamber view, at both lateral and septal side of the mitral annulus, measuring early diastolic (e') and late diastolic (a') velocities and estimating the E/e' ratio accordingly.

Cardiopulmonary exercise test

All patients performed symptom-limited treadmill exercise testing (Mortara X-Scribe, Mortara Instruments, Milwaukee, WI, USA) according to a modified Bruce protocol, with simultaneous respiratory gas analysis. Peak oxygen uptake (peak VO2), indexed to body weight, was determined.

Health related quality of life assessment

The Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) was used to assess general health status. This psychometric test is validated to the Portuguese population(18, 19).

Statistical analysis

Summary statistics were presented as the mean±standard deviaton (SD) for normally distributed continuous variables and median (interquartile range) for non-normally distributed continuous variables. Normality of distribution was assessed using the Shapiro-Wilk test. Data is presented as percentages for categorical variables.

For the primary endpoint, the main analysis was performed using a modified intention-to-treat approach (including all patients with at least one baseline observation that did not withdrew consent from the trial immediately after randomization visit, Figure 1). A random intercept mixed model was used to explore the influence of metformin treatment upon mean e' velocity over time, including as fixed effects the treatment arm and the variables used for minimization: age at baseline (under 55 years old or ≥55 years old), gender, treatment with drugs that affect the renin-angiotensin-aldosterone axis at baseline, presence or absence of signs/symptoms of heart failure and baseline degree of diastolic dysfunction. In addition, an independent t-test was used to compare the absolute mean difference at 6, 12 and 24 months, versus baseline, according to intervention arm.

Due to the pragmatic approach of the trial, to allow a better evaluation of the efficacy of the intervention, a per-protocol analysis was also used. Patients with at least one follow-up observation were included, and for this analysis we excluded patients from the metformin arm that were not treated with the target dose of 1000mg metformin twice a day during the full study period (n=3), and also patients from the control arm that started metformin during the study period (n=1).

All analyses were performed using STATA version 14.0 (StataCorp, College Station, TX). Statistical significance was defined as P<0.05. All reported P values are two-tailed.

Sample size

An interim analysis of the primary endpoint at 12 months was used for sample size estimates. A sample size of 21 patients for each group was estimated to allow the detection of a difference in means of 1.5 cm/s with a power of 80 % and an alpha of 0.05, assuming a standard deviation of 2.3 cm/s in each group and a conservative baseline/follow-up measurement correlation of 0.3. Considering a potential drop-out rate of 20 %, the final estimated sample size needed in each allocation arm was 27 patients.

RESULTS

Recruitment and follow-up

Of the 218 individuals who were screened for eligibility, 154 subjects did not fulfill all inclusion criteria and therefore were excluded (Figure 1). Ten patients declined to participate. At the end, 54 patients were randomly allocated to receive metformin and lifestyle counseling (metformin arm, $N = 27$) or lifestyle counseling (control arm, $N = 27$). Five patients were excluded from all study analysis, 3 in the metformin arm and 2 in the control arm, due to consent withdrawal after the randomization visit. Twenty-four patients completed the study in the metformin arm and 25 in the control arm (this was the patient population used for descriptive statistics and modified intention-to-treat analysis), as detailed in the flowchart in Figure 1. Metformin was discontinued in 1 patient due to gastrointestinal side effects and 2 patients were maintained on low dose metformin (1 patient with 500mg once a day and 1 patient with 500mg twice a day). In the control group, 1 patient was diagnosed with diabetes in a clinical visit outside the trial and was prescribed metformin.

These 4 additional patients (3 from the metformin arm and 1 from the control group) were excluded for the population used for the per-protocol analysis.

Baseline patient characteristics

The characteristics of the participants are presented in Table 1. The mean age of the final sample was 51.8±6.4 years, and 55% were males. The overall prevalence of each MetS component was: increased waist circumference (100%), increased blood pressure (92%), low HDL-cholesterol (59%), abnormal glucose homeostasis (55%), increased triglycerides (49%).

The mean e' velocity was 9.2±1.4 cm/s and E/e' ratio was 9.0±1.9. The mean LV ejection fraction, LV end-diastolic volume index, and LV mass index were 59.4±3.7%, 54.4±11.6 mL/m2 and 86.6±21.1 g/m2, and there were no significant differences between trial arms. Sixty-three percent of patients were taking ACE inhibitors or ARBs.

Primary endpoint

In the mITT analysis (Figure 2), metformin treatment was associated with an improvement in e' velocity at 6 months (absolute mean difference of 0.59 [95% confidence interval, -0.12 to 1.30], p=0.099), at 12 months (absolute mean difference of 0.57 [95% confidence interval, -0.21 to 1.35], p=0.150) and at 24 months (absolute mean difference of 1.00 [95% confidence interval, -0.03 to 2.03], p=0.056), which did not reach statistical significance (Table 2). In the adjusted analysis, using a linear mixed model adjusting for age, gender, treatment with drugs that affect the renin-angiotensin-aldosterone axis at baseline, presence or absence of signs/symptoms of heart failure and baseline degree of diastolic dysfunction, provided a significant p-value for the interaction term of metformin and time (beta-coefficient=0.28, SE=0.13, p=0.034). The effect of metformin in e' velocity was independent of changes in insulin resistance (HOMA index).

In the per-protocol analysis (Figure 2), metformin significantly improved e' velocity at the 3 time points. At 6 months change in e' velocity of +0.47±1.25 in metformin arm vs -0.29±1.10 cm/s in control arm (p=0.048), with an absolute mean difference of 0.76 (95% CI, 0.01 to 1.51). At 12 months, +0.49±1.36 cm/s in metformin arm vs -0.42±1.04 cm/s control arm (p=0.017), and an absolute mean difference of 0.91 (95% CI, 0.17 to 1.65). At 24 months, +0.80±1.99 cm/s in metformin arm vs -0.37±1.52 cm/s in control arm (p=0.039), with an absolute mean difference of 1.17 (95% CI, 0.06 to 2.28).

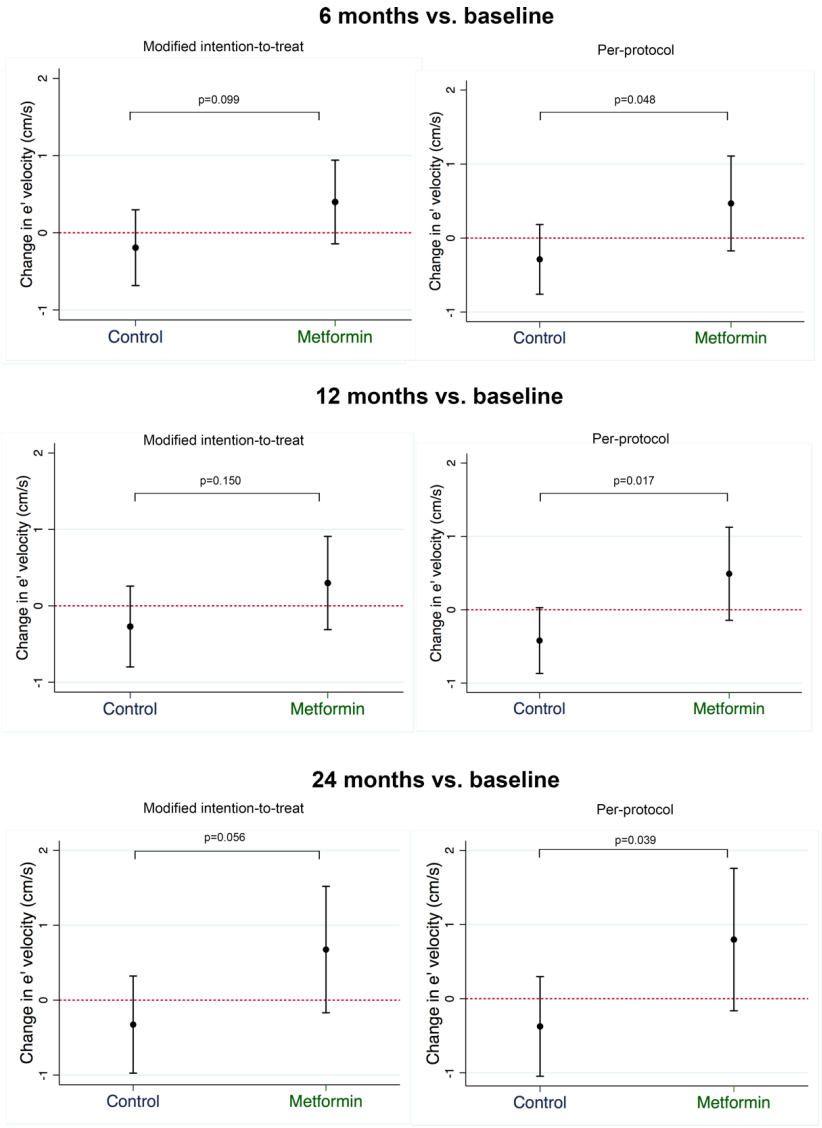

Figure 2. Change in e' prime velocity between trial arms at 6, 12 and 24 months, versus baseline.

Time-specific comparison of the change in e' velocity between trial arms showed a trend for improvement in e' velocity in the modified intention-to-treat analysis and significantly improved e' in the metformin arm according to the per-protocol analysis. Using a linear mixed model, including all longitudinal data, metformin was associated with a significant improvement in e' velocity during the full study period.

In the adjusted linear mixed model, there was a significant benefit of metformin over time in the e' velocity (beta-coefficient=0.35, SE=0.14, p=0.011), that remained significant after including HOMA index in the model.

Secondary endpoints

Metformin was associated with a decrease in the HOMA insulin resistance index, which was statistically significant at 12 months both in the mITT (absolute mean difference of -2.43, p=0.030) and per-protocol analysis (absolute mean difference of -2.37, p=0.042), and in the per-protocol population at 24 months (absolute mean difference of -1.72, p=0.044).

There was a significant decrease in body weight at 6 months (absolute mean difference of -2.47 kg in the mITT analysis, p=0.015), that was no longer significant at 12 and 24 months. When compared to lifestyle counseling only, metformin treatment significantly reduced waist circumference at all time points (absolute mean difference of -5.79 cm in the mITT analysis at 24 months, p=0.008). There was no significant between-group difference in left ventricular mass index during the full study period.

No difference in the change of hs-CRP and NTproBNP plasma levels was found according to metformin treatment. In addition, as showed in Table 2, at 24 months there was an absolute mean difference of 1.2 mL/min/kg in the peak VO2 (change in metformin arm 0.37±2.64 mL/min/kg vs control arm -0.83±1.91 cm/s), that was not statistically significant (p=0.137 in the per-protocol population). No differences in change in physical and mental health scores (according to the SF-36 questionnaire) were found, with a positive trend in both arms.

Table 1 - Demographic, clinical, laboratory and echocardiographic characteristics at baseline

	Total (n=49)	Treatment arm		P-value
		Metformin (n=24)	No metformina (n=25)	
Demographics				
Age, years	51.8±6.4	51.2±6.2	52.4±6.7	0.506
Male gender, n(%)	27 (55)	11 (46)	16 (64)	0.201
Medical history, n(%)				
Current smokers	10 (20)	5 (21)	5 (20)	0.942
Former smokers	13 (27)	7 (29)	6 (24)	0.682
Symptoms of heart failure	19 (39)	9 (38)	10 (40)	0.858
Hypertension	42 (86)	21 (88)	21 (84)	0.726
Dyslipidaemia	42 (86)	19 (79)	23 (92)	0.199
Metabolic syndrome, n(%)	49 (100)	24 (100)	25 (100)	
Increased waist circumference	49 (100)	24 (100)	25 (100)	
Increased triglycerides	24 (49)	9 (38)	15 (60)	0.115
Low HDL-C	29 (59)	14 (58)	15 (60)	0.906
Increased arterial pressure	45 (92)	23 (96)	22 (88)	0.317
Abnormal glucose homeostasis	27 (55)	12 (50)	15 (60)	0.482
Physical examination				
BMI, kg/m2	31.2 (29.0-34.3)	32.3 (30.2-35.0)	29.5 (28.7-32.4)	0.062
Waist circumference, cm	104 (100-107)	105 (100-111)	103 (101-106)	0.417
SBP, mmHg	142 (128-157)	147 (131-176)	138 (127-148)	0.101
DBP, mmHg	86.5±12.4	89.1±12.2	84.1±12.3	0.164
Heart rate, bpm	73.9±10.8	72.9±11.8	74.9±9.9	0.519
Baseline medications, n(%)				
ACE inhibitor or ARB	31 (63)	15 (63)	16 (64)	0.913
Beta-blocker	15 (31)	11 (46)	4 (16)	0.024
Calcium channel blocker	13 (27)	6 (25)	7 (28)	0.812
MRA	2 (4)	2 (8)	0 (0)	0.141
Diuretic	21 (43)	10 (42)	11 (44)	0.869
Lipid-lowering drug	23 (47)	10 (42)	13 (52)	0.469
Laboratory results				
Haemoglobin, g/dL	14.8±1.4	14.8±1.5	14.9±1.3	0.723
Creatinine, g/dL	0.8±0.2	0.8±0.2	0.9±0.2	0.259
eGFR, mL/min/1.73m2	140.1±48.1	150.5±50.0	130.2±45.1	0.151
Glucose, mg/dL	101.1±13.4	100.8±17.0	101.5±9.2	0.860
Insulin, mIU/L	14.1 (10.8-20.1)	12.9 (8.2-20.0)	15.9 (11.5-20.1)	0.285
HbA1c, %	5.7±0.4	5.7±0.5	5.7±0.4	0.873
HOMA index	3.7 (2.6-5.1)	3.3 (1.9-5.2)	4.2 (3.0-4.8)	0.254
hs-CRP, mg/L	0.3 (0.2-0.6)	0.3 (0.2-0.6)	0.3 (0.2-0.5)	0.610
NT-pro-BNP, pg/mL	46 (22-72)	52 (29-78)	42 (14-52)	0.067
Total cholesterol, mg/dL	205.3±33.8	202.6±38.3	207.8±29.7	0.598
LDL-cholesterol, mg/dL	127.1±34.0	126.7±37.8	127.4±31.0	0.951
HDL-cholesterol, mg/dL	45.3±11.2	46.8±9.8	44.0±12.4	0.398
Triglycerides, mg/dL	143 (100-225)	111 (88-166)	177 (135-238)	0.021
Echocardiographic data				
LVEF, %	59.4±3.7	58.4±3.8	60.1±3.6	0.186
LVEDV, mL	106.1±24.7	113.7±20.3	99.7±26.7	0.104
iLVEDV, mL/m2	54.4±11.6	58.1±9.9	51.3±12.2	0.088
LVESV, mL	43.1±11.9	47.3±9.7	39.6±12.7	0.061
iLVESV, mL/m2	22.1±5.5	24.2±4.4	20.4±5.9	0.047
LVMI, g/m2	86.6±21.1	88.7±23.4	84.6±18.9	0.506
Left atrial volume index, mL/m2	32.5±8.8	34.7±9.8	30.6±7.6	0.137
E wave velocity, cm/s	81.4±19.3	83.3±19.3	79.6±14.7	0.462
Mean e' velocity, cm/s	9.2±1.4	9.0±1.4	9.4±1.4	0.257
E:e' ratio	9.0±1.9	9.3±1.9	8.6±1.9	0.182
IVRT, ms	91.0±20.2	91.8±21.9	90.4±19.1	0.821
DT, ms	206.0±32.6	203.1±24.6	208.9±39.4	0.554
Grade of diastolic dysfunction				0.581
I	13 (27)	6 (25)	7 (28)	
II	35 (71)	17 (71)	18 (72)	
III	1 (2)	1 (4)	0 (0)	
Cardiopulmonary exercise testing				
Maximum exercise duration, min	9.7±2.2	9.3±2.2	10.1±2.2	0.182
Peak VO2, mL/min/kg	25.5±5.5	24.5±5.9	26.5±5.0	0.189
Health-related quality of life score				
SF-36 physical function score	46.7±9.3	45.6±8.8	47.7±9.8	0.438
SF-36 mental function score	40.8±14.7	41.1±15.7	40.6±14.0	0.905

ACE – angiotensin converting enzyme, ARB – angiotensin II receptor blocker, BMI - body mass index; DBP - diastolic blood pressure; DT – deceleration time; GFR – glomerular filtration rate; hs-CRP – high-sensitivity C-reactive protein; iLVEDV – indexed LV end-diastolic volume; iLVESV – indexed LV end-systolic volume; IVRT – isovolumic relaxation time; LVEF – left ventricular ejection fraction; LVEDV – left ventricular end-diastolic volume; LVESV – left ventricular end-systolic volume; LVMI – left ventricular mass index; MRA – mineralocorticoid receptor antagonists, SBP - systolic blood pressure.

Table 2 - Primary and secondary endpoint longitudinal analysis according to randomisation arm

	Absolute change in endpoint																			
	6 months vs. Baseline								12 months vs. Baseline								24 months vs. Baseline							
	mITT analysis				Per-protocol analysis				mITT analysis				Per-protocol analysis				mITT analysis				Per-protocol analysis			
	Metformin	Control	Difference (95% CI)	P-value	Metformin	Control	Difference (95% CI)	P-value	Metformin	Control	Difference (95% CI)	P-value	Metformin	Control	Difference (95% CI)	P-value	Metformin	Control	Difference (95% CI)	P-value	Metformin	Control	Difference (95% CI)	P-value
Primary endpoint																								
e' velocity (cm/s)	0.39 (1.16)	-0.19 (1.16)	0.59 (-0.12;1.30)	0.099	0.47 (1.25)	-0.29 (1.10)	0.76 (0.01;1.51)	**0.048**	0.30 (1.41)	-0.27 (1.25)	0.57 (-0.21;1.35)	0.150	0.49 (1.36)	-0.42 (1.04)	0.91 (0.17;1.65)	**0.017**	0.67 (1.90)	-0.33 (1.50)	1.00 (-0.03;2.03)	0.056	0.80 (1.99)	-0.37 (1.52)	1.17 (0.06;2.28)	**0.039**
Key secondary endpoints																								
Anthropometric data																								
Body weight (kg)	-3.30 (3.51)	-0.84 (3.08)	-2.47 (-4.42;-0.51)	**0.015**	-3.91 (3.39)	-1.00 (3.03)	-2.92 (-4.92;-0.91)	**0.006**	-1.81 (5.59)	-0.42 (3.40)	-1.39 (-4.09;1.32)	0.307	-2.36 (5.73)	-0.59 (3.37)	-1.76 (-4.61;1.08)	0.218	-1.69 (5.63)	-0.59 (4.30)	-1.10 (-4.07;1.86)	0.457	-2.30 (5.79)	-0.53 (4.39)	-1.77 (-4.95;1.40)	0.265
Waist circumference (cm)	-4.67 (5.29)	-1.62 (3.60)	-3.05 (-5.70;-0.39)	**0.025**	-5.39 (4.89)	-1.65 (3.67)	-3.74 (-6.41;-1.08)	**0.007**	-4.93 (7.51)	-1.71 (3.33)	-3.23 (-6.61;0.16)	0.061	-5.93 (7.28)	-1.87 (3.31)	-4.06 (-7.46;-0.65)	**0.021**	-7.52 (8.52)	-1.73 (5.31)	-5.79 (-9.97;-1.61)	**0.008**	-8.97 (7.69)	-1.80 (5.42)	-7.17 (-11.27;-3.07)	**0.001**
Analytical data																								
HOMA index	-0.50 (3.12)	-0.04 (2.34)	-0.47 (-2.19;1.25)	0.586	-0.68 (3.33)	-0.21 (2.26)	-0.47 (-2.33;1.38)	0.610	-1.53 (3.76)	0.90 (3.31)	-2.43 (-4.61;-0.25)	**0.030**	-1.70 (3.92)	0.67 (3.20)	-2.37 (-4.65;-0.09)	**0.042**	-0.42 (2.41)	0.90 (2.90)	-1.32 (-2.91;0.28)	0.103	-0.78 (2.30)	0.94 (2.96)	-1.72 (-3.41;-0.04)	**0.044**
hs-C-reactive protein (mg/dL)	-0.008 (0.645)	0.007 (0.206)	-0.015 (-0.307;0.277)	0.919	-0.058 (0.568)	-0.001 (0.207)	-0.057 (-0.326;0.213)	0.672	-0.087 (0.545)	-0.003 (0.143)	-0.084 (-0.328;0.159)	0.488	-0.086 (0.566)	-0.016 (0.131)	-0.069 (-0.327;0.188)	0.589	0.172 (0.975)	0.011 (0.244)	0.161 (-0.263;0.586)	0.448	0.099 (1.013)	0.013 (0.249)	0.086 (-0.366;0.539)	0.702
NT-pro-BNP (pg/mL)	7.38 (34.02)	-6.43 (22.40)	13.80 (-5.04;32.65)	0.146	5.50 (29.96)	-5.00 (21.98)	10.50 (-7.61;28.61)	0.246	3.25 (19.49)	3.32 (12.38)	-0.07 (-10.56;10.42)	0.990	0.27 (15.95)	4.29 (11.80)	-4.02 (-13.41;5.37)	0.390	9.71 (31.83)	1.35 (32.86)	8.37 (-11.35;28.08)	0.397	7.11 (31.32)	3.00 (32.64)	4.11 (-16.51;24.74)	0.689
Echocardiography																								
E/e' ratio	-0.69 (1.28)	0.28 (1.10)	-0.97 (-1.70;-0.25)	**0.010**	-0.64 (1.34)	0.35 (1.08)	-0.99 (-1.77;-0.22)	**0.013**	-0.31 (1.60)	0.12 (1.28)	-0.43 (-1.29;0.43)	0.317	-0.38 (1.59)	0.16 (1.29)	-0.54 (-1.44;0.36)	0.229	-0.57 (1.61)	0.02 (1.54)	-0.59 (-1.54;0.35)	0.214	-0.61 (1.71)	0.05 (1.57)	-0.66 (-1.70;0.37)	0.204
LVMI (g/m2)	4.50 (14.81)	2.57 (15.43)	1.93 (-7.67;11.53)	0.686	2.37 (10.58)	2.24 (15.74)	0.12 (-9.15;9.39)	0.979	-4.12 (17.06)	-0.20 (16.33)	-3.93 (-14.34;6.49)	0.451	-1.64 (16.37)	-0.52 (16.66)	-1.11 (-12.05;9.82)	0.837	-0.29 (15.30)	3.45 (14.75)	-3.74 (-12.88;5.40)	0.414	-0.30 (16.05)	3.45 (14.75)	-3.75 (-13.48;5.98)	0.441
CPET																								
Peak VO2 (mL/min/kg)	N/A				N/A				0.90 (2.37)	0.10 (3.57)	0.80 (-1.12;2.72)	0.405	0.79 (2.49)	0.21 (3.61)	0.58 (-1.48;2.64)	0.575	0.29 (2.56)	-0.88 (1.86)	1.17 (-0.33;2.68)	0.122	0.37 (2.64)	-0.83 (1.91)	1.20 (-0.41;2.81)	0.137
HRQoL																								
SF-36 physical function score	N/A				N/A				5.72 (7.23)	2.51 (6.08)	3.20 (-0.95;7.36)	0.127	5.31 (7.81)	2.78 (6.10)	2.53 (-2.04;7.11)	0.269	4.55 (7.93)	2.02 (9.24)	2.53 (-2.82;7.87)	0.345	4.31 (8.60)	1.82 (9.40)	2.49 (-3.43;8.42)	0.399
SF-36 mental function score	N/A				N/A				3.84 (15.02)	2.64 (10.47)	1.21 (-6.81;9.22)	0.763	1.49 (13.83)	2.94 (10.62)	-1.46 (-9.50;6.59)	0.716	10.21 (14.67)	5.50 (9.42)	4.72 (-2.78;12.21)	0.211	9.65 (13.73)	6.11 (9.16)	3.55 (-3.89;10.98)	0.340

CPET – cardiopulmonary exercise test; HRQoL – health-related quality-of-life; LV – left ventricular mass index; mITT – modified intention-to-treat;

DISCUSSION

In this pragmatic randomised clinical trial which included non-diabetic patients with MetS and diastolic dysfunction (Take-home figure), we observed that a) treatment with metformin on top of lifestyle counseling was associated with improved diastolic function (increase in e' velocity); b) this effect was independent of changes in insulin resistance; c) metformin was not associated with a significant impact on functional capacity or HRQoL.

The current integrated approach to the management of non-diabetic MetS includes an intensive lifestyle intervention beyond control of individual risk factors, and metformin represents a therapeutic option in those patients(20). When compared to patients with T2DM, non-diabetic individuals with MetS already show evidence of subclinical diastolic dysfunction with impaired relaxation that is independent of myocardial extracellular volume by cardiac MRI, a surrogate marker of diffuse myocardial fibrosis(5). Considering the progressive detrimental effect of MetS on diastolic function, that seems to be also independent of LV hypertrophy(21), the demonstration of a beneficial effect of metformin could provide a well-tolerated and widely available pharmacological weapon to tackle the cardiac impact of MetS. In the non-diabetic population with MetS, a previous small study showed that metformin administration during 6 months was associated with reduced LV mass and increased peak early diastolic strain rate, with a non-significant increase in e' velocity. In our study, metformin treatment on top of lifestyle counseling was associated with an improvement in the mitral e' velocity, an echocardiographic variable associated not only with myocardial relaxation but also with cardiac fibrosis(22). There was no significant between-group difference in left ventricular mass index, emphasizing previous data showing that diastolic impairment in MetS seems to be independent of LV hypertrophy(21).

Several previously described mechanistic pathways can contribute to the beneficial effect of metformin in diastolic function observed in this study. Metformin has a protective role in heart failure via AMPK/nitric oxide (NO) signaling, which might play a pivotal role(23). AMPK intracellular cascade has already shown several beneficial effects upon cardiac structure and diastolic function: a) it seems to be involved in titin isoform shift to more compliant isoforms(24); b) it attenuates ventricular remodeling and dysfunction following aortic banding in mice via the Sirt3/oxidative stress pathway(25); c) it influences SERCA2a and phospholamban protein content in the sedentary and exercised heart (26).

Furthermore, increased levels of NO and signaling through protein kinase G are also know to modulate myocardial relaxation properties and therefore are putative candidates to account for the beneficial effect of metformin(27).

In this study, the observed beneficial effects of metformin in diastolic function were independent of the effect on insulin resistance. Interestingly, metformin is known to exert favorable hemodynamic and cardiac remodeling effects, as well as an antioxidant and anti-inflammatory action, independent of alterations in glycaemia(28, 29). Data from the recently published MET-REMODEL trial also corroborates our findings, where metformin treatment was associated with decreased LV mass index in non-diabetic individuals, independently of changes in insulin resistance(30).

There was no significant difference in change in peak functional capacity (as assessed by peak VO2) between the 2 trial arms, although there was an absolute increase in peak VO2 in patients treated with metformin, as compared to a decrease in the control group (p=0.137 in the per-protocol population). Previous data have shown that myocardial e' velocity is correlated with peak VO2 in heart failure patients, although the most powerful predictor of the latter seemed to be the E/e' ratio (31, 32). In view of the present findings, we hypothesize that albeit having impaired myocardial relaxation, not only did most patients show normal filling pressures at baseline (mean E/E' ratio of 9.0±1.9) – suggesting early-stage diastolic dysfunction – but in addition there were no significant changes between both trial arms, thus suggesting that the improvement in myocardial relaxation fell short as to evoke a significant change in peak oxygen uptake. A longer study period or a trial targeting peak functional capacity as the primary endpoint could provide additional answers.

This study is a single-center, open-label, pragmatic, randomized clinical trial with a small sample size. However, it was adequately powered to answer the objective of the study and the pragmatic design adopted might reflect "real-world" conditions. Adherence to treatment with metformin was not strictly controlled. Furthermore, inferences regarding secondary endpoint analyses are exploratory, and should be considered as hypothesis-generating.

In conclusion, treatment with metformin of non-diabetic MetS patients with diastolic dysfunction was associated with improved diastolic function, independently of changes in insulin resistance.

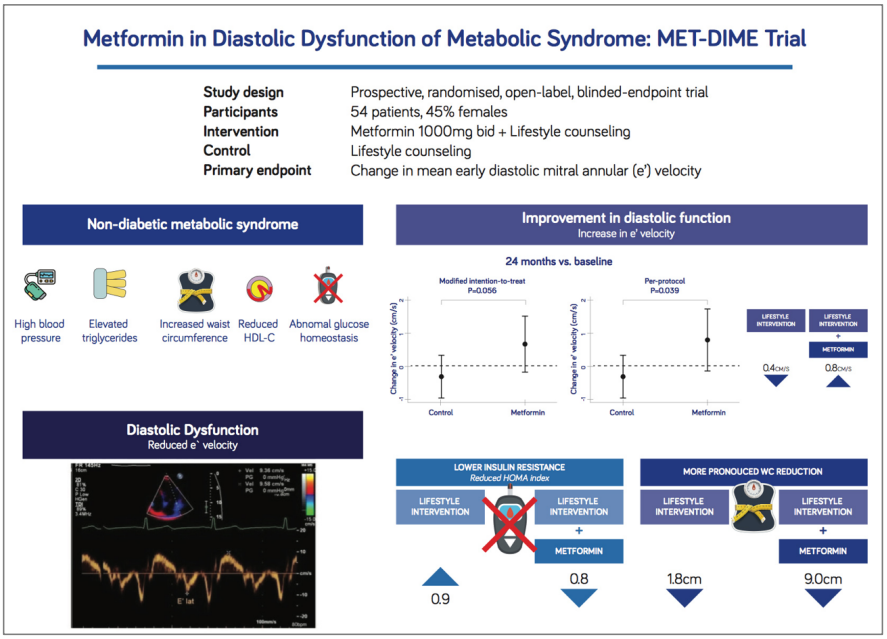

Figure 3 – **Take-home figure**

In non-diabetic patients with MetS and diastolic dysfunction, treatment with metformin was associated with improvement in diastolic function (as shown by an increase in e' velocity), as well as with reduced waist circumference and insulin resistance.

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DISCUSSION

DISCUSSION

The research studies that integrate this thesis unraveled new perspectives and provided a better understanding of the cardiometabolic risk factors and their association with CVD and cardiac structure and function (Figure 2). First, we demonstrated that the ratio of visceral to subcutaneous abdominal fat is a predictor of death and cardiovascular events and that individuals with pre-hypertension already show impaired diastolic function. In addition, cardiac magnetic resonance imaging (CMR) was used to demonstrate that non-diabetic patients with MetS already have impaired myocardial relaxation even in the absence of increased cardiac fibrosis. The utility of a MetS severity score, allowing a quantitative measurement of cardiometabolic impairment, was also shown to be a predictor of impaired diastolic function. Finally, the MET-DIME trial emphasize the potential role of metformin in the treatment of non-diabetic patients with MetS and LVDD, with a positive impact on diastolic function. The discussion will follow the three sub-chapters that were used in the Results/Publications.

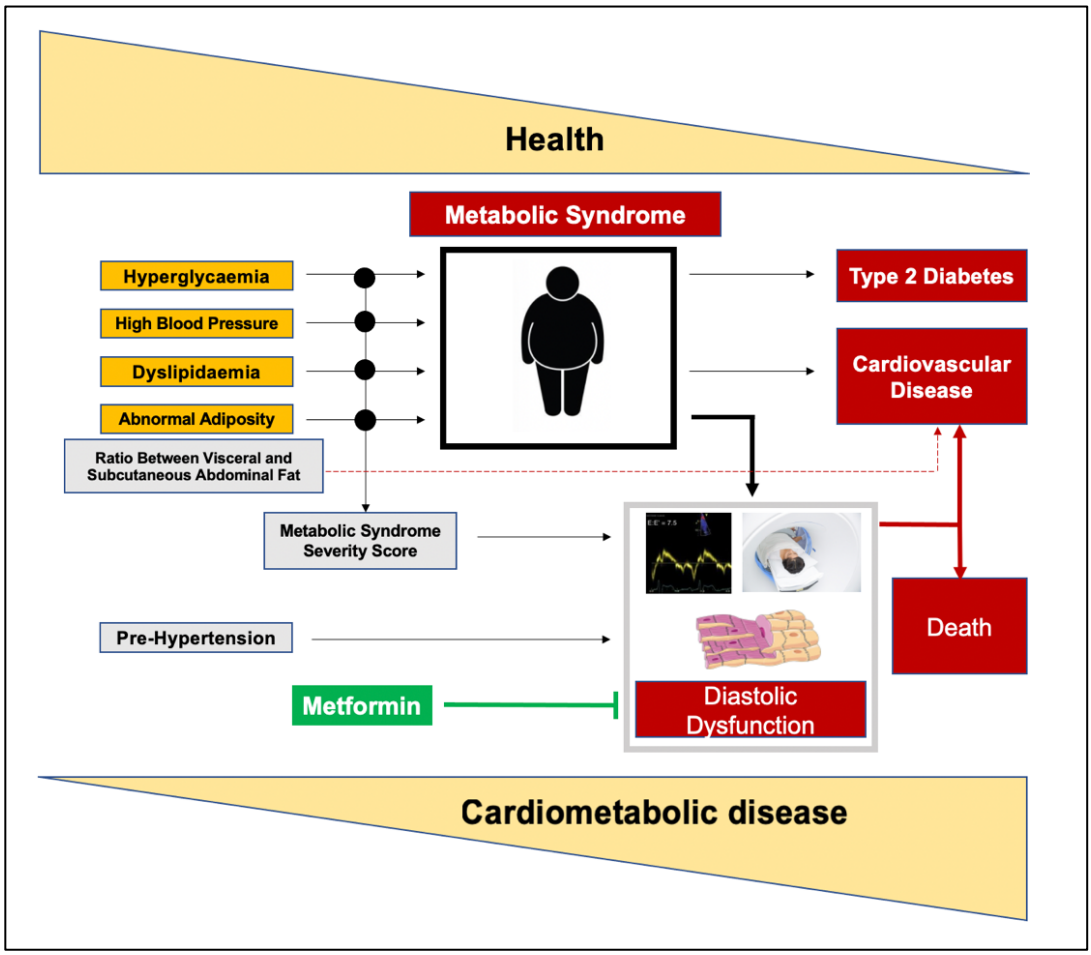

Figure 2. New perspectives of the impact of cardiometabolic risk factors in cardiovascular disease (with special emphasis on left ventricular diastolic dysfunction)

A. New perspectives on the impact of cardiometabolic risk factors in CVD – a new approach to "old" risk factors?

The successful management of cardiometabolic-based chronic disease continuum is dependent on the accurate and early identification of risk factors and subclinical structural changes, in order to prevent full-blown clinical disease. To do this, traditional risk factors should be put into perspective, while deriving new approaches to assess and quantify its impact on cardiovascular health.

The importance of the relative distribution of fat in the abdominal compartment to determine its cardiometabolic impact was explored longitudinally in Publication 1[63]. An increased visceral to subcutaneous abdominal fat ratio was associated with higher total mortality and incidence of major adverse cardiovascular events. Visceral adipose tissue is associated with a highly lipolytic state, with proinflammatory cytokine release and therefore favouring insulin resistance and endothelial dysfunction[64]. Interestingly, the association was independent of traditional cardiometabolic risk factors and coronary calcium, suggesting that preferential accumulation of fat in the abdominal visceral compartment might promote chronic, low-grade systemic inflammation, increasing the risk of plaque vulnerability, rupture and thrombosis. This deleterious impact of visceral adipose tissue also links it to the current pathophysiological paradigm of HFpEF, and obviously it is not surprising that it has a prognostic role as a predictor of hospitalization[65]. That being said, it is clear that the cardiometabolic impact of obesity is not sufficiently described by the BMI or WC alone, depending not only on its "quantity" but also on its "location". In addition, ectopic fat depots such as epicardial adipose tissue seem to be associated not only with atherosclerotic burden and risk of CVD, but also with maladaptive changes in myocardial function that increase the risk of heart failure, as highlighted in a Letter to the Editor[66] concerning Publication 1.

Out of Sight, out of Mind; Subcutaneous, Visceral, and Epicardial Adipose Tissue. Response

Ojos que no ven, corazón que no siente: el tejido adiposo subcutáneo, epicárdico y visceral. Respuesta

To the Editor,

We have read with great interest the Letter to the Editor concerning our recently published paper on the association between an increased ratio of visceral to subcutaneous abdominal adipose tissue and higher risk of major adverse cardiovascular events, independently of traditional cardiovascular risk factors and coronary calcium.[1]

Epicardial adipose tissue (EAT) is an ectopic fat storage site in direct contact with adjacent coronary arteries and myocardium; therefore, it can have a paracrine effect on coronary atherosclerosis and myocardial function through the secretion of several adipokines that might regulate insulin resistance and inflammation.[2]

The putative association between EAT and visceral or subcutaneous abdominal fat is a promising research line that should continue to be addressed in future studies. Our group has already shown that EAT volume is positively correlated to coronary atherosclerotic burden, assessed by coronary artery calcium score, independently of abdominal visceral adipose tissue.[3] Furthermore, in patients after a myocardial infarction, EAT volume was independently associated with decreased E' velocity and increased E/E' ratio, therefore suggesting impaired diastolic function.[4]

Thus, EAT seems to be associated not only with atherosclerotic burden and risk of cardiovascular disease, but also with maladaptive changes in myocardial function that increase the risk of heart failure. It is our opinion that ectopic adipose tissue, with special emphasis on EAT, greatly contribute to metabolic homeostasis and modulate activation of inflammatory cascades, therefore being a key player in cardiovascular health and disease.

Ricardo Ladeiras-Lopes,[a,b,*] Francisco Sampaio,[a,b] Nuno Bettencourt,[b] and Ricardo Fontes-Carvalho[a,b]

[a]*Department of Cardiology, Gaia/Espinho Hospital Centre, Vila Nova de Gaia, Portugal*
[b]*Unidade de Investigação Cardiovascular, Faculdade de Medicina, Universidade do Porto, Porto, Portugal*

*Corresponding author:
E-mail address: ricardoladeiraslopes@gmail.com (R. Ladeiras-Lopes).

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The changing approach to cardiometabolic risk factors discussed in this thesis includes not only different and clinically meaningful ways of measuring them but also early identification of minor derangements with clinical significance. And this is the case of hypertension, especially pre-hypertension. In Publication 2[67], in a cross-sectional study using a community-based cohort of asymptomatic individuals without known CVD, diastolic function impairment was found to be more pronounced in hypertensive individuals, but changes in diastolic function were already present in prehypertensive individuals, reflecting subclinical organ damage in this population. The deterioration of cardiac function in those patients might be explained by several mechanisms, such as increased fibrosis, hypophosphorylation of titin, altered myocardial metabolism, decreased nitric oxide availability and a low-grade pro-inflammatory milieu[68]. Of note, most are shared with the complex pathophysiology of cardiometabolic-based chronic disease and HFpEF. This highlights the critical importance of early identification of asymptomatic individuals "at risk", in order to prevent progressive deterioration in cardiac function.

CMR-derived techniques that allow a more precise and thorough evaluation of cardiac structure and function have been described in the last decades[69, 70], facilitating the detection of early subclinical changes in individuals "at risk". Those individuals are usually managed conservatively because they are still in what it is believed to be an initial stage of the cardiometabolic-based chronic disease continuum. Relying on myocardial tagging to assess diastolic function, and on extracellular volume using T1 mapping as a surrogate to interstitial myocardial fibrosis, we showed in Publication 3[71] that both higher insulin resistance and the presence of MetS, even without T2DM, were associated with impaired diastolic function (higher strain relaxation index and lower end-diastolic strain rate). This finding was independent of the myocardial interstitium (including fibrosis), and therefore the functional myocardial changes

seemed to result from intrinsic cardiomyocyte alterations. Interestingly, increased myocyte stiffness rather than increased fibrosis has been proposed as the main contributor to diastolic dysfunction in patients with T2DM who have HFpEF[72]. Likewise, the earliest changes in the heart of patients with MetS seem to have a preferential impact upon myocardial relaxation and myocyte resting tension. Overall, subclinical deleterious changes in cardiac function might help to better stratify patients with cardiometabolic-based chronic disease and MetS, and lead to earlier and more aggressive decisions in the management of these patients.

The cardiometabolic-based chronic disease model also suggests a continuum of risk, and therefore the MetS could be regarded as a continuum of metabolic derangement rather than a disease that is present or not. However, the dichotomic definition would still be needed, especially regarding classification and therapeutic decisions, but a continuous measurement of metabolic impairment would provide us a more accurate quantification of its severity. In addition, the same risk factor may contribute to overall risk with different preponderance, according to gender and race/ethnicity. A continuous gender and race/ethnicity-specific MetS severity score was described and validated in 2014 by Gurka et al.[20], and, interestingly, this score was associated with future coronary heart disease independently of individual MetS components, and showed superior predictive and discrimination ability than NCEP-ATPIII criteria[21]. In Publication 4[73], our research group demonstrated that increasing MetS severity score was associated with higher insulin resistance, increased inflammatory biomarkers and metabolically dysfunctional adipokines profile (high leptin and low adiponectin). Altogether, this implies that this score works as an integrated index of metabolic dysfunction. In addition, higher MetS score was associated with decreased *e'* velocity (impaired relaxation) and increased *E/e'* ratio (higher LV filling pressures) and patients with diastolic dysfunction showed higher MetS score and the latter was a predictor of diastolic dysfunction (defined according to a 2017 clinically-oriented algorithm[74]), independently of age and individual MetS components, although this was not the case for the definition of diastolic dysfunction according to 2009 and 2016 EACVI/ ASE recommendations[60, 61] (the controversies surrounding the definition and prognostic significance of diastolic dysfunction will be explored further in the next topic). Those findings highlight the close link between cardiometabolic risk factors and LVDD, in a continuum of higher insulin resistance, low-grade inflammation and unfavorable adipokine profile.

B. The contribution of left ventricular diastolic dysfunction to cardiovascular morbidity and mortality – a ominous cardiac "red flag"

LVDD is described as a disturbance in LV relaxation, distensibility or filling[53]. What seems to be a simple definition, encompasses a lot of controversy, not only regarding its definition but also the complex link to development of heart failure symptoms[54] and its association with all-cause mortality[46]. Notwithstanding, the close association between MetS and LVDD is well known, sharing several pathophysiological mechanisms through a chronic, low-grade systemic inflammatory state, culminating in interstitial fibrosis, increased myocyte stiffness and abnormal calcium homeostasis[75].

LVDD is usually diagnosed in clinical practice using echocardiography, a non-invasive imaging technique, and graded using Doppler echocardiographic findings. In 2009, the recommendations from the European Association of Echocardiography and the American Society of Echocardiography were published[60]. However, the heterogeneity between studies in the definition of LVDD continued and had a significant effect in its reported prevalence, ranging from 12 to 84% in the community setting[76]. The 2009 recommendations were updated in 2016[61]. However, the 2016 definition of LVDD raised several questions because it was associated with a much lower prevalence of LVDD than previous reported, detecting only the most advanced cases[77].

Our research team performed a systematic review and meta-analysis of cohort studies assessing the association between LVDD and incidence of major adverse cardiovascular events (MACE) and death (Publication 5)[78]. Overall, we included data from nineteen studies and approximately 63,000 individuals. Most studies (17 studies out of 19) showed LVDD as a significant predictor of cardiovascular events and death. LVDD was associated with a 3.53-fold higher risk of MACE or death and 3.13-fold increased risk of death. Given the consistent clinical significance and prognostic importance of LVDD, a simple and widely used definition of LVDD is urgently needed, not only for user-friendly clinical application but also for the development of new therapeutic trials specifically targeting LVDD in the subclinical, pre-symptomatic phase.

C. Improving subclinical LV diastolic dysfunction in non-diabetic metabolic syndrome: a role for metformin?

The close association between MetS and LVDD led us to hypothesize if a pharmacologic agent targeting MetS would have a beneficial effect in LVDD. Metformin is a biguanide approved for the treatment of T2DM known by its insulin-sensitizing effect. In addition, it is an option in the management of MetS (even without T2DM) and it has been demonstrated in animal models of insulin

resistance and arterial hypertension that metformin prevents cardiac remodeling and progression to heart failure with an evident benefit in time periods less than a year[79, 80].

The MET-DIME trial was a pragmatic randomised trial (Publication 6) designed to evaluate if metformin could improve diastolic function in non-diabetic patients with MetS and LVDD at rest. The results showed that treatment with metformin was associated with improved diastolic function (increase in *e'* velocity), independently of changes in insulin resistance. This improvement was not associated with a significant impact on functional capacity or HRQoL.

From previous studies exploring the cardioprotective action of metformin, two intracellular cascades were suggested as key mediators of its effect: interference with TGF-beta signaling pathway (a pro-fibrotic and pro-proliferative pathway) and activation of the AMP-kinase signaling cascade (an heterogeneous intracellular pathway that is closely linked to several other protein kinases and nitric oxide production)[81, 82]. Several mechanisms involved in the regulation of myocardial relaxation and cardiomyocyte stiffness are modulated by AMPK/nitric oxide (NO) signaling, including titin isoform shift to more compliant isoforms[83] and SERCA2a and phospholamban protein content[84], and therefore our results show biological plausibility. Interestingly, the observed beneficial effects of metformin in diastolic function in the MET-DIME trial were independent of the effect on insulin resistance. Our results are in line with the recently published MET-REMODEL trial, where metformin treatment was associated with decreased LV mass index in non-diabetic individuals, independently of changes in insulin resistance[85]. Overall, the potential direct effect of metformin on cardiovascular system, as well as its anti-inflammatory and anti-oxidant effects[86], independent of alterations in glycemia, open up new horizons for future uses of metformin in cardiometabolic-based chronic diseases. With regard to LVDD, considering its prognostic significance, patients with non-diabetic MetS should be systematically screened for the presence of LVDD and if positive, metformin seems to be a safe and effective drug to improve diastolic function. Furthermore, event-driven clinical trials might be warranted to evaluate the effect of metformin in this population.

CONCLUSIONS

CONCLUSIONS

Cardiovascular diseases are a challenging and persistent burden of disease. Despite the enormous progress in the prevention, diagnosis and management of CVD observed in the last decades, resulting in a decrease in CVD mortality, we are now starting to see some signals of an unfavorable trend. In some regions, CVD mortality curve is now flat (even with an increasing trend in some rural areas) and the direct and indirect costs of CVD are increasing[87]. The failure to actively identify and make risk factor modifications and the failure to diagnose CVD, especially at its subclinical, vastly asymptomatic stage, are key missed opportunities in the management of the global burden of CVD.

In this context, the correct identification and characterization of MetS traits represent a huge opportunity to implement an early and sustainable prevention care plan, to reduce the burden of CVD. This is in line with a patient-centric viewpoint and the cardiometabolic-based chronic disease model recently suggested. In this continuum, we propose an updated perspective of traditional risk factors in order to optimize our evaluation of cardiovascular risk. As an example, adipose tissue accumulation in visceral abdominal compartment, instead of subcutaneous location, is a marker of worse CV prognosis, and intermediate stages of risk such as pre-hypertension and non-diabetic MetS are already associated with subclinical deterioration in cardiac function, as demonstrated in this thesis. A continuous score of MetS severity is also associated with diastolic dysfunction, and the latter is a significant predictor of CVD and CV death. Therefore, systematic evaluation of diastolic function by echocardiography in individuals with cardiometabolic risk factors is suggested as part of an intensive case finding strategy in the primary prevention of stage 2 of cardiometabolic-based chronic disease, to detect pre-clinical LVDD, a significant predictor of MACE and death. In those patients, an intensive lifestyle change is paramount, and we also demonstrated a significant benefit of using metformin to improve diastolic dysfunction, irrespective of the presence of T2DM.

To conclude, the cardiovascular impact of MetS should be integrated in the continuum of cardiometabolic-based chronic disease, with an updated and meaningful re-interpretation of traditional risk factors and a thorough evaluation of cardiac structure and function using advanced cardiac imaging modalities. We should aim to modify this negative continuum as soon as possible, with the early and systematic identification of powerful prognostic predictors, still in a pre-clinical, asymptomatic stage, such as diastolic dysfunction, promoting lifestyle change and using well-known pharmacologic agents, that seem to have new therapeutic applications, like the example of metformin to improve diastolic dysfunction.

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Ricardo Ladeiras-Lopes

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