

Myocardial Mechanics in Metabolic Syndrome and Aging Populations

Submitted by

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STATEMENT OF SOURCES

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Table i. Abbreviations

LIST OF COMMON ABBREVIATIONS	
CVD	Cardiovascular disease
LV	Left ventricle/Left ventricular
RV	Right ventricle/Right ventricular
LA	Left atrial
LVED	Left ventricular end-diastolic
LVES	Left ventricular end-systolic
E WAVE	Early-diastolic transmitral blood velocity
A WAVE	Late-diastolic transmitral blood velocity
DT of E	Deceleration time of E wave
LVM	Left ventricular mass
LVMi	Left ventricular mass index
IVS	Inter-ventricular septum
E _m	Early-diastolic mitral annulus tissue velocity
A _m	Late-diastolic mitral annulus tissue velocity
S _m	Systolic mitral annulus tissue velocity
LVDD	Left ventricular diastolic dysfunction
TAPSE	Tricuspid annular plane systolic excursion
TDI	Tissue Doppler imaging
STE	Speckle tracking echocardiography
VVI	Vector-velocity imaging
SR	Strain rate
TTP	Time to peak
MetS	Metabolic syndrome
HTN	Hypertension
T2D	Type-2 diabetes
LVH	Left ventricular hypertrophy
BMI	Body mass index
DEXA	Dual-energy x-ray absorptiometry
HDL	High density lipoproteins
LDL	Low density lipoproteins
CRP	C-reactive protein
TNF- α	Tumour necrosis factor α
IL-6	Interleukin-6
PAI-1	Plasminogen activator inhibitor-1
HOMA-IR	Homeostatic model of assessment- insulin resistance
Pro-BNP	Pro- Brain natriuretic peptide
EAT	Epicardial adipose tissue
SD	Standard deviation

Thesis Abstract

Background: Cardiovascular disease (CVD) is the number one cause of death worldwide. The clustering of metabolic risk factors, defined as metabolic syndrome (MetS), and aging are two separate factors that significantly increase the risk of CVD. Both aging and MetS influence CVD by impairing heart tissue (myocardial) function. However, the exact understanding of mechanisms and evolution of these impairments are incomplete in MetS and aging populations. One of the most accessible and utilised techniques for assessing myocardial function is conventional transthoracic echocardiography, and more recently speckle tracking echocardiography (STE); a robust and sensitive modality of echocardiography, capable of assessing myocardial function in both the longitudinal and circumferential axes.

Objectives: For part 1; the aim was to examine the effects of MetS (including clinical and biological factors) and its accumulative metabolic burden on myocardial mechanics using STE. For part 2; the aim was to examine the effects of aging on myocardial mechanics using STE.

Methods: For part-one, participants were recruited from the community as part of a large project in France (RESOLVE project). Ninety-two participants with MetS (28 with type-2 diabetes and 64 without), and 50 matched controls were assessed. All participants were free from CVD. Cross-sectional analyses using conventional echocardiography, tissue Doppler imaging, and STE were conducted on all participants, in addition to clinical and biological assessment. For part-two, 45 healthy male participants aged 19 to 94 years were recruited, and underwent similar cross-sectional echocardiographic, clinical, and biological assessments.

Overview of results and conclusions: Studies 1 and 2 formed part 1, while Study 3 formed part 2. Briefly, novel findings from Study 1 included evidence of impaired longitudinal, but not circumferential, myocardial deformation in MetS participants compared with controls.

Myocardial dysfunction increased with the accumulation of MetS factors, and showed strong associations with inflammation and central obesity. Study 2 found that left ventricular (LV) dyssynchrony; a major risk factor of heart failure, was already elevated in both non-diabetic and diabetic MetS participants, compared with controls. Additionally, STE-derived LV-dyssynchrony was detected in a large proportion of MetS individuals without LV diastolic dysfunction; highlighting the potential strength of LV-dyssynchrony as an early indicator of myocardial dysfunction. Lastly, Study 3 found progressively decreased longitudinal deformation, but increased circumferential deformation and LV-twist with advancing age in healthy males, independent of blood biology. Longitudinal and circumferential dyssynchrony also increased with age, and combined with epicardial adipose tissue, explained approximately one-third of the variance in longitudinal strain and LV-twist. Both populations shared characteristic adaptations of myocardial mechanics, with declining longitudinal function (and by extension; sub-endocardial function), while circumferential function (sub-epicardial function) was preserved (MetS) and even increased (aging). LV-dyssynchrony, inflammation, epicardial fat, and abdominal fat were independent predictors and partially explained the variability of this myocardial dysfunction in the MetS and aging populations. STE may be a more sensitive technique for early detection of subclinical myocardial dysfunction in MetS and aging populations, potentially permitting earlier prevention of CVD. Overall, the thesis provides new knowledge on the myocardial mechanics of both the MetS and aging heart; with a view to better understanding its pathophysiology.

CHAPTER 1

INTRODUCTION AND OVERVIEW

1.1. Cardiovascular risk factors and statistics

Cardiovascular disease (CVD) is the number one cause of death, globally [1]. Recently, it was estimated that 17 million people world-wide die from CVD each year, equating to approximately 30% of all deaths, world-wide [1]. The incidence is expected to continue rising linearly over the next two decades. Risk factors for CVD can be classed as either modifiable or non-modifiable. The most important modifiable risk factors include poor nutritional choices, physical inactivity, tobacco use, and alcohol abuse; while aging and genetic predisposition are among the most important non-modifiable risk factors for CVD [1]. Resulting phenotypes from CVD risk factors typically include type-2 diabetes mellitus, hypertension, and obesity. When these factors combine together, the risk of CVD rises exponentially [2]. The clustering of CVD risk factors has become known as metabolic syndrome (MetS). Although the consensus definition of metabolic syndrome remains controversial [3, 4], the clustering of risk factors is becoming more prominent in the literature. Patients with MetS have a two-fold increase in the risk of CVD [2], while being aged over 65 years nearly quadruples the risk of CVD [5]. The importance of early detection of CVD in MetS and aging populations is thus critical, since detection may lead to more effective attenuation of disease progression. An understanding of the heart's regional function, or the myocardial mechanics is of primary importance when assessing risk and pathophysiology of CVD.

1.2. Overview of myocardial mechanics

1.2.1 Mechanical characteristics of the left ventricular (LV) myocardium

The architectural layout of the heart muscle, known as the myocardium, plays a critical role in the mechanical and electrical function of the ventricles [6]. To best understand the myocardium, it is important to first look at its microstructure. The LV myocardium is made up of individual myofibers, forming multiple myocyte sheet arrangements, separated by cleavage planes [6]. Extensive histological research [6, 7] has led to a description of the myocardium as a trans-mural continuum of two helical fibre organisations; whereby a right-handed helical formation in the inferior aspect of the myocardium (sub-endocardium) gradually changes into a left-handed helical formation in the superior aspect of the myocardium (sub-epicardium) (**Figure 1**).

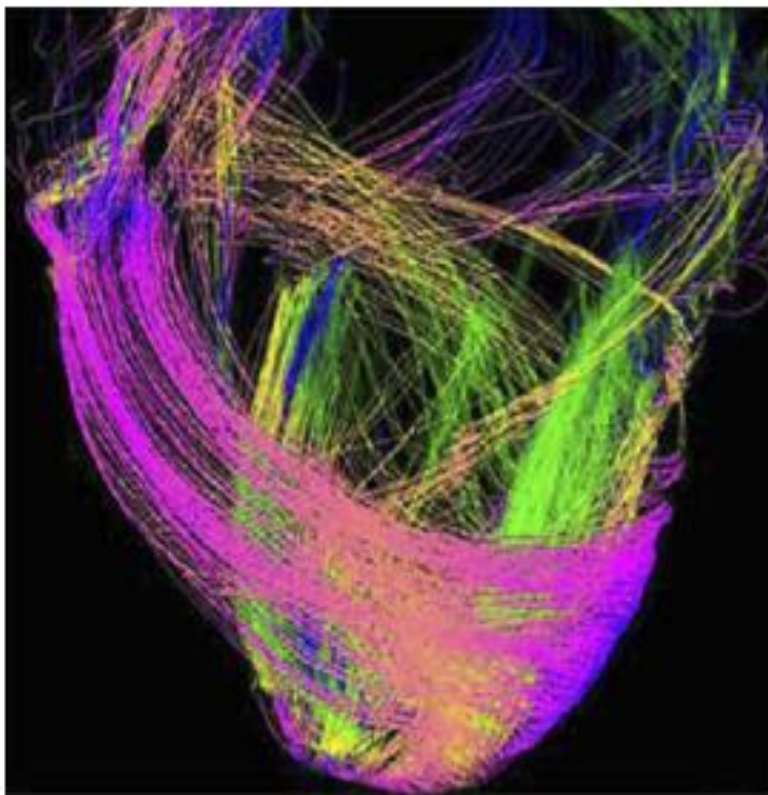


Figure 1. Muscle fibres in the LV through diffusion tensor MRI, which shows two dominant helical fibre orientations [8].

More specifically, these fibres reveal a longitudinal (vertical) orientation in the sub-endocardium, changing to a more circumferential (oblique) orientation in the LV mid-wall to sub-epicardium. This arrangement can increase efficiency of cardiac output, and ventricular filling [9]. Furthermore, electrical activation of the LV myocardium also follows a unique sequential pattern. Conduction typically spreads from the sub-endocardium outwards to the sub-epicardium, as well as from the apex to the base of the LV [6]. Electrical activation/deactivation of myofibers leads to contraction and relaxation, or deformation of myocardial segments [10]. This deformation is typically referred to as strain (the shortening of a myocardial segment relative to its original size), and occurs in three planes (**Figure 2**); circumferential, longitudinal, and radial. During the cardiac cycle, these strains have unique patterns, beginning at systole.

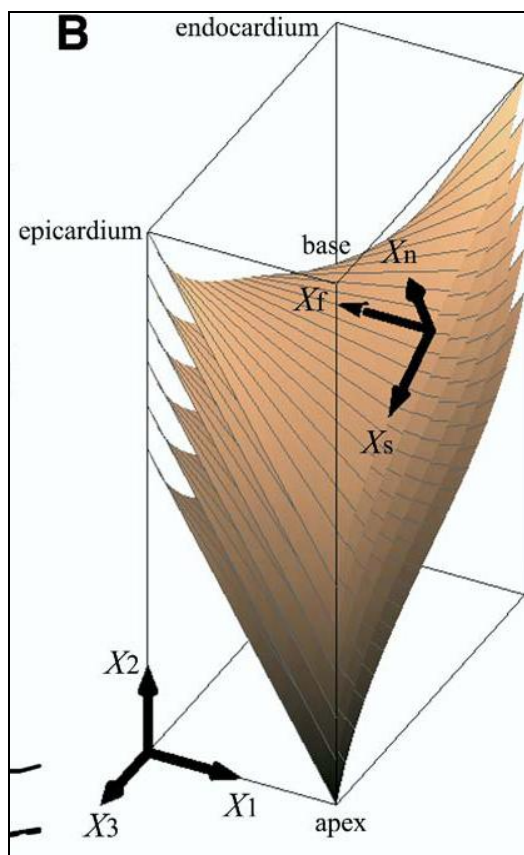


Figure 2. Three perpendicular axes forming global geometry of the LV: Circumferential (X_1), longitudinal (X_2), and radial (X_3) [10].

1.2.2. Myocardial LV Systole

Two distinct phases of systole are evident in the LV. First, isovolumic contraction; during which the LV contracts with no change in volume, and second, ejection; during which blood is propelled from the contracting LV [10]. Throughout these two phases, the LV myocardium undergoes micro-structural deformation. During isovolumic contraction, the sub-endocardial fibres (arranged in the right-handed- longitudinal axis) shorten, while the sub-epicardial fibres (arranged in the left-handed- circumferential axis) are stretched [6]. This prepares an elastic recoil of the myocardium for the subsequent ejection phase. During LV ejection, all fibres contract from base to apex; with shortening of longitudinal and circumferential fibres, and thickening of radial fibres [6]. Since the myocardial fibres are arranged in two helical orientations, as described above, contraction of these fibres causes a twisting motion of the LV around its longitudinal axis [6]. Viewed from the apex, a clockwise rotation of the base, and a counter-clockwise rotation of the apex is evident (**Figure 3**). This increases ejection efficiency from the LV, and prepares for diastolic untwisting [9].

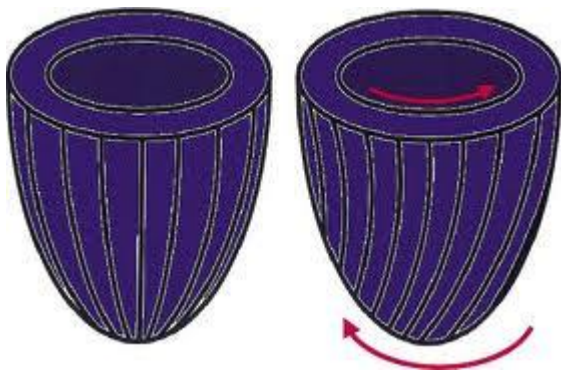


Figure 3. The LV and its torsional myocardial mechanics [11].

1.2.3. Myocardial Diastole

Three distinct phases of diastole are evident in the LV [6]: 1) Isovolumic relaxation, during which the LV relaxes with no change in volume, 2) early-filling, during which blood is suctioned into the LV due to a pressure gradient with the left atrium, and 3) active or late-filling occurs, due to atrial contraction. Myocardial strains are complex during diastole. Briefly, lengthening of the sub-endocardial fibres, with shortening of the sub-epicardial fibres occurs [6]. Longitudinally, this creates a base to apex sequence of relaxation (lengthening), while circumferentially, the relaxation is from apex to base. As the left ventricle untwists in this manner, a rapid decrease in LV pressure occurs, causing a suction of blood into the LV, and thus completing LV filling, and the cardiac cycle [6].

1.2.4. Electromechanical sequencing abnormalities in the myocardium

Myocardial function depends largely on cardiomyocyte contraction and relaxation properties, but also on electromechanical synchronicity of myocardial walls [6]. Non-uniform contraction of the LV chamber is typically referred to as intra-LV myocardial dyssynchrony, and is generating interest as an early sign of myocardial dysfunction, even in populations asymptomatic of CVD [12]. Myocardial dyssynchrony results from conduction delay, typically in the lateral wall of the heart. This causes early activation/contraction of the septum, while the lateral wall is still quiescent. The resulting mechanical abnormality is characterised by inefficient contraction of the ventricle, thus leading to reduced stroke volume and a greater risk of heart failure [12]. Dyssynchronous and inefficient LV function are also attributed to a process known as post-systolic shortening. This is defined as longitudinal myocardial shortening after aortic valve closure (end-systole) [13]. While post-systolic shortening typically occurs within an ischemic heart disease context, it can be present at low magnitudes in healthy individuals [13], and therefore has diagnostic potential. Post-systolic

shortening may be a result of the high energy dependency of myocardial relaxation [14]. A high amount of myocardial energy is required to pump calcium ions against a concentration gradient back into the sarcoplasmic reticulum [14]. This calcium reuptake allows myocyte relaxation, and initiates the diastolic phase of the cardiac cycle. Conversely, myocyte contraction results from a relatively inexpensive energetic process of calcium release [14]. Thus, the balance of contraction and relaxation may be skewed towards prolonged contraction in a pathological state in which myocardial perfusion is altered.

1.2.5. Assessing the myocardium with conventional echocardiography

Characteristics of the heart during cardiac cycles can be effectively assessed with a non-invasive and cost-effective imaging technique known as transthoracic echocardiography. Echocardiography is the projection of ultrasound waves into the thorax, which generates moving and still pictures of the heart. It permits assessment of heart chambers, blood dynamics (global function), and more recently, myocardial mechanics (regional function). This technique is thus highly valuable in risk-assessment and diagnosis of CVD. Recently, the discussion of early risk detection has shifted from symptomatic stages of heart failure, such as adverse remodelling of the heart (measured with grey-scale M-mode imaging) and global hemodynamic impairments (measured with pulsed Doppler), to focus more on regional mechanistic impairments [15]. Tissue Doppler imaging (TDI) and colour-TDI were introduced as a means of describing myocardial tissue movement velocities and deformation at the mitral and tricuspid annulus levels of the LV and right ventricle (RV), respectively [16, 17]. TDI enables measurement of systolic (S_m), early-diastolic (E_m), and late-diastolic (A_m) myocardial velocities, while colour-TDI permits assessment of strain and both systolic and diastolic strain-rate (the rate of segment shortening relative to its original size). The time duration between electrical depolarisation (start of the electrocardiograph QRS complex) and

the peak tissue velocity is also measurable with TDI. Comparing this duration amongst the different mitral-annulus sites permits the detection of intra-LV dyssynchrony [18].

TDI-derived information about the myocardium is limited by two factors. First, assessment of myocardial tissue can only be done in the longitudinal axis, and is restricted to mainly basal segments [19]. As such, it is insonation angle-dependent. Second, TDI is further limited by the “tethering effect”; an erroneous measurement of the passive movement of scar tissue adjacent to vital myocardial tissue [19]. More sensitive angle-independent analyses of myocardial function were therefore necessary.

1.2.6. Speckle tracking-imaging echocardiography

Insonation angle-independent assessment of LV myocardial mechanics became more accessible with the introduction of speckle tracking imaging echocardiography (STE) in 2004 [20]. STE computes the movement of “speckles” (natural acoustic markers in ultrasound images) on two-dimensional grey scale images. Since the speckles are arranged equally throughout the images, and move together with the tissue throughout the frames, they can be identified and tracked throughout a cardiac cycle [20]. The spatial change of the speckles represents tissue movement relative to its original size and shape. This thus permits the assessment of peak myocardial tissue deformation (or strain) and its rate of deformation (strain rate or SR), in the longitudinal, circumferential, and radial axes (**Figure 4**).

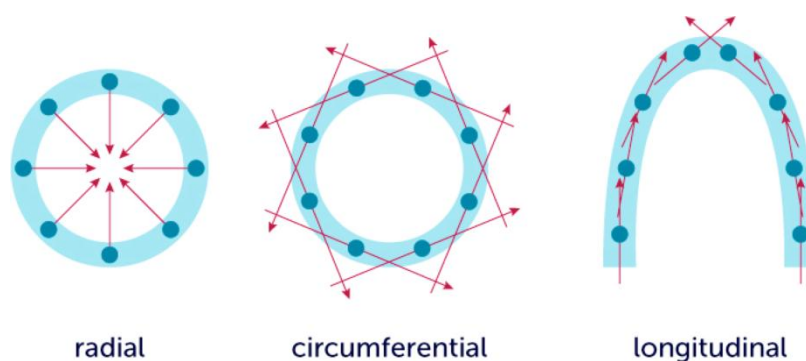


Figure 4. Three planes of myocardial deformation measurement (<http://123sonography.com/node/855>).

In addition to quantifying myocardial strain and SR, STE also permits assessment of the circumferential rotation and twist of the LV as discussed previously [21]. In the parasternal short axis, rotation is calculated as the average angular displacement of the LV segments relative to the centre of a best-fit circle drawn through the same segments [21].

Additionally, the duration between the start of the QRS complex and the peak strain, SR, or twist is also measurable with STE, thus enabling further characterisation of LV myocardial mechanics. Measuring time to peak strain in individual segments, and calculating the difference between maximum and minimum time to peak amongst all the segments allows the evaluation of intra-LV STE dyssynchrony (see chapter 2 for further details) [22].

The validity of STE in describing LV myocardial strain, SR, rotations, twist and related time to peak data has been verified extensively in the literature. STE has shown excellent agreement with gold standard heart-imaging techniques such as Sonomicrometry [23, 24] and tagged Magnetic-resonance imaging [21]. However, STE is not without limitations. Measurement of strain, SR, and twist is influenced by loading parameters within the human heart [25-27]. Preload (end volumetric pressure that stretches the LV just before contraction) and afterload (the stress developed in the myocardial wall after systole) may influence contractile responses of the myocardium, and should be considered in all STE analyses.

Nonetheless, STE has been used comprehensively in populations with cardiomyopathies, enabling better characterisation of myocardial mechanics in these patients [28, 29]. The use of STE has also become popular in the early risk detection of *subclinical* myocardial dysfunction in populations with risk-factors for CVD but who are not diagnosed with CVD, , such as individuals with advanced age or metabolic disorders [30, 31]. Early identification of myocardial dysfunction in these populations may lead to more timely and effective intervention. Since the literature is new in this area, a number of research gaps exist, forming the rationale behind this thesis. The following review of literature therefore aims to explore the research relating to 1) myocardial mechanics in populations with individual and clustered cardiovascular risk factors (i.e. metabolic syndrome) using TDI and STE, and 2) myocardial mechanics in aging populations using TDI and STE.

CHAPTER 2

LITERATURE REVIEW

2.1. PART 1: METABOLIC SYNDROME & MYOCARDIAL FUNCTION

2.1.1. Defining metabolic syndrome

Metabolic syndrome (MetS) is a relatively recent concept, first described in 1998. MetS is a collective noun for the clustering of metabolic disease risk factors, which predispose an individual to the risk of developing type-2 diabetes and CVD, with odds greater than the sum of each of the individual risk factors [32]. The clinical purpose of the MetS definition is therefore to allow for early identification of patients at greatest risk of CVD, beyond the traditional cardiac risk factors. Defining criteria for MetS have evolved over time. **Table 1** summarises the different definitions over time. The first definition was proposed by the World Health Organisation (WHO) in 1998, and defined MetS by the presence of insulin resistance, impaired glucose tolerance or type-2 diabetes, and at least two of the following factors: elevated blood pressure, decreased high density lipoprotein (HDL), increased triglycerides, microalbuminuria, and/or obesity derived from the waist-to-hip ratio or body mass index (BMI) [33]. The European Group for the study of Insulin Resistance (EGIR) updated the definition in 1999; excluding microalbuminuria, and introducing hyperinsulinemia as a required factor [34]. Waist circumference replaced BMI for measuring obesity. In 2001, the National Cholesterol Education Program (NCEP), Adult Treatment Panel III (ATPIII) established a whole new set of criteria for diagnosing MetS; removing insulin resistance as a factor, and including dyslipidemia (high triglycerides, low HDL), elevated blood pressure,

elevated fasting glucose, and elevated waist circumference as the criteria [35]. The MetS definition was again modified in 2004 and 2005, with both the American Heart Association/National Heart, Blood, & Lung Institute (AHA/NHBLI), and the International Diabetes Federation (IDF) publishing updated criteria for better clinical risk assessment [36, 37]. The AHA/NHBLI and IDF definitions included the same criteria as the NCEP: ATP III definition, with the exception of an elevated waist circumference; which became an essential criterion for the diagnosis of MetS. Controversy remained however, regarding the specific cut-off values for measuring elevated waist circumference, with ethnicity-sensitive values required. Finally, in 2009, a joint interim statement from the AHA/NHBLI & IDF convened that there should be no essential component for MetS but rather, all individual components should be considered as important risk factors [3]. **Table 2** presents the cut-off values for each component, from the latest consensus statement defining MetS.

Table 1: Historical overview of the definitions and criteria for MetS [4].

Year	Organisational Body	Inclusion Factors	Inclusion Criteria
1998	World Health Organisation (WHO)	Insulin resistance, abdominal obesity (WHR), ↑Triglycerides, ↑blood pressure, microalbuminuria	Insulin resistance, PLUS any two other factors
1999	European Group for the study of Insulin Resistance (EGIR)	Insulin resistance, abdominal obesity (WC), ↑Triglycerides, ↑blood pressure	Insulin resistance, PLUS any two other factors
2001	National Cholesterol Education Program: Third Adult Treatment Panel (NCEP:ATPIII)	↑Fasting glucose, abdominal obesity (WC), ↑Triglycerides, ↓HDL, ↑blood pressure	Any three of the factors
2004	American Heart Association/National Heart, Blood, & Lung Institute (AHA/NHBLI)	↑Fasting glucose, abdominal obesity (WC), ↑Triglycerides, ↓HDL, ↑blood pressure	Any three of the factors
2005	International Diabetes Federation (IDF)	↑Fasting glucose, population specific abdominal obesity (WC), ↑Triglycerides, ↓HDL, ↑blood pressure	Abdominal obesity (ethnic value) PLUS any two other factors
2009	AHA/NHBLI & IDF joint statement	↑Fasting glucose, abdominal obesity (WC), ↑Triglycerides, ↓HDL, ↑blood pressure	Any three of the factors (ethnic value for WC)

(WC: Waist Circumference, WHR: Waist to Hip Ration, HDL: High density Lipoprotein)

Table 2. Risk threshold (cut-off) values for classification of MetS, according to latest consensus [3].

MetS Factors	Criteria for classification as MetS (most recent)
Fasting glucose	≥100 mg/dL
Triglycerides	≥150 mg/dL
High Density Lipoproteins (HDL)	<40 mg/dL in men & <50 mg/dL in women
Blood pressure	≥130/85 mmHg
Waist circumference	≥94 cm in men & >80 cm in women (Caucasian)

2.1.2. Interactions between metabolic risk-factors and CVD

The understanding of the interrelationship between MetS and CVD remains incomplete. However, recent developments highlight the importance of several metabolic risk factors as the primary causative pathways through which MetS leads to CVD [4]. **Figure 5** presents a flowchart of these interactions. MetS is shown to be at the centre of a complex multi-pathway relationship with genetic, behavioural, and environmental influences, which may ultimately lead to CVD. Amongst these are several key parameters, including abdominal obesity, inflammation and insulin resistance.

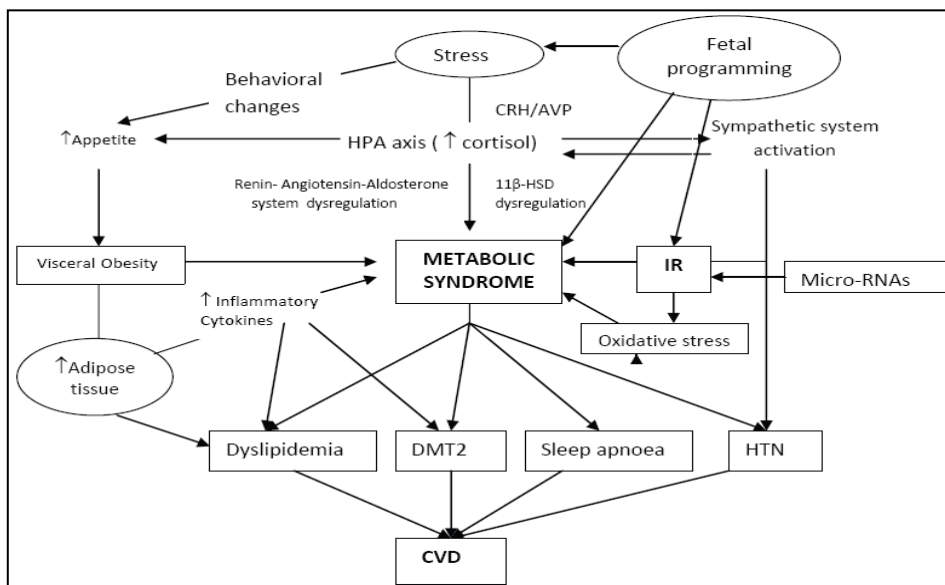


Figure 5. Schematic representation of the pathophysiology of MetS (IR: Insulin Resistance; HTN: Hypertension; HPA axis : Hypothalamic-Pituitary-Adrenal Axis; T2D: Type-2 Diabetes; CRH: Corticotropin Releasing Hormone; AVP: Arginine Vasopressin) [4].

2.1.2.1. Abdominal Obesity

Abdominal obesity is associated with the MetS phenotype, and is a major contributor to CVD. An increase in the size of fat depots may precipitate the excessive release of fatty acids into the circulation [38]. In turn, this promotes CVD through atherogenic dyslipidemia, elevated blood pressure, atherosclerosis, pro-thrombotic state, and a pro-inflammatory state [39]. In addition, increasing blood levels of fatty acids may directly affect cardiac function, through the increase in cardiac adiposity, characterised by myocardial triglyceride infiltration and an accumulation of fat surrounding the heart (epicardial fat) [38]. Epicardial fat is considered a surrogate measure of abdominal obesity [40], and along with myocardial fat, was recently linked to LV dysfunction in moderately obese individuals [41]. It is postulated that cardiac adiposity may have a protective effect on cardiac function, with some adipocytes, such as adiponectin, exerting anti-inflammatory action [42]. However, excessive accumulation of cardiac adiposity, related to abdominal adiposity, may incite a lipotoxic effect on the myocardium, and damage a number of cellular functions, described below.

2.1.2.2. Inflammation and insulin resistance

Significant interest has emerged involving the role of systemic inflammation on CVD [43, 44]. Chronic low-grade inflammation, observed in obese MetS populations, promotes pro-atherogenic profiles, endothelial dysfunction, insulin resistance and ultimately coronary artery disease, type-2 diabetes, or heart failure [4]. Strong links exist among abdominal obesity, cardiac adiposity (e.g. epicardial adipose tissue), and inflammatory adipocytokines [43]. Adipose tissue is considered a highly active organ of the endocrine system, and involved in the production of inflammatory markers, such as tumour necrosis factor-alpha (TNF- α), C-reactive protein (CRP), interleukin 6 (IL6), leptin, and plasminogen activator inhibitor (PAI-1) [44]. Insulin resistance is another important means through which MetS impacts negatively on cardiac function. In prospective studies, insulin resistance was closely associated with CVD risk, and had an independent influence on atherogenesis [45].

Additionally, insulin resistance was shown to have a greater influence than insulin sensitivity on precocious changes in LV morphology in individuals with uncomplicated obesity [46].

2.1.2.3. Possible cellular mechanistic connections

Central obesity, inflammation, and insulin resistance are thus considered key risk-factors of CVD, and are primary characteristics of MetS. While these associations are promising, the underlying mechanistic connection between MetS and CVD remains unclear. The mechanisms through which a pro-inflammatory state associated with obesity and other components of MetS, such as insulin resistance, expedite CVD remain inconclusive. However, as suggested above, increasing evidence supports the role of abdominal adiposity in active endocrinological functions, via dysfunctional adipocytes [47]. Myocardial function may thus be impaired via the known deleterious impact of adipokines myocardial lipid infiltration on 1) macro and micro-circulatory structure and function, leading to reduced coronary myocardial blood flow and sub-endocardial hypo-perfusion, 2) cell apoptosis and tissue fibrosis from lipotoxic activity and inflammation, impairing signal conductance, 3) β -oxidation and glucose utilisation in the myocardium, and 4) calcium-handling within the myocardium, leading to excitation-contraction coupling abnormalities [38, 43, 48].

Traditionally, conventional echocardiography such as ejection fraction (M-mode) and transmitral flow (pulsed Doppler) has been used to describe cardiac dysfunction in pathologies such as MetS. However, these methods lack the sensitivity to detect more subtle changes in contractility and relaxation [49]. The following section therefore aims to explore the specific effects of MetS on myocardial function, using more sensitive [50] echocardiographic techniques (TDI and STE).

2.1.2. Effects of individual and clustered metabolic risk-factors on TDI and STE-derived myocardial mechanics

This section summarises the literature describing the impact of three key individual metabolic risk factor (type-2 diabetes, hypertension, and obesity), and the clustering of these risk factors (metabolic syndrome) on systolic and diastolic myocardial function. The search strategy consisted of the following online databases: PubMed, EMBASE, and Web of Science. Data bases accessed were limited to literature between 1993 and May 2013. This timeframe was chosen to ensure only recent echocardiographic data was utilised. The timeframe also encompassed all literature since the introduction of STE in 2004. The general search strategy used in the review is presented in **Table 3**.

Specific search refinements were made in individual databases, involving MeSH terms in PubMed, and search terms in EMBASE, and Web of Science. Reference lists of included studies were manually searched for additional manuscripts.

Studies involving both male and female humans were selected for inclusion. Adult and adolescent participants were included, but paediatric populations were excluded due to maturational confounders and often complex genetic issues involved in observing metabolic disorders and heart function at this young age [51]. Only studies using 2-dimensional echocardiography were included. Specific studies were sought where investigators had used TDI-derived S_m and E_m , as well as colour-TDI-derived longitudinal strain and SR. This review also focuses on STE-derived longitudinal, radial, and circumferential strain and diastolic and systolic SR. Studies using alternative imaging techniques such as coronary catheterization, intravascular ultrasound, positron emission tomography, computed tomography angiography, magnetic resonance imaging, sonomicrometry, or 3-dimensional echocardiography were excluded. Only studies examining myocardial function at rest were included. Stress-test echocardiograms in MetS populations were not included for analysis. Studies primarily examining the LV were included. Studies investigating the right ventricle or atria, but to the exclusion of the LV were also rejected.

Table 3. Outline of general and specific search terms for part 1.

General Terms	Specific Terms	Limits
<ol style="list-style-type: none"> 1. Metabolic syndrome 2. Type-2 diabetes 3. Hypertension 4. Obesity 5. Myocardial function 6. Tissue Doppler imaging echocardiography 7. Speckle tracking imaging echocardiography 8. Dyssynchrony 9. 1 & 5 10. 2 & 5 11. 3 & 5 12. 4 & 5 13. 1, 5, 6 & 7 14. 2, 5, 6 & 7 15. 3, 5, 6 & 7 16. 4, 5, 6 & 7 17. 1, 2, 3, 4 & 8 	<p>MeSH Major Topic (PubMed) & Search term/SmartText (EMBASE/Web of science)</p> <ul style="list-style-type: none"> • Myocardial dysfunction • Myocardial dyssynchrony • Cardio-metabolic syndrome • Metabolic risk factor • Echocardiography 	<ol style="list-style-type: none"> 1. Humans 2. After 1993 3. Adults and adolescents 4. 2-D echocardiography 5. Left ventricular 6. Tissue doppler imaging 7. Speckle tracking echocardiography

Table 4. Type-2 diabetes and myocardial function.

Authors	Population	Gender	Age (years)	Technique	Myocardial systolic function	Myocardial diastolic function
Stefanidis et al (2009)	160 T2D Vs. 110 Ctrl	M & F (53% M)	56 ±13	TDI & Colour-TDI	↓ (S _m , Strain, & SR)	↓ (E _m , SR)
Stahrenberg et al (2010)	513 T2D Vs. 343 Ctrl	M & F (61% M)	66±5	TDI	Unreported	↓ in all T2D (E _m)
Andersson et al (2010)	31 T2D Vs. 31 Ctrl	M & F (52% M)	58±12	TDI & Colour-TDI	↓ (S _m & Strain)	↓ (E _m)
KoSmała et al (2004)	4 groups: 27 with T2D – SH Vs. 36 with DM + SH Vs. 38 with SH – DM Vs. 33 Ctrl	M & F (52% M)	59±11	TDI	All groups ↓ (S _m)	All groups ↓ (E _m)
Fang et al (2003)	93 T2D Vs. 93 Ctrl	M & F (53% M)	58±13	TDI & Colour-TDI	↓ (Strain & SR)	↓ (E _m)
Ng et al (2009)	47 T2D Vs. 53 Ctrl	M only	57±6	STE	↓ L / ↔ C & R (Strain, SR)	↓ L / ↔ C & R (SR)
Ernande et al (2010) & (2011)	114 T2D Vs. 88 Ctrl	M & F (61% M)	52±5	TDI & STE	↓ L & R (Strain)	↓ (E _m)
Soldatos et al (2010)	55 T2D Vs. 66 Ctrl	M & F (71% M)	62±9	TDI	↔ (S _m)	↔ (E _m) BUT increased prevalence of LVDD in T2D
Cosson et al (2007)	48 T2D Vs. 30 Ctrl	M & F (71% M)	50±6	TDI	↔ (S _m)	Unchanged (E _m)
Muranaka et al (2008)	39 T2D Vs. 16 Ctrl	M & F (63% M)	63±10	TDI & Colour-TDI	↔ (S _m) / ↓ SR	↓ (E _m and SR)
Yazici et al (2008)	72 T2D Vs. 50 Ctrl	M & F (50% M)	49±10	TDI	↔ (S _m)	↓ (E _m)
Fang et al (2003)b	41 T2D Vs. 41 Ctrl	M & F (56% M)	59±9	TDI	↓ (S _m)	↓ (E _m)

Boyer et al (2004)	61 T2D	M & F (51% M)	49±10	TDI	Unreported	63% had LVDD
Alpaydin et al (2011)	40 T2D Vs. 40 Ctrl	M & F (50% M)	52±8	TDI & Colour-TDI	↔ (S _m , Strain, & SR)	↓ (E _m)
Vinereanu et al (2003)	35 T2D Vs. 35 Ctrl	M & F (63% M)	57±10	TDI	↓ (S _m)	↓ (E _m)
Loimaala et al (2006)	49 T2D Vs. 15 Ctrl	M only	52±6	TDI	↓ (S _m)	↓ (E _m)
Charvat et al (2010)	82 T2D	M & F (76% M)	62±5	TDI	Unreported	r=0.5 correlation E _m and diabetes duration
Von Bibra (2005)	43 T2D Vs. 33 Ctrl	M & F (74%M)	61±7	TDI	↔ at rest (S _m) / ↓ with stress test	↓ (E _m)
Andersen et al (2003)	32 T2D Vs. 32 Ctrl	M & F (66% M)	53±7	TDI & Colour-TDI	↓ (S _m , SR)	Unreported (Only global LVDD present - 43%)
Seyfeli et al (2008)	57 T2D Vs. 25 Ctrl	M & F (42% M)	49±6	TDI	↔ (S _m)	↓ (E _m)
Nakai et al (2009)	60 T2D Vs. 25 Ctrl	M & F (57% M)	63±12	TDI & STE	↓ (S _m , L, C & R strain)	↓ (E _m)
Baldi et al (2005)	13 T2D Vs. 15 Ctrl	M & F (54% M)	45±6	TDI	↔ (S _m)	↓ (E _m)
Ballo et al (2010)	36 isolated T2D Vs. 70 Ctrl	M & F (55% M)	65±14	TDI	↓ (S _m)	Unreported
Zahiti et al (2013)	150 T2D Vs. 150 Ctrl	M & F (50% M)	51±10	TDI	↓ (S _m)	↓ (E _m)

T2D: Type 2 diabetes mellitus, M: Male, F: Female, TDI: Tissue Doppler imaging, STE: Speckle tracking echocardiography, L: Longitudinal, C: Circumferential, R: Radial, & SR: Strain rate.

2.1.2.1. Type-2 Diabetes and myocardial function

From the literature search, 24 studies were identified as having examined effects of type-2 diabetes (T2D) on myocardial function (**Table 4**). Twenty-three used TDI or colour-TDI [52-74], and two of these also used STE [60, 66]. One used STE alone [75]. The mean age of populations observed ranged from 45 to 66 years, with sample sizes ranging from 13 to 513 participants with T2D. Collectively, 1,898 participants with T2D were studied.

In the longitudinal axis (TDI and STE), two of 24 studies failed to report diastolic myocardial velocities [53, 56]. Of the 22 remaining studies, 18 found impaired early diastolic myocardial velocity [52, 54, 55, 57, 58, 60-67, 69-74], and one found impaired diastolic SR [75]. Two found unchanged diastolic myocardial velocity [59, 68], although both demonstrated impaired global diastolic function from pulsed Doppler. In the circumferential and radial axes (STE), one study found unchanged diastolic SR [75]. Thus, the evidence surrounding diastolic myocardial function impairment in individuals with T2D appeared relatively strong.

The evidence around systolic myocardial function and T2D was not as convincing. Three studies did not report systolic myocardial velocity [57, 58, 69]. In the longitudinal axis (TDI and STE) 11 of 21 remaining studies reported impaired systolic myocardial velocity [53, 54, 56, 61-64, 66, 70, 71, 74], and three observed impaired longitudinal strain and systolic SR [60, 66, 75]. In contrast, eight studies found no change in myocardial systolic velocity between T2D individuals and controls [52, 55, 59, 65, 67, 68, 72, 73]. In the radial axis (STE), two studies found impaired strain [60, 66], while in the circumferential axis (STE), one showed impaired strain [66], but another reported unchanged strain and SR [75].

The degree to which systolic myocardial function is impaired in T2D individuals thus requires further investigation.

Table 5. Hypertension and myocardial function.

Authors	Population	Gender	Age (years)	Technique	Myocardial systolic function	Myocardial diastolic function
Keser et al (2005)	48 HTN Vs. 20 Ctrl	M & F (50% M)	56±7	TDI	↓ (S _m)	↓ (E _m)
Leggio et al (2007)	145 HTN Vs. 69 Ctrl	M & F (54% M)	55±6	TDI	↓ (S _m)	↓ (E _m)
Ballo et al (2010)	84 isolated HTN Vs. 70 Ctrl	M & F (56% M)	67±12	TDI	↓ (S _m)	Unreported
Andersen et al (2005)	70 HTN+T2D Vs. 35 Ctrl	M & F (80% M)	57±7	TDI & Colour TDI	↓ (S _m & SR)	Unreported (60% with LVDD)
Atilgan et al (2010)	57 HTN Vs. 48 Ctrl	M & F (53% M)	49±10	TDI & Colour TDI	↔ (S _m)/ ↓ (Strain & SR)	Unreported
Narayanan et al (2009)	52 HTN Vs. 52 Ctrl	M & F (46% M)	53±12	TDI & STE	↓ (S _m , L velocity)/ ↔ (L, C & R strain)	Unreported (E _m was correlated with S _m)
Vinereanu et al (2011)	208 HTN Vs. 38 Ctrl	M & F (44-65% M)	55±12	TDI	↓ (S _m)	↓ (E _m)
Kim et al (2008)	51 HTN Vs. 21 Ctrl	M & F (55% M)	57±9	TDI & Colour TDI	↓ (S _m & SR)	↓ (E _m & SR)
Galderisi et al (2010)	18 HTN Vs. 19 Ctrl & 22 Athletes	M only	Young	TDI and STE	↓ (S _m & L Strain)/ ↔ (C & R strain, & Torsion)	↓ (E _m)
Bountiukos et al (2006)	269 HTN Vs. 128 Ctrl	M & F (55% M)	57±11	TDI	↓ (S _m)	↓ (E _m)
Pela et al (2001)	54 HTN Vs. 31 Ctrl	M & F (65% M)	50±14	TDI	↓ (S _m)	↓ (E _m)
Sengupta et al (2013)	34 HTN Vs. 25 Ctrl	M & F (71% M)	36±3	TDI & STE	↓ (S _m , L Strain, & C strain)	↓ (E _m septal)
Takeuchi et al (2007)	49 HTN ± LVH	M & F (63% M)	63±11	STE	↔ LV twist	↑LV untwisting with severity of LVH

HTN: Hypertension, LVH: Left ventricular hypertrophy, M: Male, F: Female, TDI: Tissue Doppler imaging, STE: Speckle tracking echocardiography, L:

Longitudinal, C: Circumferential, R: Radial, & SR: Strain rate.

2.1.2.2. Hypertension and myocardial function

From the literature search, 13 studies were identified as exploring the effects of hypertension (HTN) on myocardial function using TDI [56, 76-86], with four observing these effects using STE [79, 83, 85, 87] (**Table 5**). The mean age of populations observed ranged from 36 to 67 years, with sample sizes ranging from 18 to 208 participants with HTN. In total, 1,139 participants with HTN were studied.

In the 13 studies, four did not report diastolic myocardial velocities [56, 76, 77, 87]. However, one of these found 60% of HTN individuals had global diastolic dysfunction [76]. In the longitudinal axis (TDI and STE), the nine remaining studies observed impaired diastolic myocardial velocity [78-86]. One also reported impaired diastolic SR in HTN individuals [81]. Additionally, in the circumferential axis, one found increased untwisting in parallel with worsening symptoms of HTN (i.e. increasing LVH) using STE [87]. No studies examined radial diastolic function with STE. Thus, there was reasonable evidence suggesting that diastolic myocardial function was impaired in individuals with HTN.

All studies reported systolic myocardial function. In the longitudinal axis (TDI and STE), 11 of 13 studies reported impaired systolic myocardial velocities [56, 76, 78-86], but one found preserved systolic myocardial velocity in HTN individuals [77]. However, this study found impaired strain and systolic SR from colour-TDI. Additionally, two studies found impaired systolic SR from colour-TDI [76, 81], and two found impaired longitudinal strain from STE [79, 85], while one found no change in longitudinal strain [83]. In the circumferential axis (STE), one found impaired circumferential strain [85], while two reported no difference in strain between HTN individuals and controls [79, 83]. Two studies found no change in systolic LV twist [79, 87]. Lastly, in the two studies assessing radial function (STE), both found unchanged strain between HTN and control individuals [79, 83]. Thus, although impaired systolic myocardial velocity and unchanged LV twist were strongly supported, evidence of impairments in strain and systolic SR was inconclusive, and may be axis-specific.

Table 6. Obesity and myocardial function.

Authors	Population	BMI	Gender	Age (years)	Technique	Myocardial systolic function	Myocardial diastolic function
Wong et al (2004)	109 OBESE/ OVERWEIGHT Vs. 33 Ctrl	(BMI>35 kg/m ² ; n=46), (BMI: 30-34.9 kg/m ² ; n=37), (BMI: 25-29.9 kg/m ² ; n=26)	M & F (49% M)	43±10	TDI & Colour-TDI	↓ (S _m & Strain)	↓ (E _m)
Deng et al (2010)	30 OBESE Vs. 30 Ctrl	BMI>30 kg/m ²	M & F (53% M)	54±7	STE	↓ (Apical rotation & Twist)	Unreported
Tumuklu et al (2007)	33 OBESE Vs. 34 Ctrl	BMI>30 kg/m ²	M & F (33% M)	41±7	TDI & Colour-TDI	↓ (S _m , Strain, & SR)	Unreported (Only global)
Orhan et al (2010)	29 OBESE Vs. 20 Ctrl	BMI>30 kg/m ²	M & F (10% M)	49±8	TDI & Colour-TDI	↔ (S _m)/ ↓ (L SR)	↓ (E _m)
Ingul et al (2010)	10 OBESE Vs. 10 Ctrl	BMI>30 kg/m ²	M & F (60% M)	14±2	TDI & Colour-TDI	↓ (S _m , Strain, & SR)	↓ (E _m)
Di Bello et al (2006)	48 SEVERELY OBESE Vs. 48 Ctrl	BMI>35 kg/m ²	M & F (23% M)	31±7	TDI & Colour-TDI	↔ (S _m)/ ↓ (Strain & SR)	↓ (E _m & SR)
Kosmala et al (2008)	295 OBESE/ OVERWEIGHT Vs. 98 Ctrl	(BMI>35 kg/m ² ; n=143), (BMI: 30- 34.9 kg/m ² ; n=113), (BMI: 25-29.9 kg/m ² ; n=39)	M & F (26% M)	41±11	TDI & Colour-TDI	↔ (S _m)/ ↓ (Strain & SR)	↓ (E _m)
Barbosa (2013)	50 OBESE Vs. 46 Ctrl	BMI>30 kg/m ²	M & F (Unknown)	Adolescent	TDI, Colour-TDI, & STE	↓ (L Strain)/ Unreported (S _m)	Unreported (Only global)
Wierzbowska et al (2013)	27 SEVERELY OBESE Vs. 27 Ctrl	BMI>35 kg/m ²	M & F (30% M)	37±9	TDI, Colour-TDI, & STE	↓ (S _m , C & R Strain & SR)/ ↔ (L Strain)	↓ (E _m , C & R SR)
Obert et al (2012)	37 OBESE Vs. 24 Ctrl	BMI>30 kg/m ² (BMI-Z score>97 th percentile)	M & F (28% M)	14±2	TDI & STE	↓ (S _m , L Strain & SR,)/ ↔ C strain & SR/ ↑ (Apical rotation & Twist)	↓ (E _m , L SR)/ ↑ (Untwist rate)

Shah et al (2011)	223 OBESE Vs. 232 Ctrl	BMI>30 kg/m ² (BMI-Z score>95 th percentile)	M & F (29% M)	18±3	TDI	Unreported (Only global)	↓ (E _m)
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BMI: Body mass index, M: Male, F: Female, TDI: Tissue Doppler imaging, STE: Speckle tracking echocardiography, L: Longitudinal, C: Circumferential, R:

Radial, & SR: Strain rate.

2.1.2.3. Obesity and myocardial function

From the search strategy, 11 studies examining the effects of obesity on myocardial function were identified [88-98] (**Table 6**). Ten used TDI or colour-TDI [88, 90-98], and three also included STE [88, 93, 97]. One study used STE alone [89]. The mean age of populations observed ranged from 14 to 54 years, with sample sizes varying from 10 to 295 participants with obesity. A total of 891 participants with obesity were studied. The severity of obesity, derived from BMI, ranged from overweight participants (BMI: 25-29.9) [92, 98], to severely/morbidly obese (mean BMI: 46.5) [90]. Only one of the 11 studies reported the duration of obesity [94], which ranged from 4-18 years. Difficulties in reporting the duration of obesity arose from dependence on participant recall and the lack of accuracy of BMI calculation in the past. The absence of duration of obesity was an acknowledged limitation in the remaining 10 studies.

Three of the 11 studies did not report regional diastolic function [88, 89, 96], although two of these reported impaired global diastolic function [88, 96]. In the longitudinal axis (TDI and STE), all eight remaining studies found impaired diastolic myocardial velocity [90-95, 97, 98]. Additionally, three studies reported impaired diastolic SR [90, 93, 97]. In the circumferential axis (STE), one study found impaired SR [97], while another showed prolonged LV untwist [93] in the obese individuals. In the radial axis (STE), one study reported impaired SR in obese individuals compared with controls [97]. Thus, diastolic myocardial function impairment in individuals with obesity was well supported by the literature.

Three of the 11 studies did not report systolic myocardial velocity [88, 89, 95]. In the longitudinal axis (TDI and STE), five of eight remaining studies reported impaired systolic myocardial velocity [91, 93, 96-98], but three observed no change in systolic myocardial velocity [90, 92, 94]. Furthermore, eight studies found impaired longitudinal strain and/or systolic SR in obese individuals compared with controls [88, 90-94, 96, 98]. Only one study found no difference between the groups in longitudinal strain [97]. In the circumferential axis (STE), one study demonstrated impaired strain in obese individuals compared with controls [97], while another reported no difference between groups [93]. Contrasting findings

emerged from evaluation of rotational mechanics; with one study showing increased apical rotation and LV twist [93], while another showed decreased apical rotation and LV twist [89] in obese individuals. The only study assessing radial function found impaired strain in the obese individuals compared with controls [97]. Thus, systolic myocardial function appeared to be impaired in some but not all individuals with obesity. Although several studies showed preserved systolic myocardial velocities, each of these additionally reported impaired strain or systolic SR. It is possible that the detection of systolic dysfunction may be more related to the technique used. Evaluation of rotational LV mechanics in obese individuals warrants further attention.

Tables 7. MetS and myocardial function.

Authors	Population	Gender	Age (years)	Technique	Myocardial systolic function	Myocardial diastolic function	Factor effect
Mahmud et al (2009)	46 MetS Vs. 114 Ctrl	M & F (54%)	47±2	TDI	Unreported	↓ (E _m)	Yes
Kosmala et al (2012)	172 MetS Vs. 61 Ctrl	M & F (81% M)	50±13	TDI & Colour-TDI	↓ (S _m , Strain & SR)	↓ (E _m)	
Ivanovic (2011)	204 MetS Vs. 88 Ctrl	M & F (47% M)	52±8	TDI	↔ (S _m)	↓ (E _m)	
Tadic et al (2012)	204 MetS Vs. 76 Ctrl	M & F (47% M)	53±10	TDI	↔ (S _m)	↓ (E _m)	
Paneni et al (2013)	84 MetS Vs. 120 Ctrl	M & F (68% M)	53±10	TDI	↓ (S _m)	↓ (E _m)	Yes
De las Fuentes et al (2007)	186 MetS Vs. 110 Ctrl	M & F (42% M)	51±11	TDI	↓ (S _m septal only)	↓ (E _m)	
Dinh et al (2011)	97 MetS Vs. 69 Ctrl	M & F (49% M)	65±10	TDI & Colour-TDI	↔ (S _m)	↓ (E _m)	Yes
Wong et al (2005)	173 MetS Vs. 220 Ctrl	M & F (48% M)	53±12	TDI & Colour-TDI	↓ (S _m , Strain & SR)	↓ (E _m)	Yes
Seo et al (2011)	42 MetS Vs. 20 Ctrl	M & F (19% M)	58±7	TDI & Colour-TDI	↓ (S _m , Strain & SR)	↓ (E _m & SR)	
Mizuguchi et al (2008)	70 RISK Vs. 30 Ctrl	M & F (61% M)	57±7	TDI & STE	↓ (S _m , L, R & C strain, L & C SR)/ ↔ Twist	↓ (E _m , L & R SR)	
Karakurt et al (2011)	192 MetS Vs. 20 Ctrl	M & F (23% M)	54±9	TDI	Unreported (S _m)/ ↔ global systolic	↓ (E _m)	
Gong et al (2009)	200 Vs. 197 Ctrl	M & F (44% M)	50±9	TDI & Colour-TDI	↓ (Strain & SR)	↓ (SR)	Yes
Koc et al (2010)	44 MetS+ Vs. 21 Ctrl	M & F (36% M)	46±7	TDI	Unreported	↓ (E _m)	

MetS: Metabolic syndrome, M: Male, F: Female, TDI: Tissue Doppler imaging, STE: Speckle tracking echocardiography, L: Longitudinal, C: Circumferential, R: Radial, & SR: Strain rate.

2.1.2.4. Metabolic Syndrome (MetS) and myocardial function

The literature search identified 13 studies investigating the effects of MetS on myocardial function in a total of 1714 individuals [99-111] (**Table 7**). All 13 studies used TDI, but only one study also included STE to examine these effects [107]. Of these 13 studies, 11 used the National Cholesterol Education Program: Adult Treatment Panel III (NCEP: ATP III) criteria to diagnose MetS [99, 100, 102-106, 108-111] and one used the International Diabetes Federation (IDF) definition [101]. Only one of the 13 studies did not use an established definition of MetS, but rather included a population with multiple risk factors corresponding to those of MetS [107]. The mean age of populations observed ranged from 46 to 65 years, with sample sizes ranging from 42 to 204 participants with MetS.

All 13 reviewed studies reported diastolic myocardial velocities in MetS participants [99-111]. In the longitudinal axis (TDI and STE), all 13 studies reported impaired early diastolic myocardial velocity in MetS individuals compared with controls [99-111]. Additionally, two studies found impaired diastolic SR from colour-TDI in MetS individuals [101, 109], while another study also reported impaired longitudinal SR in the pseudo-MetS group using STE [107]. Five showed progressive deterioration with an accumulating number of factors of MetS [100, 101, 106, 108, 111]. No diastolic parameters were reported in the circumferential or radial axes (STE). Thus, across a robust age-range and in studies with relatively large sample sizes, there is strong agreement on the deleterious effects of MetS on diastolic myocardial function.

Effects of MetS on systolic function however, are more controversial. Of the 13 MetS studies, two did not assess systolic function with TDI [104, 106]. In the longitudinal axis, seven of 11 remaining studies found deteriorating systolic myocardial velocities in MetS individuals compared with controls [99, 101, 105, 107-109, 111]. All colour-TDI studies found decreased longitudinal strain and systolic SR in MetS participants [101, 105, 109, 111], while the sole STE study confirmed this finding in the pseudo-MetS group [107]. In contrast, four of 11 studies found preserved systolic myocardial velocities in MetS individuals [100, 102, 103, 110]. Furthermore, in the circumferential and radial axes (STE), strain and systolic SR were

also impaired in the pseudo-MetS individuals compared with controls [107]. LV torsion and both twist and untwist rates did not differ between the pseudo-MetS and control groups [107].

Discrepancies in the association between MetS and systolic dysfunction may be linked to several factors: First, the populations investigated in the 13 studies may be at various stages of pathology, or by definition, MetS individuals may also have other confounding co-morbidities. One of the six studies reporting impaired systolic myocardial velocity had a large percentage of diabetic participants [111], while another had a large percentage of smokers [108]. Second, the individuals with impaired systolic myocardial velocities may be slightly older than those with preserved function [107, 109]; so the confounding factor becomes age. However, all studies using the more sensitive regional analysis of myocardial function with colour-TDI or STE found impaired systolic function [100, 101, 105, 107, 109, 111], highlighting the potential limitation of TDI-derived myocardial velocities in detecting more subtle systolic myocardial changes. Colour-TDI and STE may be more sensitive than TDI alone in detecting early signs of systolic myocardial dysfunction in individuals with MetS [6, 16, 61, 75, 93]. Preserved, or even increased rotational mechanics have been previously demonstrated in individuals with metabolic pathology [93], highlighting a potential compensatory mechanism at play to maintain LV function despite declining longitudinal deformation.

Table 8. Various metabolic disorders and myocardial dyssynchrony.

Authors	Population	Gender	Age (years)	Technique	Myocardial systolic dyssynchrony	Myocardial diastolic dyssynchrony
Purushottam et al (2011)	74 OBESE Vs. 62 Ctrl	M & F (29% M)	53±13	STE	↑ (L, C & R dyssynchrony)	Unreported
Korosoglou et al (2006)	24 T2D Vs. 15 Ctrl	M & F (50% M)	55±7	TDI	↑ (S _m dyssynchrony)	↔ (E _m dyssynchrony)
Li et al (2009)	135 MetS Vs. 100 Ctrl	M & F (50% M)	Adults	TDI	↑ (S _m dyssynchrony)	↑ (E _m dyssynchrony)
Tan et al (2008)	115 HTN Vs. 30 Ctrl	M & F (50% M)	Adults	TDI	↑ (S _m dyssynchrony)	↑ (E _m dyssynchrony)

T2D: Type-2 diabetes mellitus, HTN: Hypertension, MetS: Metabolic syndrome, M: Male, F: Female, TDI: Tissue Doppler imaging, STE: Speckle tracking echocardiography, L: Longitudinal, C: Circumferential, R: Radial, & SR: Strain rate

2.1.2.5. Various metabolic disorders and myocardial dyssynchrony

Four studies were identified examining the prevalence and extent of LV dyssynchrony in populations with metabolic disorders but without CVD [22, 112-114] (**Table 8**). Three of these used TDI [112-114], while one used STE [22] to assess dyssynchrony. Dyssynchrony was evaluated using the maximum delay (absolute difference between shortest and longest time to peak value amongst all segments/sites observed) and standard deviation (standard deviation of all time to peak values amongst the segments/sites observed) techniques [115]. Observed numbers of participants in studies ranged between 24 and 135 adults, with a total size in the four studies of 348 individuals.

Increased diastolic dyssynchrony in the longitudinal axis (TDI only) was found in individuals with MetS and HTN [113, 114], but was unchanged in T2D individuals [112]. One of the four studies, observing an obese population, did not report diastolic dyssynchrony [22]. Diastolic dyssynchrony was not reported with STE, thus no data were available for the circumferential or radial axes (STE).

In the longitudinal axis (TDI and STE), all studies found increased systolic dyssynchrony in the various populations with metabolic pathologies [22, 112-114]. Additionally, systolic dyssynchrony in the circumferential and radial axes (STE) was also reported in the obese population [22]. While the accumulative effect of MetS on dyssynchrony was not assessed, the independent effect of hypertension was less significant than other risk factors such as blood glucose, and waist-to-hip ratio [113]. The extent and prevalence of both diastolic and systolic dyssynchrony in populations with metabolic risk-factors remain largely unexplored, and warrant further investigations.

2.2. PART 2: AGING & MYOCARDIAL FUNCTION

2.2.1. Defining aging

Aging is described in calendar years, and represents the accumulation of physiological, psychological, and social changes in a person over time [116]. Aging is complex and multi-dimensional, but is generally accepted as a time-dependent decline in intrinsic physiological function, leading to an increase in age-specific mortality rate [117]. The World Health Organisation categorises older age (senility) as greater than 60 years. Global statistics indicate that between the years 2000 and 2050, the percentage of the world's population over 60 years will rise from 11 to 22% [118] (**Figure 6**). Additionally, the number of people aged over 80 years is estimated to quadruple between 2000 and 2050, with a sharper increase in low and middle-income countries. The leading causes of death in older people are still non-communicable diseases such as CVD, cancer, and type-2 diabetes, regardless of socio-economic status. Subsequently, there is a growing need for older-age care related research into primary prevention and management of chronic diseases, incorporating the development of age-sensitive services and standards in our aging population.

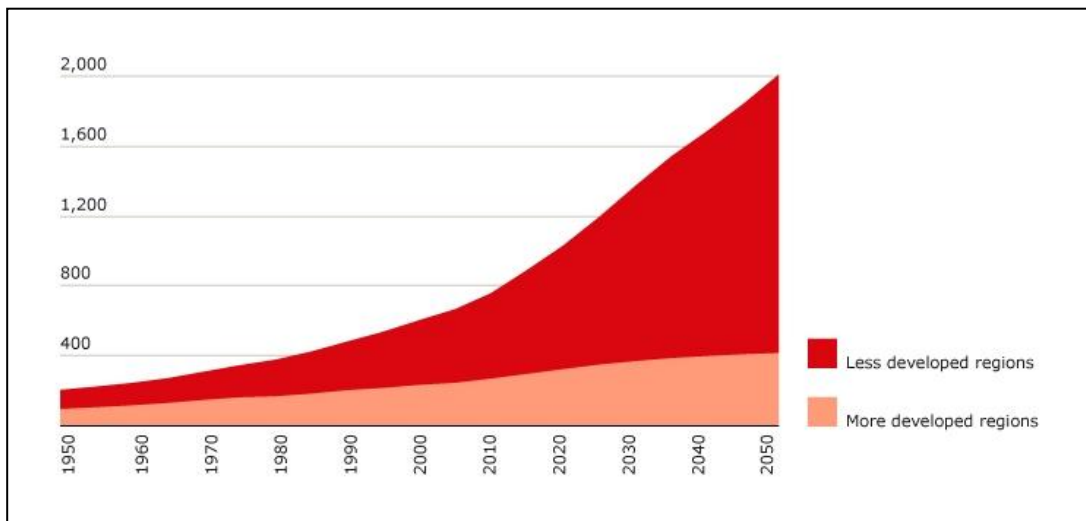


Figure 6. Source: World population prospects, The 2004 Revision Population Database, UN Department of Economic and Social Affairs
 (www.un.org/esa/population/publications/WPP2004/2004Highlights_finalrevised) [5].

2.2.2. Interactions between aging and CVD

The incidence of CVD rises linearly with advancing age [119]. Over 70% of men and women aged over 65 years demonstrate clinical symptoms of CVD, the condition that remains the primary cause of death within this cohort [119]. The influence of aging on heart disease is multifactorial. The process of aging impacts both cardiovascular structure and function via several pathways. These can be summed up with the following: 1) arterial stiffness, 2) concentric hypertrophy, 3) diastolic dysfunction, 4) conduction abnormalities, and 5) valvulopathy. **Figure 7** below shows the interrelationship between these parameters and declining heart function with advancing age.

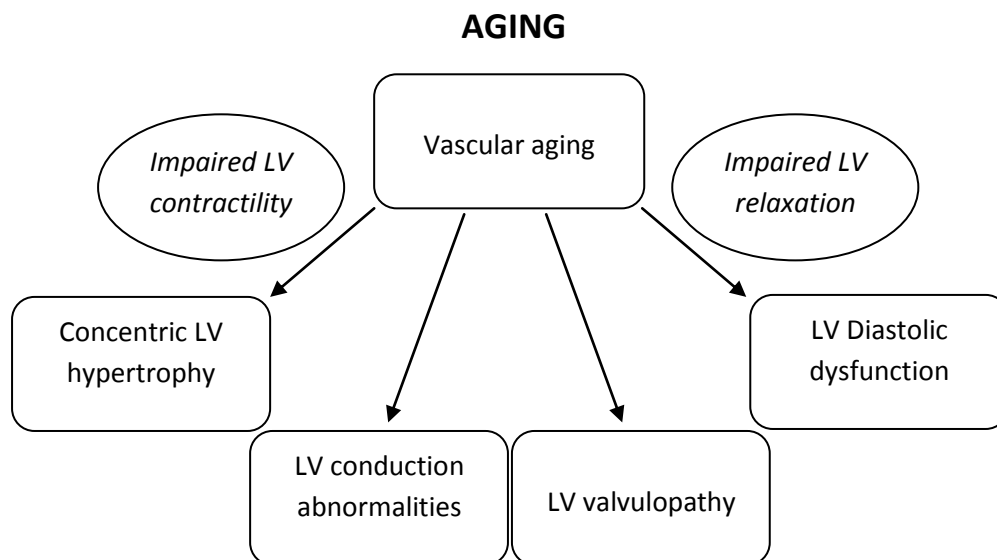


Figure 7. Coupling of deleterious vascular and LV myocardial changes with advancing age.

2.2.2.1. Vascular aging

Advancing age causes microscopic and macroscopic vascular changes, which influence heart function and structure [120]. Recent evidence highlights key associations between age-related vascular stiffening and myocardial stiffening [121]. Microscopically, normal aging is frequently associated with progressive endothelial dysfunction, and is manifested earlier in males than in females, due to the protective effect of estrogen [122]. Endothelium-dependent vascular responses may decline at a rate of 0.21-0.49% per year following the fourth decade [122]. Still on a microvascular level, endothelial dysfunction may underlie the reduction in coronary myocardial perfusion associated with aging [123, 124]. Studies in rats and humans confirm declining coronary hemodynamics with advancing age, particularly in the subendocardial region [125, 126]. Declining coronary hemodynamics may be related to age-related increases in oxidative stress [127]. On a macroscopic level, endothelial dysfunction is also responsible for stiffening and hardening of major arterial vessels, engendering systolic hypertension [128]. An increased pulsed wave velocity is typically seen in older, yet healthy adults [120]. The decrease in elasticity of the major vessels causes a faster return of the reflected pulsed-wave, which in turn places significantly greater afterload stress on the LV myocardium [129]. This abnormality forces the myocardium to adapt by adding new

sarcomeres in parallel formation along the myocardial walls, hence leading to LV hypertrophy.

2.2.2.2. Concentric hypertrophy

Even in the absence of hypertension, older age is classically associated with hypertrophic myocardial remodelling [130]. Several mechanisms may underlie this change. As discussed above, the increased afterload is a main contributor to the concentric remodelling (enlargement of the walls without dilating of the chambers) of the LV. However, cellular changes in the myocardium may also be responsible for this remodelling. In aging individuals, a significant decrease in the number of myocardial cells has been reported [131], presumably due to cell apoptosis (death of the cell). This cell loss is more pronounced in males than in females, likely due to the protective influence of estrogen [131]. Neighbouring myocardial cells thus become hypertrophied to compensate for the loss, but this leads to irregular patterns of calcium handling, and ultimately impacts myocardial contractility and relaxation [132]. Additionally, an age-dependent accumulation of collagen based fibrotic-tissue in the myocardium has also been previously reported in humans [133]. Findings from autopsy studies have revealed that myocardial collagen increases by approximately 50% between the age of 30 and 70 years [134]. Several hypotheses contend that molecular pathways involving pro-inflammatory, pro-fibrotic, and lipotoxic agents may be responsible for this remodelling of the myocardium [135]. Additionally, the issue of increasing myocardial fibrosis may be more a result of reduced collagen degradation capacity in aging hearts, rather than synthesis of new myocardial fibrotic tissue [133]. Furthermore, similar to the discussion in Part 1 of this review of literature, evidence suggests the aging-heart is also associated with increased epicardial fat and myocardial triglyceride content [136, 137], both of which may be implicated in the age-related decline in diastolic function. Substantial evidence therefore confirms a causative link between the age-dependent accumulation of myocardial fibrosis and fat, and progressively increased ventricular stiffness and thickness, as well as impaired diastolic function [138].

2.2.2.3. Diastolic dysfunction

Filling efficiency, or diastolic function, is typically impaired with advancing age [120]. This is characterised by prolonged relaxation and impaired compliance of the LV myocardium following contraction. As mentioned above, this largely stems from increased myocardial stiffness and thickness resulting from complex cellular pro-inflammatory and pro-fibrotic pathways [133]. In addition, the disrupted myocardial metabolism may result in the prolongation of calcium release/reuptake from the sarcoplasmic reticulum (and thus prolonged action potential) [139]. Delayed myocardial relaxation causes a deceleration in early (passive) diastolic filling velocity, due to the reliance of early-diastolic filling on a pressure gradient between the LV and left atrium (and an incomplete relaxation at the time of mitral valve opening). Past studies have observed a 50% decline in early diastolic velocity between the age of 30 and 90 years [120]. Subsequently, atrial contribution with late (active) diastolic filling increases with advancing age. The inverted ratio of early to late diastolic-filling velocity that consequently arises from aging may predispose an individual to diastolic heart failure [120].

2.2.2.4. Conduction abnormalities and valvulopathy

Other mechanisms through which the aging heart becomes impaired are through a progressive decrease in pacemaker cells within the myocardium, as well as deterioration of major valves in the heart [132]. By the age of 70 years, there may be less than 10% of the number of pacemaker cells found in a young adult [140]. The age-related decrease in pacemaker cells affects the responsiveness of heart-rate to imposed stress. For example, in response to exercise, the aging-heart may resemble that of young adult undergoing β -blocker therapy [141], revealing a much lower tolerance to stress. Aging also impacts valvular function, due to an increase in fibrosis and calcification of the fibrous composition of the heart [142]. The aortic and mitral valves are most commonly affected, with some studies showing evidence of aortic valve sclerosis in 80% of people over the age of 60 years, and a

progressive linear increase in mitral valve calcification with age [143]. These valvular changes may predispose an aging individual to heart failure and other CVD events.

2.2.2.5. Aging, blood biology and CVD

While an in-depth discussion of the biological changes occurring with advancing age is outside the scope of this review, a targeted understanding of the interrelationship between biological age-related changes and heart function is warranted. As described in the Review of literature: Part 1 (**Figure 5**); several key blood-markers may have substantial influence on myocardial mechanics. Briefly, inflammation, hyperglycemia, dyslipidemia, and insulin-resistance have well-established pro-atherogenic roles [43, 45].

The aging-heart is similar to the diabetic heart [144]. The resulting macroscopic and microscopic stiffening of the vascular system leads to impairments in myocardial mechanics, via mechanisms discussed above. Findings from various studies report a rising systemic manifestation of inflammation with advancing age [145, 146]. The exact reason for this change remains inconclusive, but is likely related to CVD risk factors from lifestyle and genetic origins [145]. The age-related rise in systemic inflammation is characteristically associated with increasing dyslipidemia, glucose intolerance, and insulin resistance across the lifespan [147-149]. Whether inflammation is the cause or result of an age-related increase in these metabolic risk factors is not entirely known. However, blood glucose levels can be directly linked to age-related pro-inflammatory changes through the formation of advanced glycation end-products, which in turn expedite vascular disease [149]. Thus, an evaluation of the aging-heart may be strengthened through accompanying blood biochemistry analyses.

2.2.2.6. Possible impairments in LV contractility and relaxation with age

Impaired myocardial contraction and relaxation is therefore elementary in the age-related decline in cardiac function. These myocardial mechanics are elemental to the structural and functional changes observed in the aging-heart, and the resultant increased risk of CVD.

Nonetheless, the precise mechanistic understanding of how myocardial contractility and relaxation evolve with the aging-heart is incomplete. Previously discussed developments in the understanding of myocardial mechanics suggest that changes occur in the longitudinal, circumferential, and radial directions, involving myocardial deformational, rotational, and electromechanical characteristics [6]. Therefore, assessing these changes requires more sensitive echocardiographic imaging techniques, such as colour or pulsed-wave TDI, and the more robust STE technology.

2.2.3. Effects of aging on TDI and STE-derived myocardial mechanics

This section summarises the literature describing the impact of natural human aging on myocardial systolic and diastolic function. The search strategy consisted of the following online databases: PubMed, EMBASE, and Web of Science. Data bases accessed were limited to literature between 1993 and August 2013. This timeframe was chosen to ensure only recent echocardiographic data was utilised. The timeframe also encompassed all literature since the introduction of STE in 2004. General search strategies are presented in **Table 9**. Specific search refinements were made in individual databases, involving MeSH terms in PubMed, and search terms/smart text in EMBASE, and Web of Science. Reference lists of included studies were manually searched for additional manuscripts. Studies involving both male and female humans were selected for inclusion. Studies observing participants ranging from adolescent age to elderly age were included, but paediatric populations were once again excluded due to maturational confounders and often complex genetic issues involved in observing heart function at this young age [51]. Only studies using 2-dimensional or 3-dimensional echocardiography were included. Specific studies were sought where investigators had used TDI-derived S_m and E_m , as well as colour-TDI-derived longitudinal strain and SR. The current review also focuses on STE-derived longitudinal, radial, and circumferential strain and diastolic and systolic SR. Additionally, studies using

tagged magnetic resonance imaging were included, due to several landmark studies observing the effects of aging on myocardial function being conducted with this technique. However, studies using alternative imaging techniques such as coronary catheterization, intravascular ultrasound, positron emission tomography, or computed tomography angiography were excluded. Primarily, studies examining the LV were included. Studies investigating the right ventricle or atria alone (to the exclusion of the LV) were excluded.

Table 9. Outline of general and specific search terms for part 2.

General Terms	Specific Terms	Limits
<ol style="list-style-type: none"> 1. Aging 2. Ageing 3. Myocardial function 4. Dyssynchrony 5. Echocardiography 6. 1 & 3 7. 2 & 3 8. 1 & 4 9. 2 & 4 10. 1, 3 & 5 11. 2, 3 & 5 12. 1, 4 & 5/2, 4 & 5 	<p>MeSH Major Topic (PubMed) & Search term/SmartText (EMBASE/Web of science)</p> <ul style="list-style-type: none"> • Myocardial dysfunction • Myocardial dyssynchrony • Aging/Ageing • Tissue doppler imaging • Speckle tracking echocardiography 	<ol style="list-style-type: none"> 1. Humans 2. After 1993 3. Adults and adolescents 4. Echocardiography and tagged MRI 5. Tissue doppler imaging and speckle tracking echocardiography 6. Left ventricular

Table 10. Aging and myocardial dysfunction/dyssynchrony.

Authors	Population	Gender	Age categories (years)	Technique	Myocardial systolic function	Myocardial diastolic function
Cacciapuoti et al (2009)	357	M & F (57% M)	41-62 Vs. 70-84	TDI	↔ (S _m /IVCT)	Unreported (IVRT prolonged with age)
Chahal et al (2010)	453	M & F (47-65% M per group)	35-44 Vs. 45-54 Vs. 55-64 Vs. 65-75	TDI	↓ (S _m lateral only)	↓ (E _m)
Henein et al (2002)	60	M & F (43% M)	23-88	TDI	↔ (S _m)	↓ (E _m +global diastolic parameters)
Yamada et al (1999)	80	M & F (60% M)	24-81	TDI	Unreported	↓ (E _m negative correlation with age)
Innelli et al (2008)	246	M & F (65% M)	7 decade groups (10-19 Vs. 20-29 Vs. 30-39 Vs. 40-49 Vs. 50-59 Vs. 60-69 Vs. >70)	TDI	↓ S _m (lateral only)	↓ E _m (+global diastolic parameters)
Cardim et al (2001)	45	M & F (58% M)	<45 Vs. >45 (2 groups)	TDI	↓ S _m & TTP S _m	↓ E _m & TTP E _m (+global diastolic parameters)
Munagala et al (2003)	1012	M & F (46% M)	45-49 Vs. 50-54 Vs. 55-59 Vs. 60-64 Vs. 60-69 Vs. >70	TDI	Unreported (only global- ↓ with age)	↓ E _m (+global diastolic parameters)
Nikitin et al (2003)	123	M & F (unknown)	22 - 89 (4 age groups)	TDI and Colour TDI	↓ (S _m)	↓ (E _m)
Nikitin et al (2005)	118	M & F (unknown)	<60 Vs. >60 (2 groups)	TDI and Colour TDI	↓ (S _m)	↓ (E _m)
Sun et al (2004)	100	M & F (52% M)	18-76	TDI and Colour TDI	↓ S _m /Less affected strain & systolic SR	↓ E _m /diastolic SR

Lee et al (2008)	109	M & F (45% M)	Mean age quartiles (35±8 Vs. 51±4 Vs. 60±3 Vs. 73±5)	TDI & STE	↓ S _m / ↑ twist & basal rotation	Unreported (only global- impaired with age)
Gruner-Svealv et al (2006)	82	M & F (50% M)	20-29 Vs. 50-59 Vs. 60-69	AVP movement	↓ (AVP- systolic velocity/amplitude)	↓ (AVP- diastolic velocity/amplitude)
Slotwiner et al (1998)	464	M & F (73% M)	16-41 Vs. 41-54 Vs. 54-88	Myocardial M-mode imaging	↓ mid-wall shortening/Increased ESWS	Unreported
Takeuchi et al (2006)	118	M & F (42-54% M per group)	Young (29±6) Vs. Middle (49±6) Vs. Old (65±4)	STE	↑ twist	↑ untwist time
Zhang et al (2007)	121	M & F (47-60% M per group)	Young (19-45) Vs. Middle (46-64) Vs. Old (>65)	STE	↑ twist	↑ untwist time
Van Dalen et al (2010)	75	M & F (47% M)	16-35 Vs. 36-55 Vs. 56-75	STE	↑ twist	↓ (E _m)/ ↑ untwist time
Kim et al (2007)	56	M & F (71% M)	<31 Vs. 31-40 Vs. 41-50 Vs. 51-60 Vs. >60	STE	↔ twist/↓ basal rotation	Unreported (only global- ↓ with age)
Van dalen et al (2008)	61	M & F (51% M)	16-35 Vs. 36-55 Vs. 56-75	STE	↑ twist + apical rotation/ ↓ basal rotation + rotational delay	Unreported
Sun et al (2012)	228	M & F (48% M)	18-78 (18-39 Vs. 40-59 Vs. >60)	STE	↑ twist, basal & apical rotation/↓ L & C strain	↓ E _m (+global diastolic parameters)
Zghal et al (2011)	90	M & F (30% M)	Mean age groups (83±5 Vs. 34±8)	STE	↓ L strain/ ↔ C strain	Unreported (only global- ↓ with age)
Tavakoli et al (2013)	124	M & F (unknown)	21-82	STE-3D	↑ twist + apical & basal rotation	Unreported

Hollingsworth et al (2012)	49	M & F (51% M)	20-40 Vs. 40-60 Vs. >60	MRI tagging	↑ torsion-shortening ratio	↓ early filling percentage
Lumens et al (2006)	31	M & F (75% M)	19-26 Vs. 60-74 (2 groups)	MRI tagging	↑ torsion-shortening ratio	Unreported
Foll et al (2011)	58	M & F (50% M)	20-40 Vs. 40-60 Vs. >60	MRI tagging	↑ L & R dyssynchrony	↑ L dyssynchrony
Fonseca et al (2003)	31	M & F (75% M)	19-26 Vs. 60-74 (2 groups)	MRI tagging	↓ with age (L & C strain - apex only)	↓ with age (L & C relaxation)
Rosen et al (2009)	1100	M & F (54% M)	45-54 Vs. 55-64 Vs. 65-74 Vs.75-85	MRI tagging	↑ C dyssynchrony (TTP systolic SR)	Unreported
Oxenham et al (2003)	31	M & F (68% M)	19-26 Vs. 60-74 (2 groups)	MRI tagging	↑ torsion-shortening ratio & apical rotation	↑ apical rotation, torsion, L & C strain during relaxation + slower rates of L & C lengthening

M: Male, F: Female, TDI: Tissue Doppler imaging, STE: Speckle tracking echocardiography, MRI: Magnetic resonance imaging, AVP: Atrio-ventricular plane, ESWS: End-systolic wall stress, TTP: Time to peak, L: Longitudinal, C: Circumferential, R: Radial, & SR: Strain rate.

2.2.3.1. Aging and myocardial function/dyssynchrony

The literature search identified 27 studies examining the effects of aging on LV myocardial function and/or dyssynchrony in a total of 5,422 individuals [31, 144, 150-174] (**Table 10**). All studied, populations included both males and females. Eleven of these studies used TDI/colour-TDI [144, 150, 151, 154, 156, 159-161, 165, 168, 174], eight used STE [31, 157, 164, 166, 167, 169, 170, 172], six used tagged MRI [152, 155, 158, 162, 171, 173], and two used other means of assessing myocardial function with echocardiography [153, 163]. Comparisons ranged from correlation analysis of a single population [154, 165, 167, 168], to two-way analyses of seven age-stratified groups [156]. The overall age range observed was from 16 to 89 years.

Nine of the 27 studies did not report diastolic myocardial function [144, 157, 158, 162, 163, 167, 169, 172, 174], although four of these found impaired global diastolic parameters, such as prolonged isovolumic relaxation time and decreased E/A ratio [144, 157, 169, 174].

In the longitudinal axis (TDI/STE/tagged-MRI), the remaining 18 studies observed age-related changes in diastolic myocardial mechanics; including decreasing myocardial velocities [150, 151, 153, 154, 156, 159-161, 164, 165, 168], decreasing diastolic SR [165], impaired early filling percentage [155], increased longitudinal dyssynchrony [152], and delayed diastolic relaxation in the longitudinal axis [171, 173]. In the circumferential axis (STE and tagged-MRI), three studies found delayed diastolic LV untwisting [31, 166, 170], and one reported decreased circumferential strain relaxation [173]. No diastolic parameters were assessed in the radial axis. Thus, there is strong agreement in the literature from heterogeneous samples across the lifespan regarding the deleterious effect of aging on diastolic myocardial function.

Two studies failed to report systolic myocardial function [159, 168], although one of these found increasing global systolic function (LV ejection fraction) with age [159]. In the longitudinal axis, two of the 25 studies found unchanged systolic myocardial velocities [144, 154]. Fourteen of the remaining studies observed an impairment in longitudinal systolic myocardial function with age; including impaired systolic myocardial velocities [150, 151,

156, 160, 161, 165, 174], impaired atrio-ventricular plane myocardial movement [153], impaired longitudinal strain and SR [164, 165, 169, 173], reduced mid-wall shortening [163], and increased longitudinal dyssynchrony [152]. In the circumferential axis, 15 studies reported age-related changes, including; increased twist [31, 164, 166, 167, 170, 172, 174], increased torsion-shortening ratio [155, 158, 171], increased apical rotation [164, 167, 171, 172], and increased circumferential systolic dyssynchrony [152, 162]. Conversely, one study found no change in circumferential strain [169], and another reported no change in LV twist, although it was able to demonstrate an age-related decline in basal rotation [157]. In the radial axis, one study found increased dyssynchrony with advancing age [152]. Therefore, evidence supporting impairment and compensatory changes in systolic function with aging is well established, but no studies have collectively observed the interrelationship between these variables within the same aging population.

CHAPTER 3

EXTENDED METHODOLOGY

For the following extended methodology chapter, part 1 for each subheading relates to the study of MetS and myocardial function (Studies 1 and 2). Part 2 relates to the study of aging and myocardial function (Study3).

3.1. RATIONALE

3.1.1. Part 1: The RESOLVE trial

3.1.1.1. Issues in the literature on myocardial mechanics and MetS

Early CVD risk detection in high-risk populations such as MetS individuals is of significant priority, since it may enable more timely and effective prevention. More robust and sensitive imaging technologies (such as STE) may enable this earlier detection by identifying more subtle sub-clinical changes. However, no studies have assessed myocardial function using STE in a clinically defined MetS population. The influence of MetS and the accumulative impact of its factors on parameters such as longitudinal and circumferential strain, SR, rotation, and twist are currently uncertain. Additionally, no studies have assessed the interrelationships of these myocardial indices, along with longitudinal and/or circumferential myocardial dyssynchrony (an independent predictor of LV dysfunction) using the STE technique in a MetS population asymptomatic of CVD. Lastly, the association between biological components of MetS, such as markers of insulin resistance and inflammation, and the sensitive myocardial dysfunction and dyssynchrony parameters lacks clarity. The understanding of underlying mechanisms linking MetS to CVD remains incomplete.

3.1.1.2. Aims

Therefore, with part 1 of this research, the following aims are presented:

- 1) To characterize LV myocardial longitudinal, circumferential, and twist mechanics in adults with MetS compared with healthy matched controls, and elucidate the effects of multiple risk-factors on myocardial function using STE (Study 1).
- 2) To explore associations between STE-based myocardial function indices and obesity-related insulin resistance and inflammation (Study 1).
- 3) To compare longitudinal and circumferential LV-dyssynchrony in MetS individuals with and without type-2 diabetes, and healthy controls (Study 2).
- 4) To explore associations between other cardiovascular risks factors (such as impaired longitudinal deformation and inflammation) and LV-dyssynchrony (Study 2).

3.1.1.3. Hypotheses for Study 1

Based on reviewed literature, it was expected that MetS patients would experience deteriorating conventional and TDI-derived diastolic function, and that this deterioration would occur in a dose-dependent manner, i.e. worsening with accumulating MetS factors.

Since the present MetS population was free from confounding factors such as CVD and smoking, it was expected that conventional and TDI-derived systolic function would be preserved in MetS patients.

Based on the previous STE study [107], it was also expected that STE-derived longitudinal and circumferential myocardial deformation mechanics would be impaired in MetS individuals. However, hypothesised changes in LV twist and rotational mechanics in MetS individuals were less obvious. Previous findings revealed no change in LV twist in MetS

[107] and HTN individuals [79, 87], while in obese adults and adolescents; one study reported increased apical rotation and LV twist [93], and yet another reported a decrease in apical rotation and LV twist [89]. Since this discrepancy may be due to the duration of obesity; whereby increased twist is a transient phenomenon in young populations that disappears with increased duration of obesity, it was expected that the present MetS population, who have been obese for a moderate duration, would have no changes in LV twist or rotation mechanics.

Indices of myocardial function such as longitudinal and circumferential strain were also hypothesised to be closely associated with markers of glycaemia (fasting blood glucose), insulin-resistance (HOMA-IR), central obesity (waist circumference), and inflammation (e.g. CRP), since the underlying role of these factors are becoming increasingly recognised in the literature [175].

3.1.1.4. Hypotheses for Study 2

Following the critical appraisal of the literature, it was expected that both systolic and diastolic TDI-derived LV dyssynchrony would be increased in all MetS patients, compared with healthy controls. For STE-derived LV dyssynchrony, a confirmation and extension of known TDI findings [113]; with increased longitudinal and circumferential dyssynchrony in MetS patients was expected. It was also hypothesised that more advanced MetS, i.e. MetS in combination with T2D, would exacerbate LV dyssynchrony, since the accumulative effect of MetS factors on myocardial dysfunction is well established [100, 101, 106, 108, 111], and the previous study on T2D and LV-dyssynchrony found increased systolic dyssynchrony in this population [112]. Similar to Study 1, a close association between STE-derived myocardial dyssynchrony and markers of insulin resistance (HOMA-IR), central obesity (waist circumference), and inflammation (e.g. CRP), was predicted [113, 175].

3.1.2. Part 2: The Aging Study

3.1.2.1. Issues in the literature on myocardial mechanics and aging

The effects of aging on global heart function using conventional echocardiography have been extensively investigated in the literature. The consensus from the literature reviewed was that normal aging promotes LV hypertrophy and declining diastolic function, while systolic ejection remains preserved [132, 140, 141]. However, an understanding of age-related changes in regional cardiac function, or myocardial mechanics, remains in its early stages.

Similar to the MetS heart, identification of early subclinical impairments in myocardial mechanics may be important to allow more timely and effective CVD risk prevention. Thus far, no studies have combined multiple indices of TDI and STE-derived systolic and diastolic myocardial function (strain, twist, etc) with LV-dyssynchrony in the circumferential and longitudinal axes, using STE. A comprehensive profile of the aging-heart is hence incomplete.

Furthermore, the degree to which underlying LV-dyssynchrony relates to myocardial dysfunction is unknown, and may be critical in understanding the mechanisms behind LV myocardial dysfunction in the longitudinal axis, circumferential basal and apical axes, and rotational mechanics of the aging-heart. Additionally, no studies have evaluated the aging-heart in a single sex population, despite evidence of gender differences in cardiac aging [131].

Deleterious trends in biological parameters such as inflammation, glycemia, and dyslipidemia across the lifespan are well documented [145], and likened to the diabetic heart [144], but their association with, and role in declining myocardial function in a healthy aging population is inconclusive. Finally, the evolution of epicardial fat across the lifespan in healthy males, and its role in declining myocardial function is so far unexplored, yet remains an important consideration in completing the description of the aging-heart.

3.1.2.2. Aims

Therefore, with part 2 of this research (Study 3), the aims were:

1) To form a comprehensive profile of the aging-heart (including LV systolic and diastolic tissue velocities, deformation, twist, and dyssynchrony) in a single-sex aging-population, using TDI and STE echocardiography.

2) To determine the explanatory role of longitudinal/circumferential LV-dyssynchrony and epicardial adipose tissue, on STE-derived indices of myocardial function (longitudinal and circumferential mechanics) in the aging-heart, independent of the influences from blood biology.

3) To explore associations between blood biological parameters (inflammation, glycemia, and dyslipidemia) and the comprehensive profile of myocardial mechanics in the aging-heart.

3.1.2.3. Hypothesis for Study 3

Evidence of LV hypertrophy and declining global diastolic, but not systolic function was expected in the healthy aging males, confirming the literature. Diastolic and systolic TDI-derived myocardial velocities were expected to be impaired with age. It was also hypothesised that the more sensitive longitudinal STE strain and SR would likely deteriorate with age, but LV circumferential function, such as circumferential strain, SR, rotation, and twist are likely to increase with age, as reported in a number of aging studies [146, 164, 166, 170, 172]. Increasing circumferential function is likely to be a compensatory mechanism aimed at preserving LV systolic function, despite declining longitudinal function. Another hypothesised expectation was the increase in TDI and STE LV dyssynchrony, as well as epicardial adipose tissue with advancing age. Since surrogates of these parameters are

known to increase with age and their deleterious role on myocardial function is well established [136, 152, 162], it was expected that these parameters would have significant explanatory power on indices of myocardial function in the aging-heart.

Lastly, since the aging-heart may be similar to the diabetic-heart [144], healthy aging males would likely have increasing blood-glucose levels and inflammation. These biological parameters were expected to associate significantly, yet independently, with LV dyssynchrony, and other TDI and STE indices of myocardial function (such as longitudinal strain).

3.2. DESIGN AND RECRUITMENT

3.2.1. Part 1: The RESOLVE trial

The same MetS population was observed for part 1 (Studies 1 and 2). These two studies (observing MetS and myocardial function) formed part of a large scale national project in France called the RESOLVE trial (**REversing metabolic SyndrOme by Lifestyle and Various Exercise**). Although this trial was designed as a long-term prospective follow-up, studies 1 and 2 were derived from the baseline analyses, and were therefore cross-sectional in design. Briefly, the purpose and layout of the RESOLVE trial was to conduct a one-year follow-up randomised trial to examine the effects of intensive lifestyle intervention on MetS parameters in obese middle-aged individuals. The follow-up was initiated by a three-week residential (live-in, lifestyle education) program. The design of the RESOLVE study and the integration of Studies 1 and 2 into this layout are outlined in **Figure 8** overleaf.

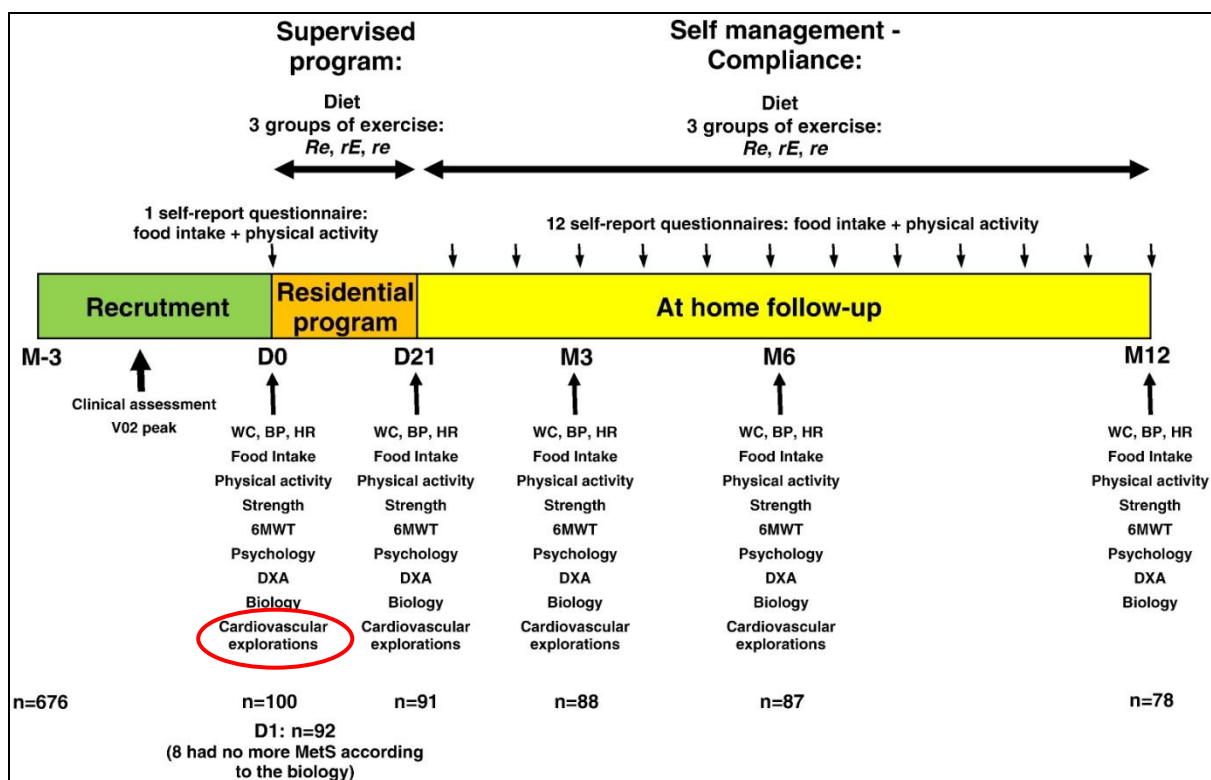


Figure 8. Study design for the RESOLVE clinical trial conducted in Clermont-Ferrand, France between 2010 and 2012 [176]. Red circle represents time-point of cross-sectional studies 1 and 2. WC: Waist circumference, BP: Blood pressure, HR: Heart rate, 6MWT: 6-minute walk, D0: Day 0 (baseline), and DXA: Dual-energy X-ray absorptiometry.

Studies 1 and 2 thus compared various cardiovascular parameters of MetS individuals with those of healthy age and gender-matched controls, at the same point in time. All data collection occurred in Clermont-Ferrand (France) at a common residential facility. The process of recruitment of participants is described in **Figure 9** overleaf. Ethical approval was obtained from the local (French) hospital-based human research ethics committee before any testing commenced, and all participants provided informed consent prior to participation.

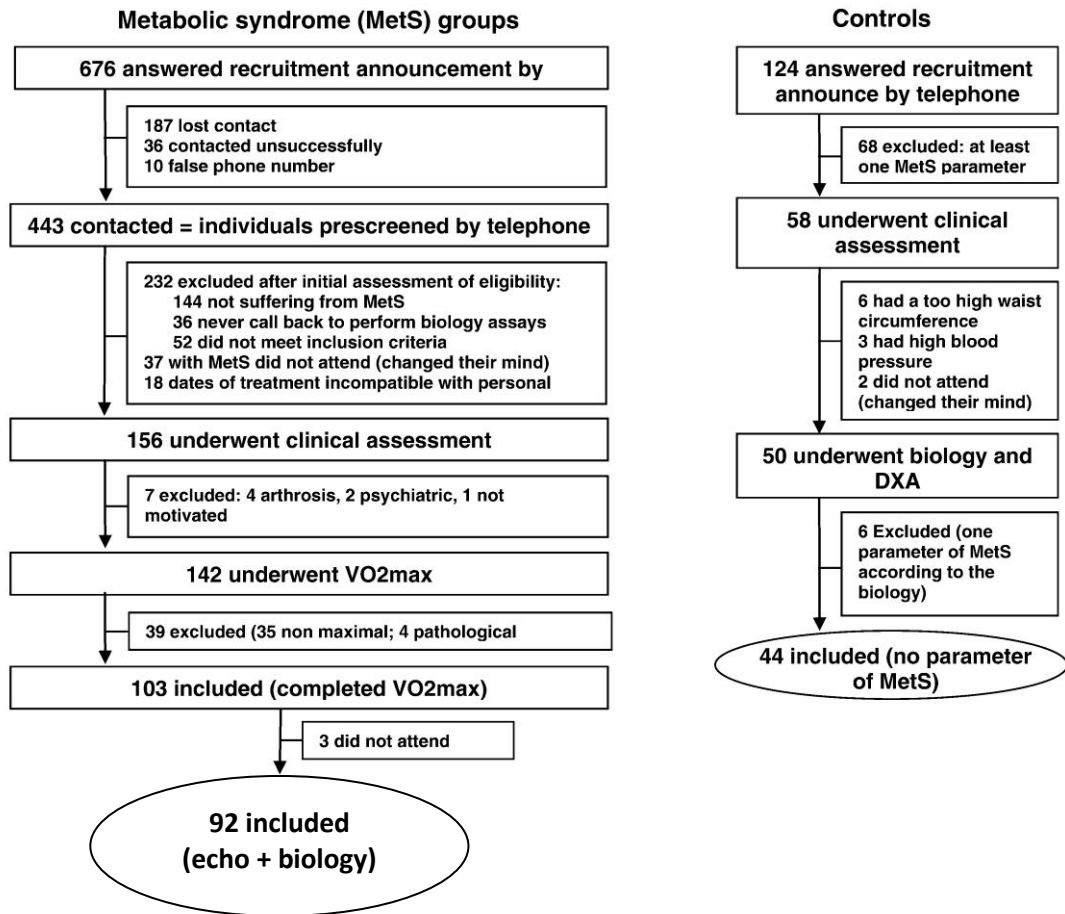


Figure 9. Recruitment flow-chart of MetS and control participants for RESOLVE trial.

3.2.2. Part 2: The Aging Study

A separate population was observed for part 2 (Study 3). This third study was designed as a cross-sectional exploration of cardiac parameters in a healthy aging population. A male-only population was selected to minimise heterogeneity of cardiac changes with aging [131]. The study was designed to maximise multiple-comparisons of cardiac variables amongst three different age categories, as well as trend analyses (correlations) in the one collapsed sample. The sample size required in each age category was determined from power analysis, and is further described in the statistical analyses within this chapter. All participants included were healthy volunteers, recruited from the community. **Figure 10** below represents the recruitment process, while **Figure 11** represents the distribution of the study population.

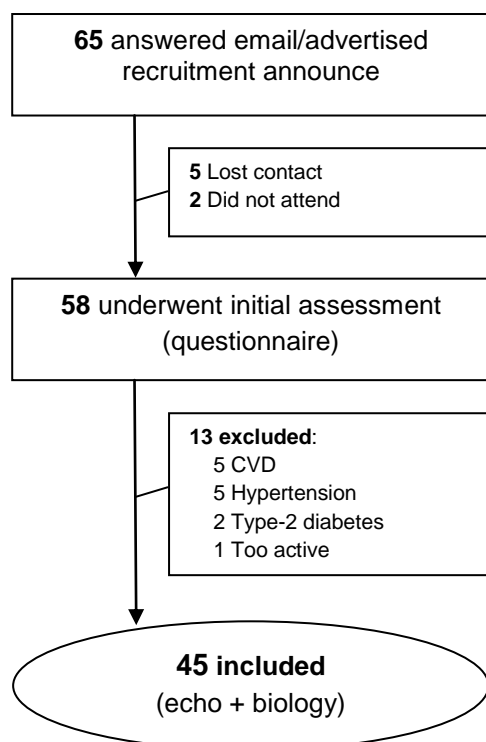


Figure 10. Recruitment flow-chart of aging population for Study 3.

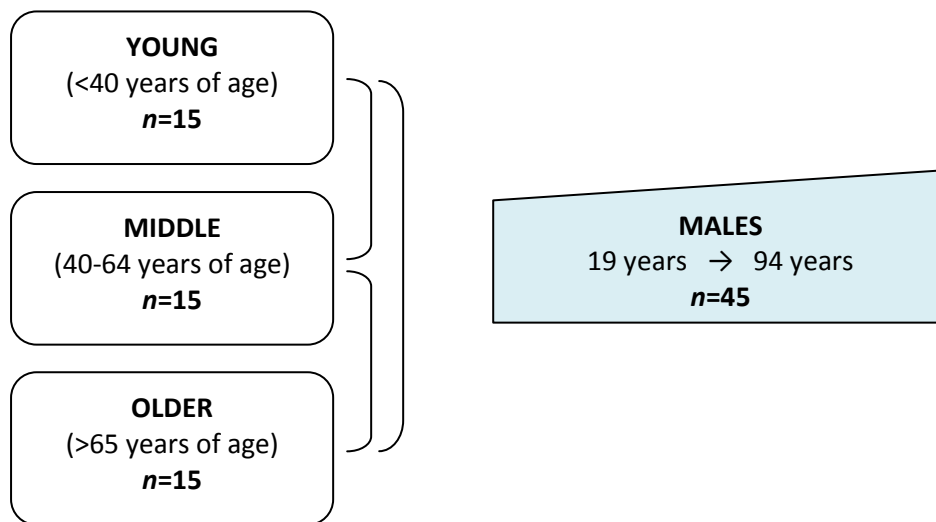


Figure 11. Distribution of the study population into three age categories, and the age-range of the collapsed sample.

Recruitment and testing of older participants was conducted at the *Retired Soldier's League for the Australian and New Zealand Army Corps Village*, Narrabeen, Sydney, Australia, and the Australian Catholic University's Strathfield campus. Recruitment and testing of middle and young participants occurred at the Australian Catholic University's Melbourne campus. Ethical approval was obtained from the local (Australian) University-based human research ethics committee before any testing commenced, and all participants provided informed consent prior to participation.

3.3. CLINICAL AND BIOLOGICAL DATA COLLECTION

3.3.1. Part 1: The RESOLVE trial

Clinical data were obtained from all participants (MetS and controls) for the RESOLVE study (studies 1 and 2). Height in metres was assessed using a standard stadiometer; while body mass in kilograms was assessed using digital weight scales (Tanita®). Weight/height² was subsequently calculated to obtain body mass index (BMI). Waist circumference was measured at the midpoint between sub-costal and supra-iliac landmarks. Blood pressure and heart rate were measured after 15 minutes rest using a digital upper arm sphygmomanometer (SunTech Medical, Model 222). Fat mass, lean body mass, bone mineral content, and central (abdominal) fat were assessed using dual-energy X-ray absorptiometry (DEXA), as previously described [177]. Participants were scanned in light clothing, in the supine position. Central fat was measured by constructing a window of the total abdominal region, which extended from the diaphragm to the superior iliac spine (or between the L2 and L4 lumbar vertebrae). The *in vivo* coefficients of variation (CV) were 4.2, 0.4 and 0.5% for fat, lean and bone masses, respectively. A CV of 1.6% in central fat measures was also reported in 20 participants.

Blood biology was also obtained from all participants (MetS and controls) by an experienced phlebotomist. Fasting blood samples were drawn between 7.00 and 7.30 am, aliquoted and stored at -80°C until further analyses (with the exception of HbA_{1c} which was measured directly after the blood draw). Basic biological assays were performed in the biochemistry laboratory of the University Hospital of Clermont-Ferrand (France) and comprised serum concentrations of triglycerides, high-density lipoprotein (HDL), low-density lipoproteins (LDL), fasting glucose, HbA_{1c}, and high sensitivity C-reactive protein (CRP). Insulin, Tumor necrosis factor α (TNF- α), Interleukin-6 (IL-6), Plasminogen activator inhibitor-1 (PAI-1), and Brain natriuretic peptide (Pro-BNP) were assayed by ELISA with commercial kits (Millipore,

Billerica, MA, USA). Sensitivity, intra- and inter-assay CV were respectively; 3.0 ng/ml, 2.6%, 7.2% for insulin, 0.7 pg/ml, 6.0% and 9.0%, for TNF- α , 1.3 pg/ml, 7.0% and 10.0% for IL-6, 1.3 pg/ml, 6.6% and 10% for PAI-1 active, and 0.16 ng/ml, 6.1% and 7.1% for Pro-BNP. Insulin resistance was estimated by the calculation of the homeostasis model assessment-insulin resistance (HOMA-IR) index (fasting plasma glucose x fasting plasma insulin)/22.5 [178].

3.3.2. Part 2: The Aging Study

Clinical data were obtained from all participants (young, middle, and older) for Study 3. Height in metres was assessed using a standard stadiometer; while body mass in kilograms was assessed using digital weight scales (Tanita®). Weight/height² was subsequently calculated to obtain BMI. Waist circumference was measured at the midpoint between the sub-costal and supra-iliac landmarks. Blood pressure and heart rate were measured following 15 minutes of supine rest using a digital upper arm sphygmomanometer (Carescape-V100, Dinamap, GE technology, USA). Participant categorisation of weekly physical activity was determined using the International Physical Activity Questionnaire modified for the Elderly [179]. All participants (including young and middle) were administered this questionnaire to minimise heterogeneity, and were categorised as participating in low, moderate, or high weekly levels of physical activity [179]. Participants involved in high were excluded, to minimise confounding influences of exercise on cardiac morphology/function. Only one participant (young-aged) was excluded for being involved in high levels of weekly physical activity.

Blood biology was also obtained from all participants (young, middle, and older) by a qualified phlebotomist. Fasting blood samples were drawn between 8.00 and 9.00am, and collected in a serum separator gold-top vacutainer and a sodium fluoride grey-top vacutainer. Samples were then centrifuged, aliquoted in duplicate, and stored at -80°C. Frozen serum samples were later taken to a local pathology laboratory (Melbourne Pathology, Fitzroy, Australia), where basic biological assays were conducted for concentrations of triglycerides, high-density lipoprotein (HDL), low-density lipoproteins (LDL), total cholesterol, fasting glucose, and high sensitivity C-reactive protein (CRP). Sensitivity and CV of biological testing conformed to Australian pathology laboratory standards [180].

3.4. ECHOCARDIOGRAPHIC ANALYSES

3.4.1. Part 1 and 2: All three Studies

Echocardiographic data acquisition for part 1 (Studies 1 and 2) and part 2 (Study 3) were similar, and are therefore described within the same section in this chapter. However, there were slight differences in the STE analyses, which are outlined below. The following section presents details on the procedures used in all echocardiographic testing. A detailed table of abbreviations is available at the start of the current thesis to assist with this section (**Table i**).

3.4.1.1. Image acquisition

All transthoracic echocardiographic analyses for part 1 (Studies 1 and 2) and part 2 (Study 3) were conducted in accordance with American Society of Echocardiography standards [181], and acquired by an experienced operator. Participants were positioned in the left lateral decubitus position in a dark room, and connected to a 3-lead electrocardiogram. For studies 1 and 2, participants were examined using an Esaote ultrasound equipment-pack (MyLab30, Esaote, Firenze, Italy). For study 3, participants were examined using a GE ultrasound equipment pack (Vivid-i, General Electric, Milwaukee, WI, USA). Both packs operated with a 3.5-Mhz sector scanning electronic transducer. Images were acquired in cine-loops triggered to the electrocardiogram-derived QRS complex. A minimum frame-rate of 60 Hz was used when acquiring cine-loops. All data were recorded digitally for subsequent blinded off-line analysis with specific software (MyLab desk 9.0, Esaote, Florence, Italy for studies 1 and 2; EchoPAC PC, Version 5, GE Healthcare for study 3). An average of three cardiac cycles was recorded.

3.4.1.2. Conventional Echocardiography

Motion-mode (M-mode) measurements were obtained in the parasternal long-axis view (**Figure 12**). Left atrial (LA) and LV dimensions were assessed at both end-diastole and end-systole. LV mass was calculated by the Devereux formula [182] and indexed for height (Cornell adjustment). Pulsed-Doppler LV transmitral flow velocity, including early-diastolic (E) and late-diastolic (A) waves, were measured in the apical four-chamber view (**Figure 13.a**). Isovolumic relaxation time (where the LV relaxes with no change in volume) was measured by pulsed Doppler in the apical five-chamber view (**Figure 13. b**), along with aortic ejection velocity. Pulsed Doppler pulmonary venous flow was measured at the base of the LA in the four-chamber view (pulmonary A-wave duration or transit time was measured here). LV ejection fraction was calculated from semi-automatic quantification of LV volumes, as previously described [183].

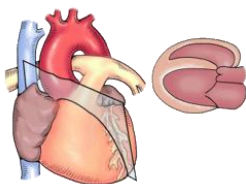
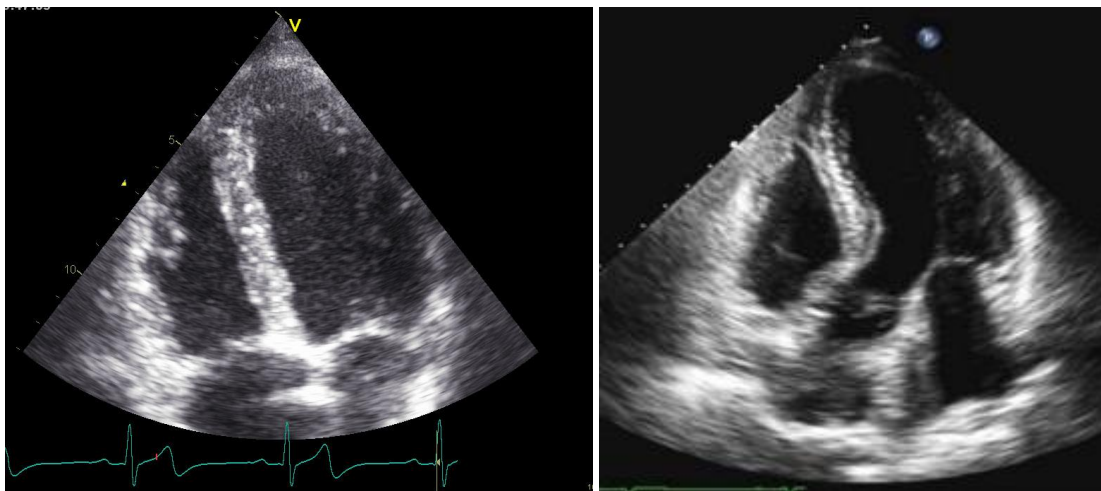


Figure 12. Parasternal long axis view of the heart.



a)

b)

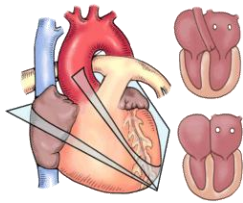


Figure 13(a). Apical 4-chamber view of the heart, and **(b).** Apical 5-chamber view of the heart.

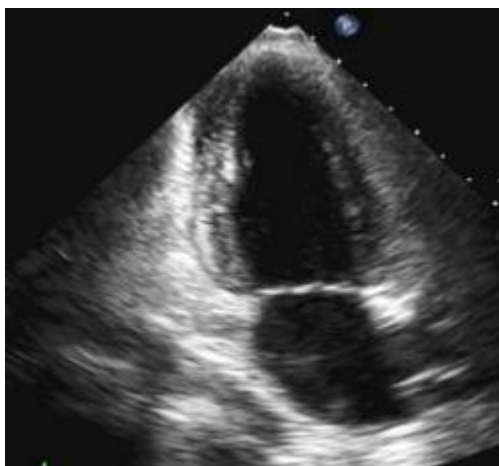


Figure 14. Apical 2-chamber view of the heart (same insonation angle as 4-chamber but rotated 90° about its longitudinal axis).

3.4.1.3. Pulsed tissue Doppler imaging (TDI)

Pulsed-TDI measures of myocardial systolic (S_m), early-diastolic (E_m), and late-diastolic (A_m) tissue velocities were assessed at the mitral annulus of the left ventricle, in the apical 4-chamber view (septal and lateral walls) and apical 2-chamber view (inferior and anterior walls) (Figure 14). Acquisition of pulsed-TDI was conducted in accordance with recommended procedures [184]. Briefly, the sample volume was positioned at the septal, lateral, inferior, and anterior insertion sites of the mitral leaflets and adjusted to cover the longitudinal excursion of the mitral annulus at both systole and diastole.

Using diastolic velocities from transmitral flow and TDI, as well as isovolumic relaxation time and A-wave transit time, several estimates of diastolic function were made. These included the E/A ratio; indicative of LV diastolic filling efficiency, the E/E_m ratio; indicative of LV diastolic filling pressure, E-wave deceleration time (DT); indicative of myocardial relaxation capacity, and mitral A – pulmonary A wave duration; indicative of LV stiffness. LV diastolic-dysfunction grading was determined using these indices and LA volume, according to well-documented criteria [184] (Figure 15).

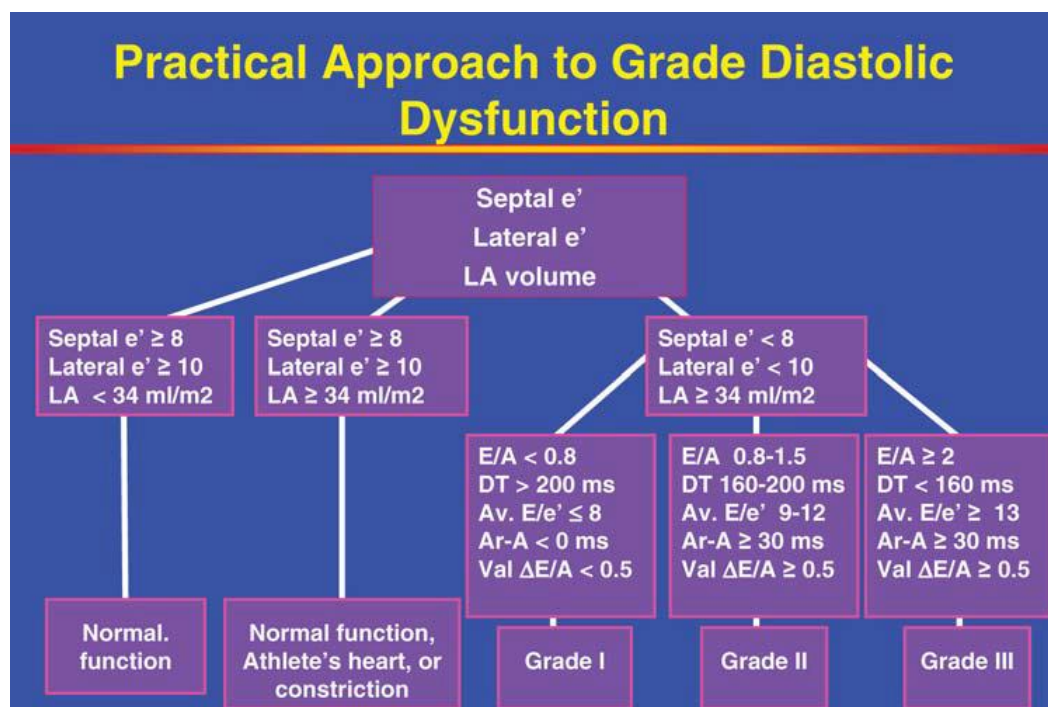


Figure 15. Flow-chart for grading LV diastolic dysfunction [184].

3.4.1.4. Right ventricular echocardiography

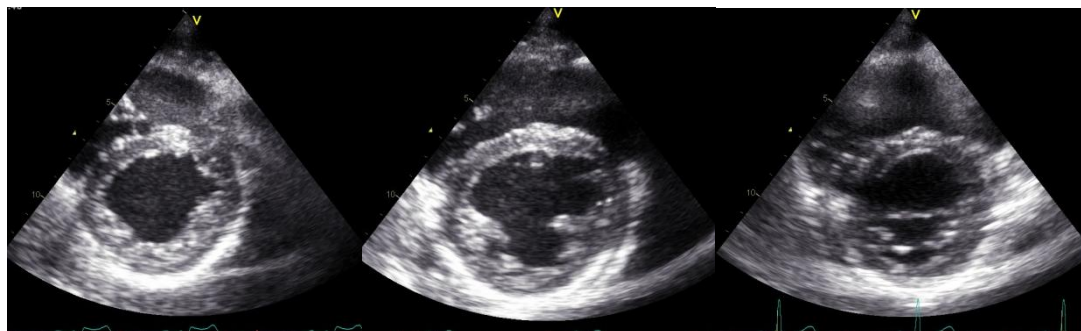
Select parameters of right ventricular (RV) function were assessed from the apical 4-chamber view (**Figure 13.a**). The tricuspid annular plane systolic excursion (TAPSE) was assessed on the lateral (free) wall of the RV. TAPSE represents longitudinal function of the RV in the same way as LV mitral annular TDI velocities. TAPSE was acquired by placing the sample volume through the lateral myocardial wall at the tricuspid annulus, and measuring longitudinal motion of the annulus at peak-systole using M-mode imaging. Peak RV S_m velocity was also assessed at this site, using TDI.

Epicardial adipose tissue (EAT) thickness was observed in Study 3 only, in agreement with recommended procedures [185]. EAT thickness was measured on the RV free wall in the parasternal long-axis view (Figure 12), and was identified as the echo-free or hyper-echoic space just superior to the lateral border of the RV.

3.4.1.5. Speckle tracking echocardiography (STE)

STE was used to quantify myocardial wall motion through a series of manually placed B-mode pixel-tracking markers. These markers analyse myocardial motion through frame-by-frame tracking of natural acoustic “speckles” (reflections, interference, scattering between ultrasound and myocardium) in a region of interest. STE measures Lagrangian strain using different tracking algorithms. The STE modality of vector velocity imaging (VVI) is a more sophisticated approach that involves endocardial border tracking performed with Fourier algorithms [24]. VVI assumes the coherence of the tracked geometry and follows reference points, which guide the detection of adjacent points, using snake contours. This technique was used for Studies 1 and 2. On the other hand, 2-D strain is based on an autocorrelation algorithm of a larger constant acoustic marker, called a “kernel” (20-40 pixels) [186]. EchoPAC automatically defines six anatomic segments of the myocardium, which contain a large number of kernels. An average of all kernels in a defined region of interest is tracked throughout a cardiac cycle, allowing the measurement of sub-endocardial and sub-epicardial

fibre motion. This modality was used for Study 3. Reasonable agreement between the two techniques is established in the literature [186, 187]. The validity and reliability of STE was discussed in Chapter 1. Using both modalities, longitudinal LV characteristics were obtained from 2-D harmonic grey scale images in the apical 4-chamber view, whilst circumferential LV characteristics were obtained from the parasternal short-axis view (at apical, mid, and basal levels of the LV) (**Figure 16**).



a)

b)

c)

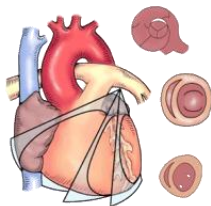


Figure 16. Parasternal short-axis view of the heart at the **a)** apex, **b)** mid, and **c)** base levels of the LV. Note: The animation image (short-axis aortic root) was not used in this research.

Data were recorded and stored for subsequent off-line analyses using specific software (X-Strain software, Esaote, Florence, Italy for studies 1 and 2; and EchoPAC PC, Version 5, GE Healthcare for study 3). In an offline setting, an optimal frame was selected for each cine-loop (e.g. 2-D cardiac-loop of the LV in 4-chamber view), and the endocardial border was manually traced. Using the Esaote software (studies 1 and 2), endocardial tracing was assisted by the Aided-Heart-Segmentation option. Using the GE software (study 3), following endocardial tracing the software automatically tracked myocardial motion. Whenever the

software signalled poor tracking efficiency, the endocardial trace was readjusted by the observer until a better tracking score could be obtained.

LV Lagrangian strain and SR, and their time to peak values, were automatically obtained from the automatic anatomic segments (2-D strain- six myocardial segments) or a six (longitudinal, circumferential-basal, and circumferential-mid-LV) and four (circumferential apical) segment model (VVI). The LV rotations at basal and apical short-axis views were determined as average angular displacement of the six (basal) and four (apical) circumferential myocardial segments (VVI) or the apical and basal automatic anatomic segments (2-D strain- six myocardial segments). Care was taken to ensure that the basal short-axis view contained the mitral valve, and that the apical short-axis view was acquired distally to the papillary muscle. LV twist was calculated as the maximal instantaneous difference between basal and apical rotations. All measurements were averages derived from three consecutive cardiac cycles, and were paired with real-time electrocardiogram. To adjust all STE data for inter-participant differences in heart rate, specific software (Scilab 4.1-developed at Avignon University, Avignon, France) was used to normalise time sequence as a percentage of systolic duration. Only cine-loops with adequate endocardial border definition, adequate frame rate (>60 Hz), and good image tracking were used. Due to the lack of echogenicity in the study populations (MetS and older-aged individuals), clear imaging of the epicardial border was difficult. This precluded accurate estimation of radial deformation. From the 92 MetS and 50 control participants recruited for the RESOLVE trial (Studies 1 and 2); 24 MetS and 10 control participants were excluded due to indistinct endocardial border definition. From the 45 (15 young, 15 middle, and 15 older) participants recruited for Study 3; five were excluded (one young, two middle, and two older) due to indistinct endocardial border definition.

3.4.1.6. Intra-LV dyssynchrony

Intra-LV dyssynchrony was evaluated in the longitudinal axis using TDI, and both the longitudinal and circumferential axes using STE. The following is a step-by-step description of how dyssynchrony was measured.

1) Time taken in milliseconds from the start of the QRS complex to peak TDI-derived S_m velocity (systolic intra-LV dyssynchrony), and peak TDI-derived E_m velocity (diastolic intra-LV dyssynchrony) was measured manually and recorded for septal, lateral, anterior, and inferior annulus sites.

2) Time taken in milliseconds from the start of the QRS complex to peak strain in the six LV segments was measured in both the longitudinal (apical 4-chamber) and circumferential (parasternal short-axis; mid-LV level for Studies 1 and 2, both basal and apical LV levels for study 3) axes.

3) Three separate methods were thus utilised to assess LV-dyssynchrony. These included:

a. Maximum delay – The difference between the shortest time to peak (minimum) and longest time to peak (maximum) duration amongst the four sites (TDI) or six segments (STE- longitudinal and circumferential, respectively) was calculated.

b. Standard deviation (SD) – The standard deviation of the mean of the four sites (TDI) or six segments (STE- longitudinal and circumferential, respectively) was calculated.

c. Opposing wall delay – The difference in time to peak duration between opposing basal walls (TDI; septal vs. lateral, inferior vs. anterior), and opposing segments for longitudinal (STE; apical-septal vs. apical lateral, mid-septal vs. mid-lateral, and basal-septal vs. basal-lateral) and circumferential (STE; anterior vs. inferior, antero-septal vs. infero-lateral, antero-lateral vs. infero-septal) axes was calculated (**Figure 17**)

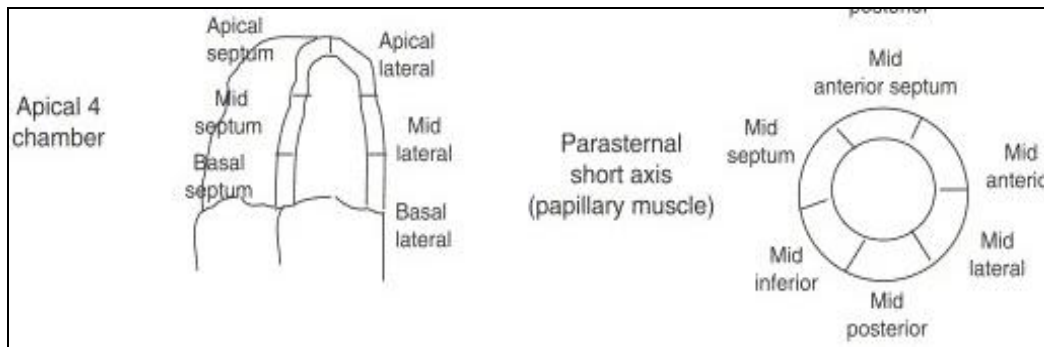


Figure 17. LV segmentation in the apical 4-chamber and short-axis mid-LV views (<http://crashingpatient.com/wp-content/images/part1/rwma.jpg>).

4) Clinical diagnosis of LV-dyssynchrony was made if the maximum delay was greater than 65 ms [18] or 100 ms [22] for TDI and STE respectively, or if TDI SD was greater than 33 ms [18]. No cut-off criteria currently exist for STE longitudinal or circumferential SD. Additionally, dyssynchrony was also diagnosed if the opposing wall delay was greater than 65 ms or 75 ms for TDI and STE, respectively. Due to the higher error involved in measuring opposing wall delay [188], this method was not used for measuring clinical incidence of dyssynchrony in the three studies.

3.5. RELIABILITY ANALYSES

3.5.1. Part 1 and 2: All three Studies

Inter and intra observer reliability evaluation was conducted for both populations, in order to demonstrate the offline analyses were reproducible. Three classic indicators of reliability were used: Coefficient of variation (CV), intra-class correlation coefficient (ICC), and the lower and upper limits of agreement (LOA).

For part 1 (Studies 1 and 2), 20 randomly selected participants were analysed for reliability, whereas for part 2 (Study 3), 10 randomly selected participants were analysed. Major cardiac variables (from conventional, TDI, and STE analyses) were evaluated twice by the same observer (O1 and O2), and once by another experienced observer (O3). Results were tabulated, and the three datasets were compared as follows: O1 vs. O2, and O1 vs. O3. The calculated mean and SD of the two data-points were established. CV was then calculated by SD/mean, and expressed as an absolute percentage. The ICC was calculated using SPSS with a two-way mixed model with 95% confidence interval. Limits of agreement were calculated from the mean difference $\pm 1.96 \times$ the SD of the mean difference, according to medical statistics protocol [189]. These indicators of reliability are presented in **Table 11** and **Table 12** below (for part 1 and 2, respectively).

Briefly, for part 1; the variable with the strongest CV for both intra and inter-observer reliability was time to peak S_m , while the variable with the weakest CV for both intra and inter-observer reliability was LV-twist. For ICC, the strongest variables were longitudinal strain (intra) and E_m (inter), while the weakest variables were time to peak circumferential strain (intra) and LV-twist (inter). The variable with the lowest range of limits of agreement for

both intra and inter-observer reliability was longitudinal strain, while the variable with the highest range of limits of agreement was LVM.

For part 2; the variable with the strongest CV for both intra and inter-observer reliability was S_m , while the variables with the weakest CV were time to peak longitudinal strain (intra), and time to peak circumferential strain (inter). For ICC, the strongest variable for both intra and inter-observer reliability was circumferential strain, while the weakest variable was E_m . Lastly, the variables with the lowest range of limits of agreement for both intra and inter-observer reliability was S_m , while the variable with the highest range of limits of agreement was time to peak longitudinal strain.

Table 11. Reliability analyses for major echocardiographic parameters (Studies 1 and 2).

	INTRA			INTER		
	CV	ICC	LOA	CV	ICC	LOA
CONVENTIONAL/TDI						
LVM	2.900	0.950	-7.446 to 13.665	3.970	0.895	-8.034 to 16.999
Lateral Em	3.100	0.945	-2.379 to 3.244	4.250	0.977	-2.877 to 3.787
Lateral Sm	3.800	0.911	-1.555 to 5.432	3.900	0.931	-1.566 to 4.998
Time to peak Sm	1.990	0.921	-3.544 to 7.324	2.800	0.966	-5.999 to 8.655
STE						
Longitudinal strain	3.250	0.975	-1.172 to 2.880	4.200	0.941	-1.731 to 3.980
Time to peak strain	9.511	0.858	-3.439 to 8.765	9.934	0.921	-3.356 to 7.892
Circumferential strain	2.992	0.971	-1.152 to 3.126	7.870	0.919	-1.178 to 6.979
Time to peak strain	9.124	0.841	-3.711 to 8.242	10.433	0.930	-2.421 to 5.519
LV-twist	14.300	0.884	-1.701 to 3.830	16.940	0.825	-1.931 to 4.958

CV: Coefficient of variation, ICC: Intra-class correlation coefficient, LOA: Limits of agreement, TDI: Tissue Doppler imaging, STE: Speckle tracking echocardiography. Time-to-peak measures were used as indicators of LV-dyssynchrony reliability.

Table 12. Reliability analyses for major echocardiographic parameters (Study 3).

	INTRA			INTER		
	CV	ICC	LOA	CV	ICC	LOA
CONVENTIONAL/TDI						
LVM	2.657	0.968	-6.748 to 15.980	2.72	0.96	-13.21 to 14.140
Septal Em	2.206	0.562	-0.011 to 0.017	4.028	0.562	-0.014 to 0.221
Septal Sm	1.409	0.74	-0.007 to 0.013	2.356	0.74	-0.091 to 0.097
Time to peak Sm	5.43	0.858	-6.353 to 18.765	7.791	0.69	-13.25 to 39.860
STE						
Longitudinal strain	5.89	0.914	-2.389 to 4.675	5.09	0.903	-2.467 to 5.344
Time to peak strain	10.24	0.899	-10.566 to 25.677	9.3	0.849	-18.006 to 39.192
Circumferential strain	4.89	0.989	-1.556 to 2.144	5.5	0.988	-0.556 to 2.016
Time to peak strain	10.23	0.854	-7.865 to 13.533	11.06	0.789	-9.034 to 18.159
LV-twist	7.95	0.985	-1.134 to 2.102	10.04	0.982	-1.241 to 2.726

CV: Coefficient of variation, ICC: Intra-class correlation coefficient, LOA: Limits of agreement, TDI: Tissue Doppler imaging, STE: Speckle tracking echocardiography. Time-to-peak measures were used as indicators of LV-dyssynchrony reliability.

3.6. STATISTICAL ANALYSES

3.6.1. Part 1: The RESOLVE trial

The following section is a description of the statistical tests, including power analyses and normality testing, conducted for Studies 1 and 2. All statistics were assessed using SPSS Version 19.0 for windows (SPSS Inc).

In calculating the required sample size for RESOLVE, the primary judgement criteria selected was the measurement of visceral adipose tissue from DEXA, and based on the work of two selected publications [177, 190]. They found a reduction in abdominal fat mass of 3.6 ± 0.6 kg in females and 3.9 ± 0.6 kg in males to be significant [177], and a reduction in total fat mass of 35.5 ± 9.4 kg to be significant [190]. Thus, to determine the optimal sample size for detecting a minimum significant difference of >600 g (1 SD) between the groups, a bilateral comparison of the two independent means was conducted (**Figure 18**) [176].

$$2n = \left(Z_{\alpha/2} - Z_{1-\beta} \right)^2 \times \frac{4\sigma^2}{\Delta^2}$$

Figure 18. Mathematical formula for determining sample size with SD [189].

A minimum of 30 participants per group (MetS and control) were required to detect a difference between groups of approximately one standard deviation in the major variable (80% power, at an alpha level of 0.05), assuming equal variances within groups (**Table 13**).

Table 13. Estimating sample size from SD in established literature [177, 190].

	Abdominal fat mass (kg) ($\alpha = 0.6$)
If sample size is 30	$\Delta = 0.561$
If sample size is 45	$\Delta = 0.354$
If sample size is 90	$\Delta = 0.251$

Gaussian distribution was verified for all variables using the following criteria [189]:

- 1) Data are continuous, interval, or ratio
- 2) Difference between mean and median is <10%
- 3) Mean is >SD x 2
- 4) Skewness and Kurtosis scores are within ± 1.00
- 5) Kolmogorov-Smirnov test is >0.05 (since total observed population was >100)

Variables which did not satisfy the above Gaussian distribution criteria were log-transformed before being evaluated with parametric statistical analyses. In order to exclude outliers, data-values which were more than two SDs from the population mean were removed.

Differences in descriptive (clinical, biological, and echocardiographic) characteristics of MetS and control participants were assessed using an independent-samples *t*-test, and presented as means \pm SD.

Differences in descriptive characteristics among MetS subcategories (e.g. MetS with accumulating number of risk-factors [study 1], or diabetic-MetS vs. non-diabetic-MetS [study 2]) and controls were determined using one way analysis of variance (ANOVA), with Tukey's and Bonferroni post-hoc tests.

The effect size of differences between MetS and control participants was determined for major variables. Effect size was calculated using the following formula (Cohen's *d*):

$$d = \frac{\bar{x}_1 - \bar{x}_2}{SD_{pooled}}$$

The SD pooled was derived from the following formula, where *n*₁ and *n*₂ represent the sample sizes for the two populations, and SD₁ and SD₂ represent the standard deviation of the mean in the variable compared between each population:

$$SD_{pooled} = \sqrt{\frac{(n_1 - 1)SD_1^2 + (n_2 - 1)SD_2^2}{n_1 + n_2}}$$

Due to uneven group sizes, Hedge's *g* effect size was calculated. Following the calculation of Cohen's *d*, the Hedge's *g* was determined with the following conversion:

$$g = \frac{d}{\sqrt{\frac{n_1 - n_2}{n_1 + n_2 - 2}}}$$

An effect size of >0.2 was considered small, >0.5 moderate, and >0.8 large [191].

Chi-square analysis was used in the comparison of categorical data, and evaluation of incidence of specific parameters. For example, the incidence of clinically-diagnosed LV-dyssynchrony in MetS and control participants was compared using Chi-Square. The incidence and distribution of LV diastolic-dysfunction in MetS and control participants was also assessed using Chi-square.

Pearson's r correlation coefficient analysis was used to measure associations between clinical, biological, and echocardiographic variables in the total population. Based on the correlation analyses; a series of multiple stepwise linear-regression models were explored to assess the strongest independent predictors of a selected dependent variable (e.g. how much of the variability in longitudinal strain is explained by LVM, abdominal obesity, and TNF- α). The regression model was consistently verified for a tolerance level >0.05 to avoid co-linearity, and the standardised residuals were plotted and checked for normal distribution [189].

3.6.1. Part 2: The Aging Study

The following section is a description of the statistical tests, including power analyses and normality testing, conducted for study 3. All statistics were assessed using SPSS Version 19.0 for windows (SPSS Inc).

In calculating the required sample size for the aging research (Study 3), the primary outcome selected was the measurement of longitudinal strain from STE (a major myocardial function parameter). Considerations of sample size were based on the work of two related publications [164, 169]. Previously, a between group difference of $1.3 \pm 2\%$ [169] and $2.1 \pm 3\%$ [164] has shown significance using the alpha value of $p < 0.001$. Thus, to determine the optimal sample size for detecting a minimum significant difference of $>3\%$ (1 SD) between the groups, a bilateral comparison of the two independent means was conducted (see **Figure 18** above). A minimum of 13 participants per group (young, middle and older) was required to detect a difference between groups of approximately one standard deviation in the major variable (80% power, at an alpha level of 0.05), assuming equal variances within groups (**Table 14**).

Table 14. Estimating sample size from SD in established literature [164, 169].

	Longitudinal strain (%) ($\alpha = 3.0$)
If sample size is 13	$\Delta = 2.98$
If sample size is 15	$\Delta = 2.34$
If sample size is 20	$\Delta = 1.89$

Gaussian distribution was verified using the same criteria as described above for part 1. Variables failing to satisfy Gaussian distribution were log-transformed before being

evaluated with parametric statistical analyses. In order to exclude outliers, data-values more than two SDs from the population mean were removed.

Differences in descriptive (clinical, biological, and echocardiographic) characteristics among the Young, Middle, and Older-aged sub-groups were determined using one way analysis of variance (ANOVA), with Tukey's, Bonferroni, and LSD post-hoc tests. Effect sizes were calculated with Cohen's *d* (equal groups) described above. Chi-square analysis was conducted to compare categorical data, and the frequency of specific parameters. For example, the incidence of clinically-diagnosed LV-dyssynchrony and LV diastolic dysfunction grading was compared amongst the young, middle, and older-aged sub-groups. Results from Chi-square testing were not reported in the study as the differences in frequencies failed to reach statistical significance. Pearson's *r* correlation coefficient analysis was used to measure associations between clinical, biological, and echocardiographic variables within the whole population (males aged 19-94 years). Following the correlation analyses, a univariate analysis of covariance (ANCOVA) was conducted to test the strength of the relationship between age and selected cardiac parameters (e.g. STE longitudinal LV dyssynchrony) after controlling for the effects of confounding variables (e.g. total cholesterol, CRP) that were also significantly associated with the selected cardiac parameter.

3.7. STRENGTHS AND WEAKNESSES

3.7.1. Part 1: The RESOLVE trial

The following section is a description of the potential study weaknesses and associated strengths of Studies 1 and 2, when considering the respective designs, populations, and statistical methodologies.

For Study 1, the aim was to examine the effect of MetS, and its accumulative impact, on conventional, TDI, and STE-derived cardiac parameters. The aim of Study 2 was to compare LV-dyssynchrony in MetS individuals with and without type-2 diabetes, and healthy controls (study 2). Several weaknesses and strengths were present and common in both studies since the same population and a similar study-design were used. These included:

- **(Weakness 1)** The cross-sectional design of both studies was a limitation. The design did not permit prospective (longitudinal) assessment of myocardial mechanics in the MetS individuals, and is lower on the scale of evidence hierarchy than a randomised-control trial or systematic review, according to the National Health and Medical Research Council (NHMRC).
- **(Strength 1)** Nonetheless, both studies were still classified in the quality of evidence rating as a level III-2 comparative studies with concurrent controls, and represent a reasonably strong level of evidence; a necessary step towards larger, multi-disciplinary studies
- **(Weakness 2)** A large percentage of the MetS (including diabetic and non-diabetics) individuals were hypertensive (78%). The presence of hypertension may confound

the findings, suggesting that hypertension, not MetS, is responsible for differences found between the populations.

- **(Strength 2)** However, further investigation found no statistical difference in the major cardiac-parameters reported (e.g. longitudinal strain, twist) between hypertensive and non-hypertensive individuals. This confirms that although many MetS individuals were hypertensive, it is more likely that MetS (and/or diabetes), not hypertension, was a stronger contributor to the changes in cardiac function.
- **(Weakness 3)** Due to the higher fat mass of the MetS population and thus reduced echogenicity of the participants, high-quality images were difficult for certain views of the heart. Since STE inherently relies on high-quality images for analysis, this resulted in a number of images (and thus participants- 24/92 MetS and 10/50 control) being excluded from STE analyses. The smaller sample size weakens statistical power, and the discarding of poor-quality images precluded some STE assessments (e.g. radial deformation, 2-chamber view). This also limited the possibility of assessing LV dyssynchrony in more LV segments (e.g. 12 or 18 instead of six), potentially underestimating dyssynchrony.
- **(Strength 3)** Nonetheless, the participant exclusion rate for STE analyses is not dissimilar from other studies [93, 192], and sample size estimates prior to recruitment were upheld despite reduced numbers. Additionally, removal of unclear images strengthens Studies 1 and 2 by ensuring only valid images were analysed. Notwithstanding the use of fewer segments and potentially underestimating dyssynchrony, incidence of dyssynchrony in the study population remained significant.
- **(Weakness 4)** Inter and intra-observer variability was high (approximately 9-16% CV) for some of the more sensitive STE parameters (e.g. LV dyssynchrony, LV twist).

- **(Strength 4)** However, inter and intra-observer variability was comparable to other studies reporting similar variables [93, 192]. Additionally, a minimum of three cycles/images were averaged for all echocardiographic analyses, helping to minimise measurement error.
- **(Weakness 5)** Some of the MetS participants were receiving β -blocker medication (17%). This drug has been previously shown to sometimes mask and alter LV morphology and function, and may be a confounder in the findings [193].
- **(Strength 5)** However, further comparisons of MetS participants receiving or not receiving β -blocker medication revealed no differences in the main cardiac parameters studied (e.g. longitudinal strain, twist, LVM).

3.7.1. Part 2: The Aging Study

The following section is a description of the weaknesses and associated strengths of Study 3, when considering its design, population, and statistical methodology.

The aim of Study 3 was to form a comprehensive profile of the aging-heart in a single-sex aging-population, using TDI and STE echocardiography, as well as explore interrelationships between the clinical, biological, and echocardiographic parameters. Several weaknesses and related strengths were also evident, and included:

- **(Weakness 1)** Similar to Studies 1 and 2, Study 3 was also cross-sectional in its design. Longitudinal outcome measures were not investigated. Additionally, Study 3 was designed without concurrent controls, since it only involved age-stratification of healthy males for comparisons.
- **(Strength 1)** Nonetheless, Study 3 is classified as a Level III-3 comparative study without concurrent controls according to the NHMRC, and maintains a reasonable level of evidence. A longitudinal follow-up of the effects of aging would require a substantial time-frame, and was not feasible in the time restrictions imposed on this thesis.
- **(Weakness 2)** Due to the lack of echogenicity in the middle and older-aged participants, high-quality images were sometimes difficult for certain views of the heart. As mentioned above, since STE depends on good-quality images for analysis, several images (and thus participants- one young, two middle, and two older-aged) were discarded. Again, statistical power was weakened by this, and certain assessments were not possible (e.g. radial deformation, 12 or 18 segment dyssynchrony).

- **(Strength 2)** However, the participant exclusion rate was comparable to other similar studies [169], while the removal of dubious images ensures validity of the STE analyses. Samples size requirements were estimated before recruitment, and took into consideration the possibility of participant exclusion for STE analyses. The ability to detect significant differences between the three age-groups still existed despite the smaller sample size (which was maintained at the minimum of 13 participants per age group). Furthermore, while the sample size may appear small (total $n=45$), included participants met strict inclusion criteria (no CVD, diabetes, hypertension, dyslipidemia or other confounding risk factors) and their classification as 'healthy-aging males' cannot be contested.
- **(Weakness 3)** Inter and intra-observer variability was also moderately high (9-11%) for some of the sensitive STE parameters (e.g. LV dyssynchrony, twist).
- **(Strength 3)** However, this is comparable to other similar studies [164], and a minimum of three cycles/images were averaged for all echocardiographic analyses to reduce error.
- **(Weakness 4)** Due to financial limitations, the biological analyses for Study 3 included only a few blood-markers (basic lipid markers, fasting glucose, and high-sensitivity CRP) and lacked the elaborate variety of markers observed in part 1. Observing insulin, and other inflammatory markers such as TNF- α , and PAI-1 may have strengthened the assessment of age-related changes in biology accompanying myocardial changes.
- **(Strength 4)** Nevertheless, the most clinically important markers were chosen, and thus presented a selective but comprehensive profile of basic blood biology across the lifespan.

CHAPTER 4

ACTUAL AND POTENTIAL PUBLISHED MANUSCRIPTS

4.1. STUDY 1

4.1.1. Preamble

The following is a presentation of the manuscript for Study 1 (from the RESOLVE trial) in the format that was accepted for publication in Obesity Journal (Silver Spring). Briefly, this first study examined the effects of MetS, and its accumulative metabolic burden, on conventional, TDI, and sensitive STE-derived parameters. It also included parallel observations of systemic blood biology.

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MYOCARDIAL DEFORMATION AND TWIST MECHANICS IN ADULTS WITH METABOLIC SYNDROME: IMPACT OF CUMULATIVE METABOLIC BURDEN

Short Title: CRENDAL, WALTHER - MYOCARDIAL MECHANICS IN METABOLIC SYNDROME

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1. a. WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Transthoracic conventional echocardiography is a valid and reliable tool for the detection of subclinical cardiac dysfunction in high risk populations (diabetes, hypertension, and metabolic syndrome).
- Conventional and Tissue Doppler Imaging (TDI) echocardiography typically reveal decreased diastolic function, with preserved systolic function in individuals with metabolic syndrome, but lack sensitivity in detecting more subtle changes.
- Metabolic syndrome is associated with risk factors that predispose individuals to insulin-resistance, diabetes, and a pro-inflammatory state. The role of abdominal obesity in this pathophysiology is fast becoming recognised as critical.

1. b. WHAT THIS STUDY ADDS

- The use of STE extends earlier research detecting myocardial dysfunction in a high risk MetS population, revealing more subtle changes than with conventional and TDI echocardiography alone (systolic impairments *as well* as diastolic).
- This impairment in myocardial function was observed in the longitudinal axis, but not the circumferential axis, or twist/untwist mechanics. We also showed evidence of increasing longitudinal impairment as risk factors accumulated.
- Abdominal obesity, glucose intolerance, inflammation, and systolic intra LV-dyssynchrony explained a large proportion of the variability in longitudinal myocardial dysfunction.

2. ABSTRACT

Objective: We aimed to characterize left ventricular (LV) myocardial mechanics in adults with metabolic syndrome (MetS), and elucidate the effects of multiple risk-factors on myocardial function using speckle tracking echocardiography (STE); a more sensitive method than conventional echocardiography for detecting subclinical myocardial dysfunction.

Design and Methods: Cross-sectional analyses of 92 adults (50-70 years) with MetS, and 50 healthy controls included conventional echocardiography, blood biochemistry, and STE-derived myocardial longitudinal, circumferential, and twist mechanics.

Results: Using conventional measures, MetS participants revealed LV hypertrophy and reduced diastolic function compared with controls, while systolic function was preserved. From STE, MetS participants showed attenuated longitudinal strain (-16.8 ± 2.8 vs. $-20.6 \pm 2.7\%$), and both diastolic (1.1 ± 0.2 vs. 1.4 ± 0.3 s.s⁻¹) and systolic (-1.0 ± 0.1 vs. -1.2 ± 0.2 s.s⁻¹) strain rate (SR). Circumferential strain, SR, and twist mechanics did not differ. Participants with the highest number of MetS factors or diabetes demonstrated the greatest reduction in longitudinal strain and SR. Abdominal obesity, TNF- α , HbA_{1c}, and systolic dyssynchrony explained 48% of impairment in longitudinal strain.

Conclusions: Impaired longitudinal myocardial diastolic and systolic function, but preserved circumferential function and twist mechanics were found in MetS participants, indicative of altered sub-endocardial function. This dysfunction was best predicted by abdominal obesity, inflammation glucose-intolerance, and systolic dyssynchrony.

KEYWORDS: myocardial dysfunction, speckle tracking imaging, metabolic syndrome, abdominal obesity, inflammation.

3. INTRODUCTION

With abdominal obesity reaching epidemic proportions [1], there are increased risks of cardiovascular morbidity and mortality with unfavorable metabolic consequences. Metabolic syndrome (MetS) is a clustering of cardio-metabolic risk factors, including abdominal obesity, insulin resistance, dyslipidemia and hypertension, and is a key phenotype leading to atherogenic and diabetogenic profiles [2, 3].

MetS is consensually associated with left ventricular (LV) hypertrophy and atrial enlargement [4-6]. Previous studies using conventional and pulsed tissue Doppler imaging (TDI) echocardiography have also demonstrated decreased diastolic function in participants with MetS, while systolic function is usually well preserved [5, 7]. However, the lack of sensitivity of these methods in detecting subtle myocardial dysfunction may have led to underestimation of systolic impairments. Additionally, since myocardial functionality results from a complex interplay between deformation (longitudinal and circumferential) and twist/untwist mechanics [8, 9], these studies only partially described the effects of MetS on myocardial function. Speckle tracking echocardiography (STE) may help to overcome these limitations by permitting angle-independent evaluation of deformation in both the longitudinal and circumferential planes, as well as twist mechanics [9]. The STE technique provides more sensitive and comprehensive indices of subclinical myocardial dysfunction in a number of pathologies [10, 11]. Attenuated global longitudinal strain and systolic and diastolic strain rate (SR) in MetS participants have been reported using color-TDI [12, 13]. However, these results suffer from similar limitations as pulsed TDI (e.g. longitudinal angle-dependency and restriction to basal and median segments), and fail to fully explain the myocardial pathophysiology associated with MetS. To our knowledge, no study has thus far examined the impact of MetS on myocardial mechanics using the more sensitive and comprehensive STE indices. The link between myocardial pathophysiology and abdominal obesity-related insulin-resistance and inflammation is an emerging interest [14]. Insulin-resistance and inflammation are known pathways leading to the development of MetS [15], but

whether they are associated with myocardial dysfunction remains uncertain, especially using newer STE indices.

Therefore, the aims were 1) to comprehensively investigate the impact of MetS on myocardial longitudinal, circumferential, and twist mechanics using STE, in middle-aged adults, and 2) explore associations between STE-based myocardial function indices and obesity-related insulin resistance and inflammation.

4. METHODS AND PROCEDURES

Participants and research design

This study formed part of the RESOLVE trial [16]. Following clinical pre-screening of 156 potential participants on a consecutive basis, 92 participants with clinically diagnosed MetS (56% males) were included. Participants with incompatible diseases such as cardiopathy and arteriopathy were excluded. Fifty aged and gender-matched controls were also tested. Within the MetS population, we identified 28 participants with type-2 diabetes (fasting glucose levels $>7\text{mmol.L}^{-1}$, or medicated for type-2 diabetes), and 72 participants with hypertension (resting blood pressure $>140/90$ mmHg, or medicated for hypertension).

Cross-sectional echocardiographic analyses were conducted using conventional, pulsed TDI and STE (vector velocity imaging) methods. Participants provided informed consent prior to commencement of testing, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by our local Hospital ethics committee.

Biochemical, clinical and Anthropometric assessment

Blood biology and anthropometric data were used to classify the MetS status of participants [17]. Serum concentrations of triglycerides, high-density lipoprotein (HDL), low-density lipoproteins (LDL) and fasting glucose were measured, as well as insulin, homeostatic-model of assessment-

insulin-resistance (HOMA-IR), and the following inflammatory markers: Interleukin-6 (IL-6), high sensitivity C-reactive protein (CRP), pro-brain natriuretic peptide (BNP), and tumour-necrosis factor- α (TNF- α).

Stature and body mass were assessed, and body mass index was subsequently calculated. Central fat was measured using dual-energy x-ray absorptiometry (DEXA), and waist circumference was measured at the level of the umbilicus. Blood pressure was obtained with a digital sphygmomanometer (Carescape-V100, Dinamap, GE technology, USA). Diagnosis of MetS was made according to the international diabetes federation definition [18], using waist circumference, HDL cholesterol, triglycerides, blood pressure, and fasting blood glucose as diagnostic variables.

Echocardiographic image acquisition

Images were acquired by the same experienced operator (GW) according to standard procedures recommended by the American Society of Echocardiography, and based on the average of 3 cardiac-cycles. The participant was positioned in the left lateral decubitus position, and examined using an Esaote ultrasound equipment-pack (MyLab30, Esaote, Firenze, Italy), with a 3.5-Mhz sector scanning electronic transducer. Images were acquired in cine loops triggered to the QRS complex. All data were recorded digitally for subsequent blinded off-line analysis with specific software (MyLab desk 9.0, Esaote, Florence, Italy).

Conventional, pulsed Doppler, and Tissue Doppler imaging echocardiography

M-Mode measurements were obtained in the parasternal long-axis view, and left atrial (LA) and LV dimensions were measured at both end-diastole and end-systole. LV mass was calculated by the Devereux formula and indexed for height (Cornell adjustment). LV ejection fraction was calculated from semiautomatic quantification of LV volumes, using vector velocity imaging.

Pulsed-Doppler LV trans-mitral velocity, including early (*E*) and atrial (*A*) waves, were measured in the apical four-chamber view. Isovolumic relaxation time was measured by pulsed Doppler in the apical five-chamber view, along with aortic ejection velocity. Pulsed Doppler pulmonary venous flow was measured at the base of the LA in the four-chamber view. Pulsed-TDI measures of myocardial

systolic (S_m), early diastolic (E_m), and Atrial (A_m) velocities were assessed at the mitral annulus of the left ventricle, in apical four (septal and lateral) and two (anterior and inferior) chamber views. The E/E_m ratio, recorded from the mitral annulus lateral wall, was used as an index of LV filling pressure. LV diastolic-dysfunction grading was determined using transmitral flow, pulmonary venous flow, and TDI indices, according to well-documented criteria [19].

STE: Vector velocity imaging echocardiography

The STE modality of vector velocity imaging was used to quantify myocardial wall motion through a series of manually placed B-mode pixel tracking algorithms, which follow the endocardium throughout a cardiac cycle. This measurement technique was previously validated against magnetic resonance-imaging and sonomicrometry [10]. STE has been extensively used to investigate LV strain and twist in health and disease [20]. The strain and twist analyses performed in the present study followed the procedure recently described [11], and focused only on the endocardial border, as the epicardial border could not be clearly visualized in the majority of participants, precluding the ability to quantify LV radial strain. LV longitudinal strain and SR were obtained from 2-D harmonic grey scale images in the apical four-chamber view, whilst circumferential strain and SR measures were obtained from the parasternal short-axis view (at basal and apical levels of the LV). Data were recorded for subsequent off-line analyses (X-Strain software, Esaote, Florence, Italy). On each cine-loop, an optimal frame was selected and the endocardial border was manually traced using the Aided-Heart-Segmentation option. LV strain, SR and rotations were automatically obtained from a six or four segment model, depending on the view.

Only cine-loops with adequate endocardial border definition, adequate frame rate (>60 Hz), and good image tracking were used. Ten control and 24 MetS participants were excluded from the 142 participants initially included in the study, due to indistinct endocardial border definition. This was possibly related to the higher corpulence of these participants.

The LV rotations at basal and apical short-axis views were determined as average angular displacement of six myocardial segments. Care was taken to ensure that the basal short-axis view contained the mitral valve, and that the apical short-axis view was acquired distally to the papillary

muscle. LV twist was calculated as the maximal instantaneous difference between basal and apical rotations. All measurements were averages derived from three consecutive cardiac cycles, and were paired with real-time electrocardiogram. To adjust all vector velocity imaging data for inter-participant differences in heart rate, a specific toolbox (Scilab 4.1-developed in our laboratory) was used to normalise time sequence as a percentage of systolic duration [11].

Inter and intra-observer reproducibility were estimated in 20 randomly selected participants, and the coefficient of variation was 3.1% and 5.9%, respectively for strain, and 14.2% and 16.6%, respectively for twist.

Intra-LV dyssynchrony

Time taken in milliseconds from the start of QRS to a specified point was measured for intra-LV dyssynchrony. The specified points were peak TDI-derived S_m velocity for systolic intra-LV dyssynchrony, and peak TDI-derived E_m velocity for diastolic intra-LV dyssynchrony (averaged from septal, lateral, anterior, and inferior annulus sites). The difference between maximum and minimum diastolic and systolic durations for the four sites (*TDI S_m : Max-Min 4-sites and TDI E_m : Max-Min 4-sites*) was measured. Differences greater than previously validated criteria [21, 22] constituted a diagnosis of dyssynchrony.

Statistical analyses

Following checks for normal distribution, anthropometric and general participant data were presented as mean \pm standard deviation. Differences in descriptive cardiac variables among MetS participants with various numbers of cardio-metabolic risk factors and controls were determined using one way analysis of variance (ANOVA), with Tukey's post-hoc test. Due to uneven group sizes, Hedge's g was used to calculate effect size between MetS and control participants for specified variables. An effect size of ≥ 0.2 was considered small, ≥ 0.5 considered medium, and ≥ 0.8 considered large. Chi-square analysis allowed comparisons of the incidence of TDI and STE-derived intra-LV dyssynchrony in participants with and without MetS, as well as the distribution of LV diastolic-dysfunction in MetS and control participants. Arithmetic transformation was performed for non-

Gaussian variables. Pearson's r correlation coefficient analysis was used to identify associations between metabolic risk factors and morphological, global functional and myocardial functional cardiac measures. Multiple stepwise linear regression analyses assessed the strongest predictors of variability in longitudinal strain. Statistical analyses were made using SPSS 16.0 for windows (SPSS Inc). Statistical significance was set *a priori* at $p < 0.05$.

5. RESULTS

Study population and clinical characteristics

Demographic characteristics of MetS and control participants showed no age and gender differences (Table 1). MetS participants had a greater waist circumference (Hedge's $g = 2.2$; $p < 0.001$), systolic blood pressure ($g = 0.9$; $p < 0.001$), triglycerides ($g = 0.9$; $p < 0.001$), and fasting blood glucose levels ($g = 1.2$; $p < 0.001$), as well as lower HDL ($g = 1.0$; $p < 0.001$) than controls. All inflammatory cytokines, with the exception of pro-BNP, were greater in MetS participants than controls.

Conventional and Tissue Doppler echocardiography

MetS participants revealed greater LV mass, parietal dimensions, and LA diastolic diameter than controls (Table 2). They also demonstrated lower E/A ratio ($g = 0.59$; $p < 0.001$) and E_m ($g = 1.2$; $p < 0.001$) than controls (Table 2). However, when using a combined approach to assess diastolic dysfunction [19], 60.4% of MetS participants revealed normal diastolic function. LV ejection fraction, peak S_m , and end-systolic wall stress did not differ between MetS participants and controls. Although E/E_m was more elevated in MetS participants compared with controls ($g = 0.7$; $p < 0.001$), absolute values for both populations remained within normal limits.

Vector velocity imaging

MetS participants had lower longitudinal strain ($g=1.6$; $p<0.001$), longitudinal diastolic ($g=1.6$; $p<0.001$) and systolic ($g=1.4$; $p<0.001$) SR, and earlier time to peak longitudinal strain than controls, even after normalisation to heart rate (Table 3). All circumferential strain, SR, and respective time to peak parameters at basal and apical levels were not different between MetS and control participants.

No differences were found between MetS and control participants in apical and basal rotation, as well as resultant twist. Compared with controls, MetS participants had higher twist ($g=0.6$; $p<0.05$) and untwist ($g=0.5$; $p<0.05$) rates, but no difference remained after co-varying for heart rate. A positive association between the number of accumulated MetS parameters and longitudinal strain was observed (Table 4). Specifically, MetS with five risk factors had lower longitudinal strain than MetS participants with three and four factors ($p<0.05$).

Intra-LV dyssynchrony

Significant differences were found between MetS participants and controls regarding TDI-derived intra-LV systolic, but not diastolic, dyssynchrony indices (Table 2). Additional chi-square analyses showed that intra-LV systolic, but not diastolic, dyssynchrony was more prevalent in MetS participants (19 of 68) than controls (0 of 44), according to previously validated criteria (TDI Sm Max-Min difference >65 ms) [22].

Independent effects of diabetes and hypertension within MetS population

Non-diabetic MetS participants showed lower longitudinal strain ($g=1.5$; $p<0.001$), systolic ($g=1.1$; $p<0.001$), and diastolic ($g=1.2$; $p<0.001$) SR than controls. Diabetes exacerbated this alteration in MetS participants, leading to lower longitudinal strain ($g=2.6$; $p<0.05$) than observed in non-diabetic MetS participants. Diabetes did not impair circumferential strain, SR, and twist in MetS participants. No difference in longitudinal or circumferential parameters was observed between hypertensive and normotensive MetS participants.

Relationships between longitudinal myocardial dysfunction and clinical data

Linear regression and correlation analyses were performed to examine the relationships between longitudinal strain and clinical, inflammatory, and functional cardiac parameters. Only moderate-strong correlates of longitudinal strain ($r > 0.40$) were entered into regression analyses (Table 5). The forward stepwise method was used to predict variability in longitudinal strain. A significant regression model emerged with a tolerance > 0.05 to avoid co-linearity ($F_{4, 92} = 23.5, p < 0.001$. Adjusted $R^2 = 0.484$). Standardised residuals were normally distributed. The most significant independent predictors of longitudinal strain were central fat, HbA_{1c}, TNF- α , and TDI S_m: max-min difference of 4 sites (Table 6).

6. DISCUSSION

The major findings of the present study were as follows: 1) Asymptomatic patients with MetS revealed myocardial functional impairments in the longitudinal axis only, while other major components of myocardial performance, such as circumferential deformation and twist/untwist mechanics, remained preserved. 2) Diastolic, as well as systolic longitudinal myocardial abnormalities were identified; the latter was detected only using recent STE technology. 3) In association with abdominal obesity; inflammation, glucose intolerance, and systolic intra-LV dyssynchrony emerged as major contributors to longitudinal dysfunction.

Effect of MetS on myocardial mechanics

Several studies using conventional echocardiography and TDI have shown evidence of isolated diastolic dysfunction in MetS patients, irrespective of grading criteria, but with preserved systolic function [5, 7]. Our findings agree; showing attenuated TDI-derived indices of diastolic function along with normal ejection fraction and systolic longitudinal velocities in MetS participants, compared with controls (Table 2). Of note in the present study; when diastolic function was assessed using previously validated grading criteria [19], 60.4% of MetS participants revealed normal diastolic

function. A major limitation of conventional echocardiography and TDI is a lack of sensitivity (dependency on loading conditions, tethering effect) in detecting subtle myocardial changes [23]. This is of particular concern when evaluating systolic abnormalities. Whether systolic myocardial function is normal or not in MetS remains hitherto unclear. Additionally, due to insonation angle-dependency, TDI-based indices of myocardial performance are usually restricted to basal and median segments in the longitudinal axis, therefore allowing only partial evaluation of myocardial performance, and ignoring the important role of both circumferential deformation and twist/untwist mechanics [9]. STE offers a comprehensive and sensitive evaluation of myocardial function [24]. In contrast to TDI, it enables the assessment of all segments in the parietal walls, thus limiting erroneous readings from translational cardiac movement. In addition, STE assesses not only longitudinal, but also circumferential function and torsional mechanics, and has previously been used to detect early dysfunction in various pathologies, including diabetes [25] and obesity [11]. Impaired longitudinal strain as well as diastolic and systolic SR in our MetS patients concurs with, and extends previous findings in MetS patients that used colour-TDI to assess strain and SR in LV basal segments [7, 12, 13]. The absence of impairment in circumferential function and twist/untwist in MetS patients, despite revealing decreased longitudinal function, was another major and novel finding of our study. The reason for the decreased longitudinal but preserved circumferential function and twist/untwist mechanics cannot be ascertained, but may be related to myofiber spatial-organization within the LV. Sub-endocardial fibers, running parallel to the LV long-axis and governing longitudinal function, have greater sensitivity to ischemia and fibrosis than other layers [26]. We hypothesize that inferiorly located myofibers may be more susceptible to MetS related inflammation and interstitial tissue damage (see discussion below), leading to earlier impairment of longitudinal function [26, 27]. Meanwhile, mid to superior myocardial/epicardial fiber layers, which predominantly govern circumferential function, may be preserved despite sharing the same stage of pathology. Of note, in more advanced pathologies, such as type-2 diabetes, circumferential function often decreases while twist increases [28, 29]. Furthermore, using STE, both myocardial diastolic and systolic dysfunction in the longitudinal axis was detected in MetS patients. This highlights the importance of evaluating

diastolic and systolic coupling using the same, more sensitive, technique to gain insight into overall myocardial function.

Mechanisms responsible for depressed longitudinal function in MetS patients

Myocardial deformation is affected by increased cardiac preload and afterload [30]. However, no differences between MetS participants and controls were found in the pre-load dependent E velocity measure and the afterload dependent end-systolic wall stress index; indicating that loading conditions were unlikely to have influenced longitudinal strain and SR. Growing evidence in the literature highlights a link between cardiac abnormalities and metabolic risk factors [14]. However, their association with LV myocardial dysfunction in MetS patients remains incompletely understood. In the present study, we observed that abdominal obesity, biological markers of systemic inflammation and glucose intolerance, as well as myocardial systolic dyssynchrony, were independent predictors of longitudinal strain in MetS patients. While the exact mechanisms behind impaired longitudinal but preserved circumferential function are still unknown, this finding has been replicated in other metabolic disease states [11, 25]. Several hypotheses, which align with the present study's findings, may help elucidate these mechanisms.

Increasing evidence supports the role of abdominal adiposity in active endocrinological functions, via dysfunctional adipocytes [31]. Increased adipokine levels were observed in our MetS participants (Table 1), and significant relationships were shown between some markers of systemic inflammation, such as TNF- α and high sensitivity CRP, and longitudinal strain (Table 4). Elevated pro-inflammatory cytokines and oxidative-stress in our MetS patients could explain the depressed myocardial longitudinal strain, and diastolic and systolic SR. This may be via the known deleterious impact of adipokines on 1) macro and micro-circulatory structure and function, leading to reduced coronary myocardial blood flow and sub-endocardial hypo-perfusion, 2) cell apoptosis and tissue fibrosis, impairing signal conductance and increasing LV dyssynchrony, or 3) calcium-handling, leading to excitation-contraction coupling abnormalities [14, 32].

It is well established that exposure to an increasing number of MetS factors causes stepwise deterioration in global diastolic function [4, 7, 33, 34]. We also found a stepwise deterioration in

longitudinal strain in MetS participants with an increasing number of factors. Moreover, diabetes had a similar exacerbating effect on longitudinal function. The aforementioned mechanisms and deleterious effects of chronic hyperglycaemia on cardiomyocyte calcium cycling may explain this finding [35]. Other studies have reported evidence of LV-dyssynchrony in participants with obesity [21] and type-2 diabetes [36]. Our results extend previous findings; showing systolic LV-dyssynchrony was an independent predictor of longitudinal myocardial function in MetS participants. The mechanisms underlying this finding are speculative, but may be related to the inflammation and obesity-related LV remodelling [37], and the vulnerability of sub-endocardial fibers to ischemic tissue damage, as discussed earlier [38]. More studies in humans are needed to clarify this, and other underlying mechanisms responsible for the impairment in LV longitudinal function in MetS participants.

Study limitations

The results may be confounded by a large portion of the participants being hypertensive (78%). However, we found no significant difference in longitudinal myocardial function between hypertensive and normotensive MetS participants. Within the MetS population, 17% were medicated using beta-blockers; a drug which may mask myocardial dysfunction, or reverse LV remodelling [39]. Nonetheless, no differences in LV mass index or longitudinal and circumferential strain, SR, and twist were observed between MetS participants taking beta-blockers and non drug-taking counterparts. Lastly, we were limited to a somewhat small sample size due to strict inclusion criteria, and difficulty in acquiring adequate quality images from all participants. Generalizing the results and assuming causality in the findings should be done with caution.

In conclusion, MetS participants without a history of incompatible diseases such as CVD showed impairments in both LV diastolic and systolic myocardial function in the longitudinal axis, while circumferential myocardial function was preserved. Abdominal obesity, combined with key inflammatory, clinical, and systolic dyssynchrony markers were strongly associated with this deterioration. Participants with diabetes and a greater number of metabolic risk factors demonstrated

the most severe myocardial dysfunction, but only in the longitudinal axis. More research is needed to understand the mechanisms through which MetS affects longitudinal LV function. However, we contend that early identification of subclinical myocardial dysfunction in MetS participants, using more precise STE methods, may allow better comprehension of the pathophysiology of MetS.

7. CONFLICTS OF INTEREST

No conflict of interest is declared.

8. ACKNOWLEDGMENTS

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TABLE 1 Clinical and inflammatory characteristics of MetS and control participants

	Controls	MetS	P-value
<i>n</i>	50	92	
Demographic parameters			
Age (years)	58 ± 5	59 ± 5	NS
Gender (males/females)	26/24	40/52	NS
Medication (number of treatments)			
Hypertension	0	65 (16 β-blockers)	<0.001
Type-2 diabetes	0	27	<0.001
Dyslipidemia	0	47	<0.001
Clinical and laboratory parameters			
BMI (kg.m ⁻²)	24.1 ± 3.1	33.4 ± 3.9	<0.001
Systolic blood pressure (mmHg)	117 ± 12	130 ± 15	<0.001
Diastolic blood pressure (mmHg)	73 ± 8	77 ± 10	NS
Lean mass (kg)	50.7 ± 11.2	58.2 ± 10.6	<0.001
Fat mass (kg)	16.5 ± 4.1	30.9 ± 7.8	<0.001
Central Fat (kg)	1.2 ± 0.6	3.1 ± 0.7	<0.001
Waist circumference (cm)	82.4 ± 7.7	102.2 ± 9.4	<0.001
Fasting glucose (mmol.L ⁻¹)	4.2 ± 0.5	5.6 ± 1.4	<0.001
HbA _{1c} (%)	5.5 ± 0.4	6.2 ± 0.8	<0.001
LDL cholesterol (mmol.L ⁻¹)	3.6 ± 0.8	3.4 ± 0.9	NS
HDL cholesterol (mmol.L ⁻¹)	1.6 ± 0.5	1.2 ± 0.3	<0.001
Triglycerides (mmol.L ⁻¹)	1.2 ± 0.4	1.9 ± 0.9	<0.001

†HOMA-IR	2.5 ± 1.4	4.0 ± 2.1	<0.001
Inflammatory blood markers			
†High sensitivity CRP (mg.L ⁻¹)	1.3 ± 1.4	3.6 ± 2.5	<0.001
†TNFα (pg.ml ⁻¹)	3.6 ± 3.4	11.5 ± 8.0	<0.001
†Leptin (ng.ml ⁻¹)	11.8 ± 10.4	30.3 ± 15.9	<0.001
†IL-6 (pg.ml ⁻¹)	1.3 ± 1.3	3.0 ± 2.5	<0.001
†PAI-1 active (ng.ml ⁻¹)	8.0 ± 4.9	19.7 ± 11.4	<0.001
†Adiponectin (μg.ml ⁻¹)	31.9 ± 21.7	19.1 ± 13.5	<0.001
†Pro-BNP (pg.ml ⁻¹)	21.7 ± 36.0	29.5 ± 58.7	NS

Data are means ± standard deviations, unless otherwise stated. BMI: Body mass index, HbA_{1c}: Glycated haemoglobin, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, HOMA-IR: Homeostatic model assessment of insulin resistance, CRP: C-reactive protein, TNF-α: Tumor necrosis factor α, IL-6: Interleukin-6, PAI-1: Plasminogen activator inhibitor-1, Pro-BNP: Brain natriuretic peptide. †Data were log or square-root transformed to satisfy Gaussian distribution prior to further statistical tests.

TABLE 2 Global echocardiographic parameters of MetS and control participants

	Controls	MetS	P-value
<i>n</i>	44	92	
Cardiac morphology			
LV mass (g)	201 ± 50	267 ± 65	<0.001
LV mass indexed (g.m ^{-2.7})	46.9 ± 14.0	66.1 ± 22.4	<0.001
LVED diameter (mm)	47.7 ± 5.1	50.2 ± 5.7	<0.05
LVES diameter (mm)	29.2 ± 4.7	30.4 ± 5.5	NS
LA diameter (mm)	31.5 ± 4.1	36.4 ± 4.0	<0.001
IV septum diastolic (mm)	10.0 ± 1.3	11.4 ± 1.5	<0.001
Posterior wall diastolic (mm)	10.0 ± 1.1	11.6 ± 1.4	<0.001
Relative wall thickness (mm)	0.42 ± 0.06	0.46 ± 0.08	<0.05
End systolic wall stress (g.cm ⁻²)	54.4 ± 13.5	51.2 ± 16.6	NS
Pulsed Doppler cardiac function			
LV ejection fraction (%)	61.4 ± 5.6	61.6 ± 6.7	NS
Resting heart rate (beats.min ⁻¹)	61 ± 5	72 ± 11	<0.001
<i>E</i> velocity (cm.s ⁻¹)	63.8 ± 12.0	64.7 ± 14.9	NS
<i>A</i> velocity (cm.s ⁻¹)	51.4 ± 14.2	62.9 ± 17.5	<0.001
<i>E/A</i>	1.3 ± 0.4	1.1 ± 0.3	<0.001
Deceleration time of <i>E</i> (ms)	205 ± 33	210 ± 37	NS
IVRT (ms)	119 ± 18	120 ± 19	NS
Tissue Doppler Imaging			
<i>S_m</i> velocity (cm.s ⁻¹)	8.6 ± 1.7	8.2 ± 1.3	NS
<i>E_m</i> velocity (cm.s ⁻¹)	10.6 ± 1.8	8.7 ± 1.5	<0.001
<i>A_m</i> velocity (cm.s ⁻¹)	9.6 ± 1.7	10.6 ± 4.9	NS
<i>E/E_m</i> lateral wall	5.7 ± 1.5	6.9 ± 1.9	<0.001

Intra-LV dyssynchrony

TDI S_m : Max-Min diff of 4 sites (>65ms: yes/no)	25.3 ± 11.9 (0/48)	43.4 ± 21.6 (19/68)	<0.001
TDI E_m : Max-Min diff of 4 sites (>65ms: yes/no)	21.9 ± 10.2 (0/48)	25.6 ± 11.9 (3/68)	NS

Data are means ± standard deviations, unless otherwise stated. S_m , E_m , & A_m are means of four sites at the mitral annulus from apical 4-chamber and 2-chamber views. IVRT: Isovolumic Relaxation Time, LVED: Left Ventricular End Diastolic, LVES: Left Ventricular End Systolic, TDI: Tissue Doppler Imaging, Strain L: Longitudinal strain.

TABLE 3 STE-derived parameters of myocardial function in MetS and control participants

	Controls	MetS	P-value
<i>n</i>	40	68	
Longitudinal axis of LV			
L strain (%)	-21.2 ± 2.6	-16.8 ± 2.8	<0.001
Time to peak L strain (ms)	99.9 ± 6.1	97.6 ± 5.4	<0.05
L systolic SR (strain.s ⁻¹)	-1.2 ± 0.2	-1.0 ± 0.1	<0.001
Time to peak L systolic SR (ms)	53.6 ± 6.2	52.0 ± 7.1	NS
L diastolic SR (strain.s ⁻¹)	1.5 ± 0.3	1.1 ± 0.2	<0.001
Time to peak L diastolic SR (ms)	132.2 ± 7.0	132.2 ± 11.9	NS
Circumferential apex of LV			
Apical C strain (%)	-31.6 ± 4.7	-30.9 ± 5.3	NS
Time to peak apical C strain (ms)	95.9 ± 4.8	94.0 ± 5.4	NS
Apical C systolic SR (strain.s ⁻¹)	1.9 ± 0.4	-2.0 ± 0.4	NS
Time to peak apical C systolic SR (ms)	59.1 ± 6.9	57.2 ± 7.3	NS
Apical C diastolic SR (strain.s ⁻¹)	2.4 ± 0.5	2.3 ± 0.6	NS
Time to peak apical C diastolic SR (ms)	131.9 ± 5.1	130.8 ± 9.5	NS
Circumferential base of LV			
Basal C strain (%)	-26.5 ± 4.1	-25.3 ± 4.8	NS
Time to peak basal C strain (ms)	104.4 ± 6.7	103.7 ± 7.9	NS
Basal C systolic SR (strain.s ⁻¹)	-1.8 ± 0.3	-1.8 ± 0.4	NS
Time to peak basal C systolic SR (ms)	55.8 ± 8.2	55.2 ± 8.4	NS
Basal C diastolic SR (strain.s ⁻¹)	2.3 ± 0.5	2.0 ± 0.5	NS
Time to peak basal C diastolic SR (ms)	130.7 ± 9.1	133.8 ± 12.1	NS
Rotational mechanics of LV			
Apical rotation (°)	4.5 ± 2.2	4.6 ± 2.1	NS

Basal rotation (°)	-4.2 ± 1.7	-4.5 ± 1.9	NS
Twist (°)	8.1 ± 2.5	8.6 ± 2.4	NS
Twist rate (°·s ⁻¹)	48.7 ± 12.5	58.4 ± 16.4	<0.05
Untwist rate (°·s ⁻¹)	-58.1 ± 16.0	-69.7 ± 25.4	<0.05

Data are means ± standard deviations. SR: Strain rate, L: Longitudinal, C: Circumferential.

TABLE 4 Cumulative impact of MetS on longitudinal myocardial function

	Controls	MetS		
		3 factors	4 factors	5 factors
<i>n</i>	40	21	32	18
L strain (%)	-21.2 ± 2.6	-17.2 ± 2.5*	-17.5 ± 2.6*	-14.7 ± 2.1*†
L systolic SR (strain.s ⁻¹)	-1.22 ± 0.17	-1.04 ± 0.16*	-1.03 ± 0.11*	-0.92 ± 0.12*†
L diastolic SR (strain.s ⁻¹)	1.47 ± 0.30	1.12 ± 0.14*	1.18 ± 0.18*	0.97 ± 0.18*†

*Data are means ± standard deviations. *Significantly different from control subjects: p<0.05. †Significantly different from MetS 3 factors & MetS 4 factors. L: Longitudinal. SR: Strain rate.*

TABLE 5 Relationships between longitudinal myocardial deformation and clinical, inflammatory, and morphological cardiac parameters

	Longitudinal strain	
	<i>r</i> value	<i>P</i> -value
Central fat	0.61	<0.001
Systolic blood pressure	0.48	<0.001
Left ventricular mass	0.47	<0.001
Posterior wall diastolic thickness	0.47	<0.001
TNF- α	0.46	<0.001
High sensitivity CRP	0.45	<0.001
HbA _{1c}	0.42	<0.001
TDI S _m : Max-Min diff of 4 sites	0.40	<0.001

Only variables with moderate to strong correlation with longitudinal strain ($r > 0.40$) were included for analysis. HbA_{1c}: Glycated haemoglobin, CRP: C-reactive protein, TNF- α : Tumor necrosis factor α , TDI: Tissue Doppler Imaging.

TABLE 6 Independent predictors of longitudinal strain following multiple regression analyses

Longitudinal strain: $R^2 = 0.48$ ($p < 0.001$)		
	<i>Beta</i>	<i>P-value</i>
Central fat	0.343	<0.001
HbA _{1c}	0.249	<0.05
TNF- α	0.222	<0.05
TDI S _m : Max-Min diff of 4 sites	0.200	<0.05

HbA_{1c}: Glycated haemoglobin, TNF- α : Tumor necrosis factor α , TDI: Tissue Doppler Imaging.

4.2. STUDY 2

4.2.1. Preamble

The following is a presentation of the short-communication manuscript for Study 2 in the format that was accepted for publication in the Canadian Journal of Cardiology. Briefly, this second study examined the prevalence and extent of myocardial LV-dyssynchrony in both non-diabetic and diabetic MetS individuals, compared with controls. LV-dyssynchrony was assessed with TDI and STE methods, to provide a reasonably complete (multi-axis) assessment of myocardial LV-dyssynchrony.

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Figure 1. Extent and clinical prevalence of LV-dyssynchrony from maximum delay and standard deviation of the four sites (TDI-systolic) and six segments (STE-longitudinal) in control, MetS-NT2D, and MetS-T2D individuals _____ 125

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Supplemental Figure S1. Example of measurement of time to peak systolic and diastolic myocardial velocity (TDI) at the septal annulus site, used in calculating LV-dyssynchrony _____ 130

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Left ventricular myocardial dyssynchrony is already present in non-diabetic patients with metabolic syndrome

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Abstract

The presence of left ventricular (LV) dyssynchrony in individuals with metabolic syndrome (MetS), a predictor of type-2 diabetes (T2D), lacks clarity. We compared LV-dyssynchrony in MetS individuals with and without T2D, and healthy controls using speckle-tracking-imaging echocardiography. 92 MetS participants (64 without, 28 with T2D) and 40 controls underwent echocardiographic and clinical/biological analyses. LV-dyssynchrony in the longitudinal-axis only was present in all MetS individuals, but was not further exacerbated by T2D. Strong associations were found with systemic inflammation, abdominal obesity and LV mass. Investigations of myocardial dyssynchrony in the non-diabetic MetS stage may facilitate timely and more effective prevention.

Brief summary

We investigated left ventricular dyssynchrony in metabolic syndrome patients (MetS) with and without type-2 diabetes compared to healthy controls, using speckle-tracking echocardiography. Longitudinal but not circumferential dyssynchrony was noticed in MetS individuals, regardless of the presence of type-2 diabetes. Inflammation, abdominal obesity and left-ventricular mass were most associated with dyssynchrony.

Background

Metabolic syndrome (MetS) is defined by abdominal obesity, dyslipidemia, and elevated blood pressure and blood glucose [1]. This clustering of cardio-metabolic risk factors shares a similar phenotype to type-2 diabetes (T2D), with abdominal obesity, chronic low grade inflammation, and insulin-resistance. MetS and T2D are linked to myocardial dysfunction identified by tissue-Doppler imaging (TDI) and speckle-tracking echocardiography (STE) [2, 3]. Myocardial function depends largely on cardiomyocyte contraction and relaxation properties, but also on electromechanical synchronicity [4]. STE has the advantage over TDI of angle-independency, offering the possibility to examine not only longitudinal, but also circumferential function; a major component of left ventricular (LV) performance [4]. TDI-derived LV-dyssynchrony was previously identified in T2D patients with abdominal obesity and insulin-resistance [5]. However, research into STE-derived LV-dyssynchrony, and thus circumferential function, in T2D is absent. Similarly, TDI LV-dyssynchrony was found in individuals with MetS; a stage predictive of T2D [6]. Whether longitudinal and/or circumferential dyssynchrony is present in MetS individuals without T2D (MetS-NT2D) remains unknown.

Our aim was to compare longitudinal and circumferential dyssynchrony in MetS individuals with and without T2D, and healthy controls.

Methods

For detailed methods, please see Online Supplement.

Participants

This study formed part of the RESOLVE trial [7]. We conducted a cross-sectional analysis in 92 individuals with IDF-defined MetS [1], aged 50-70 years: 64 without T2D (Mets-NT2D) and 28 with T2D (MetS-T2D). Individuals were free from cardiovascular disease with the exception of factors defining MetS, and had satisfactorily completed an exercise-tolerance test prior to testing. MetS individuals were age and gender-matched with 40 healthy controls from the community.

Echocardiography

For echocardiographic methods, see Online Supplement.

Clinical and Biological Measures

For Clinical and Biological measurement methods, see Online Supplement.

Statistics

Following tests for normality, analysis of variance (ANOVA) with Tukey's post-hoc test was used to assess inter-group differences in descriptive variables. Chi-square analysis was used to compare incidences between groups. Pearson's r correlation coefficient analysis was used to assess associations. SPSS Version 16.0 for windows (SPSS Inc) was used for analyses.

Results

MetS individuals demonstrated higher blood glucose, lipids and inflammation levels than controls as well as higher LV mass and depressed diastolic function (Table 1). Longitudinal,

but not circumferential, strain and strain rate were lower in MetS individuals than controls. MetS-NT2D had lower fasting glucose, HbA_{1c}, PAI-1 active, and HOMA-IR than MetS-T2D. Maximum delay and SD for TDI systolic and longitudinal STE LV-dyssynchrony were significantly greater in MetS individuals than controls, but did not differ between MetS-NT2D and MetS-T2D (Table 1 & Fig. 1). The incidence of TDI systolic and STE longitudinal dyssynchrony (both maximum delay and SD) was higher in MetS-NT2D and MetS-T2D than controls (Fig. 1). No inter-group differences were observed for TDI diastolic dyssynchrony or STE circumferential dyssynchrony. In 71 MetS individuals with normal E/A ratio (>0.8), incidence of TDI-derived systolic maximum delay and SD was 18.6% and 19.7% respectively, while incidence of longitudinal STE-derived maximum delay was 16.9%. Additionally, in 47 MetS individuals with normal septal E_m (>8cm.s⁻¹), incidence of TDI-derived systolic maximum delay and SD was 19.1% and 21.3% respectively, and 13.2% for longitudinal STE-derived maximum delay. TDI systolic and STE dyssynchrony indexes both correlated with LV mass index, left atrial diameter, waist circumference and PAI-1 active. A significant correlation was also obtained between TDI systolic dyssynchrony index and systolic blood pressure (Supplemental Table). Following analysis of covariance with the above correlating variables, significantly prolonged (p<0.05) TDI and longitudinal STE-derived dyssynchrony was still evident between MetS (both diabetic and non-diabetic) and control individuals. Further correlation analyses revealed some concordance between STE and TDI dyssynchrony parameters. Significant associations were found between longitudinal STE maximum delay and both systolic TDI maximum delay (r=0.22; p<0.05) and systolic TDI SD (r=0.24; p<0.001).

Discussion

The main finding of our study was that TDI systolic and STE longitudinal dyssynchrony indices were already impaired in MetS-NT2D individuals compared with controls. Furthermore, significant inter-relationships between cardiac morphology, abdominal-obesity, inflammatory markers, and dyssynchrony were observed, independent of diabetic status. Clinically diagnosed systolic TDI and longitudinal STE dyssynchrony were present even in MetS individuals with preserved conventional diastolic parameters.

Previous studies have found evidence of dyssynchrony in MetS and T2D populations, but the isolated effect of MetS was not reported [5, 6]. Hyperglycaemia is regarded as an independent predictor of TDI-derived dyssynchrony [6], suggesting that T2D populations are at higher risk of myocardial dyssynchrony. A novel finding in our study was that dyssynchrony was already present in non-diabetic MetS individuals, with no further impairment in T2D individuals. In addition, the high incidence of clinically-diagnosed systolic TDI and longitudinal STE dyssynchrony in MetS individuals with normal conventional diastolic parameters highlights, for the first time, the significance of LV dyssynchrony in early detection of isolated systolic dysfunction.

MetS is a powerful predictor of T2D [8], and is considered less advanced than T2D. Notably, most of our population was hypertensive, which may confound the isolated effect of MetS. No differences in LV-dyssynchrony indices were observed between hypertensive and normotensive MetS individuals (Supplemental Figure). Furthermore, in our population, LV mass emerged as a significant correlate of myocardial LV-dyssynchrony. LV remodelling, not hypertension, may therefore be more relevant to the causality of dyssynchrony in MetS.

Several key clinical and biological factors also emerged as significant correlates of both TDI and STE LV-dyssynchrony. In addition to LV mass; TDI-systolic and STE-longitudinal LV-

dyssynchrony indices correlated with abdominal obesity and inflammatory markers. The pro-inflammatory status combined with hyperglycemia may lead to interstitial fibrosis in the sub-endocardial region [9]. This may explain why we observed longitudinal, but not circumferential dyssynchrony. The myocardium is perfused from epicardial to endocardial surface, with endocardial fibres being most prone to ischemia [4]. The transmural continuum of the two helical fibre organisations; whereby a more vertical right-handed helical formation in the inferior aspect of the myocardium (sub-endocardium) gradually changes into a more horizontal left-handed helical formation in the superior aspect of the myocardium (sub-epicardium) suggests that longitudinal function is governed by sub-endocardial fibres [4]. Moreover, abdominal obesity and cardiac hypertrophy are typically associated with myocardial steatosis [10]. In turn, myocardial steatosis may impair cardiac function in populations with metabolic disorders through increased myocardial fibrosis, which is a strong predictor of LV dyssynchrony [11]. Lastly, hyperglycaemia and inflammation may have a direct impact on cardiomyocytes [12]. Dysfunctional calcium-cycling may be caused by chronic hyperglycaemia and elevated cytokines [12].

Limitations

Intra and inter-observer variability was slightly high for STE-dyssynchrony, not dissimilar from other studies. However, the accuracy of assessing LV dyssynchrony was increased by using several complementary techniques (TDI, STE) and both maximum delay and SD. The percentage of poor quality cine-loops and image removal (related to the lack of echogenicity) was similar to commonly reported values. This limitation precluded accurate measurement of dyssynchrony in the more classical 12 to 18-segment model. However, using 12 to 18-segments would likely have increased, not decreased maximum delay or SD. The cross-

sectional design has limitations; however, proof of concept was important and achieved. For more detailed discussion, see Supplement.

Clinical significance and Conclusion

Early identification of intra-LV myocardial dyssynchrony in a population with high risk, but no symptoms of CVD (such as our MetS patients), is important for several reasons. First, it provides an additional and more sensitive means of detecting subclinical systolic dysfunction. Second, LV dyssynchrony may also enable very early detection of isolated systolic dysfunction. In the present study, a significant percentage of MetS individuals without impaired diastolic function manifested clinical TDI and STE LV dyssynchrony. We noted a strong interrelationship between LV dyssynchrony, systemic inflammation, and abdominal obesity in MetS participants, independent of diabetic status. A better understanding of myocardial mechanics in earlier stages of metabolic disorders may facilitate timely and thus more effective prevention.

Acknowledgements

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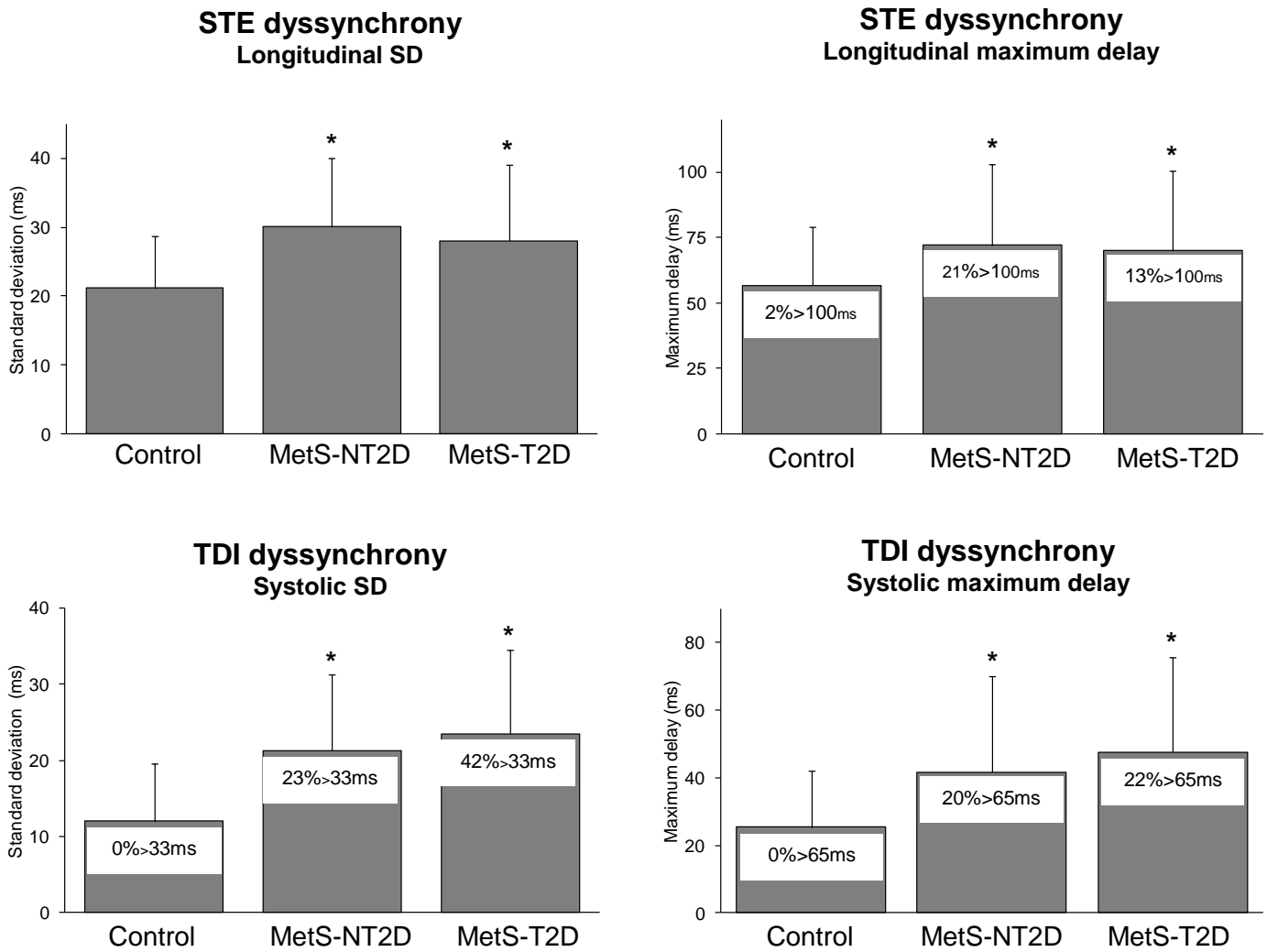
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Table 1. Clinical, biological, and echocardiographic characteristics in control, MetS-NT2D, & MetS-T2D individuals.

	Controls	MetS-NT2D	MetS-T2D	P-value
<i>n</i>	40	64	28	
Age (years)	58 ± 4.7	59 ± 4.9	59 ± 5.2	NS
Gender (males/females)	26/24	27/37	13/15	NS
Clinical & inflammatory measures				
Waist circumference (cm)	82.4 ± 7.7	100.6 ± 8.9*	106.0 ± 9.8*#	<0.001
BMI (kg.m ⁻²)	24.1 ± 3.1	32.9 ± 3.9*	34.6 ± 3.7*	<0.001
Systolic blood pressure (mmHg)	117 ± 12	129 ± 15*	134 ± 15*	<0.001
Diastolic blood pressure (mmHg)	74 ± 8	77 ± 10	77 ± 9	NS
Fasting glucose (mmol.L ⁻¹)	4.2 ± 0.5	5.1 ± 0.9*	7.0 ± 1.9*#	<0.001
HbA _{1c} (%)	5.4 ± 0.40	6.0 ± 0.51*	7.1 ± 0.85*#	<0.001
HDL (mmol.L ⁻¹)	1.6 ± 0.5	1.2 ± 0.3*	1.2 ± 0.2*	<0.001
Triglycerides (mmol.L ⁻¹)	1.2 ± 0.5	2.0 ± 0.9*	2.0 ± 1.0*	<0.001
PAI-1 active (mmol.L ⁻¹)	8.0 ± 4.9	17.6 ± 11.2*	24.5 ± 10.6*#	<0.001
High sensitivity CRP (mmol.L ⁻¹)	1.3 ± 1.1	3.5 ± 2.3*	3.8 ± 2.9*	<0.001
HOMA-IR (mmol.L ⁻¹)	2.5 ± 1.4	3.5 ± 1.5*	5.1 ± 2.8*#	<0.001
Conventional echocardiographic & TDI measures				
LAdD	31.5 ± 4.1	36.1 ± 3.9*	37.1 ± 4.2*	<0.001
LVEdD (mm)	47.7 ± 5.1	49.9 ± 5.9	50.9 ± 5.2	NS
LVEsD (mm)	29.2 ± 4.7	30.5 ± 5.6	30.1 ± 5.1	NS
PWT (mm)	9.9 ± 1.1	11.6 ± 1.5*	11.6 ± 1.3*	<0.001
LVEF (%)	63.8 ± 5.0	59.6 ± 8.9	59.4.3 ± 4.9	NS
LVMI (g.m ^{-2.7})	46.9 ± 14.0	65.0 ± 23.3*	68.5 ± 20.3*	<0.001
E/A	1.3 ± 0.4	1.1 ± 0.3*	1.1 ± 0.2*	<0.001
E _m (cm.s ⁻¹)	10.6 ± 2.0	8.7 ± 2.1*	8.6 ± 1.2*	<0.001
S _m (cm.s ⁻¹)	8.6 ± 1.7	8.3 ± 1.4	8.2 ± 1.1	NS
STE deformation measures				
L strain (%)	-21.2 ± 2.6	-17.1 ± 2.5*	-15.9 ± 2.9*	<0.001
L diastolic SR (%.s ⁻¹)	1.5 ± 0.3	1.2 ± 0.2*	1.0 ± 0.2*	<0.001
L systolic SR (%.s ⁻¹)	-1.2 ± 0.2	-1.0 ± 0.1*	-0.9 ± 0.2*	<0.001
C strain (%)	-28.9 ± 4.0	-28.2 ± 5.4	-29.6 ± 4.4	NS
C diastolic SR (%.s ⁻¹)	2.3 ± 0.5	2.2 ± 0.5	2.33 ± 0.4	NS
C systolic SR (%.s ⁻¹)	-1.8 ± 0.3	-1.9 ± 0.4	-2.1 ± 0.4	NS
LV dyssynchrony measures				
TDI systolic maximum delay	25.3 ± 16.9	41.7 ± 28.5*	47.6 ± 28.14*	0.001
TDI systolic SD	12.0 ± 7.9	21.3 ± 16.3*	23.5 ± 19.0*	0.002
TDI diastolic maximum delay	21.9 ± 13.2	26.2 ± 13.7	24.1 ± 14.6	NS
TDI diastolic SD	9.9 ± 5.6	13.0 ± 6.9	11.3 ± 6.7	NS
STE L maximum delay	56.6 ± 21.9	76.6 ± 30.2*	72.9 ± 28.3*	0.001
STE L SD	21.2 ± 8.2	30.1 ± 16.3*	28.0 ± 10.5*	0.004
STE C maximum delay	69.4 ± 26.4	81.2 ± 32.4	71.9 ± 29.0	NS
STE C SD	30.0 ± 13.5	35.2 ± 16.6	28.3 ± 13.8	NS

*Mean±standard deviations. BMI: Body mass index, HDL: High density lipoproteins, PAI-1 active: Plasminogen activator inhibitor-1 active. CRP: C-reactive protein, HOMA-IR: Homeostatic model assessment of insulin resistance, TDI: Tissue Doppler imaging, LAdD: Left atrial diastolic diameter, LVEdD: Left ventricular end diastolic diameter, LVEsD: Left ventricular end systolic diameter, PWT: Posterior wall thickness, LVEF: Left ventricular ejection fraction, LVMI: Left ventricular mass index, STE: Speckle tracking echocardiography, L: Longitudinal, SR: Strain rate, C: Circumferential, SD: Standard deviation. *: $p<0.01$ vs. controls. #: $p<0.05$ vs. controls and MetS-NT2D.*

Figure 1. Extent and clinical prevalence of LV-dyssynchrony from maximum delay and standard deviation of the four sites (TDI-systolic) and six segments (STE-longitudinal) in control, MetS-NT2D, and MetS-T2D individuals. STE: Speckle tracking echocardiography, SD: Standard deviation, & TDI: Tissue Doppler imaging. *: $p < 0.05$ vs. controls.



SUPPLEMENTAL DATA (Online Supplement)

Extended methodology: Echocardiography

Echocardiographic analyses were performed according to standard criteria [1], with a minimum frame-rate of 70 Hz. Cine-loops were triggered to an electrocardiogram-derived QRS complex, and recorded for offline analyses using specific software (MyLab desk 9.0, Esaote, Florence, Italy). Conventional echocardiographic parameters included M-Mode measurements obtained in the parasternal long-axis view. Left atrial (LA) and LV dimensions were measured at both end-diastole and end-systole. LV mass was calculated by the Devereux formula and indexed for height (Cornell adjustment). LV ejection fraction was calculated from semiautomatic quantification of LV volumes, using vector velocity imaging [2]. Pulsed-Doppler LV transmitral velocity, including early (E) and atrial (A) waves, were measured in the apical four-chamber view, with the sample volume placed at the tip of the mitral valve leaflets. Isovolumic relaxation time was measured by pulsed-Doppler in the apical five-chamber view. Pulsed-wave TDI-derived peak systolic (S_m), early-diastolic (E_m), and late-diastolic (A_m) myocardial velocities were measured at the LV annular septal, lateral, inferior, and anterior walls (apical 4-chamber and apical 2-chamber views). STE analyses were performed by manually tracing the LV endocardial border in the apical 4-chamber view, and parasternal short axis view at the mid-LV level, as described previously [3].

For pulsed-wave TDI LV-dyssynchrony, time in milliseconds was measured from the start of QRS to peak S_m (systolic dyssynchrony) or peak E_m (diastolic dyssynchrony) in the four TDI sites (Figure S1). For STE LV-dyssynchrony, time taken from the start of QRS to peak strain in the six segments (basal-septal, mid-septal, apical-septal, apical-lateral, mid-lateral, and basal-lateral) was recorded in both longitudinal (apical 4-chamber) and circumferential (parasternal short-axis; mid-LV) axes (Figure S2). LV-dyssynchrony was assessed by

calculating both the maximum delay and the standard deviation (SD) in the four LV-annulus sites (TDI) or six LV-segments (STE). Clinical diagnosis of LV-dyssynchrony was made if the maximum delay was greater than 65 ms [4] and 100 ms [5] for TDI and STE respectively, or if TDI SD was greater than 33 ms [4]. No cut-off criteria currently exist for STE longitudinal or circumferential SD. Inter and intra-observer reliability for STE LV-dyssynchrony was 9.3% and 10.2%, respectively.

Extended methodology: Clinical Measurements and Blood biology

Waist circumference was measured at the midpoint between sub-costal and supra-iliac landmarks. Blood pressure and heart rate were measured after 15 minute rest using an automated upper arm sphygmomanometer (SunTech Medical, Model 222). Blood-biology data was collected for measurement of triglycerides, high-density lipoprotein (HDL), low-density lipoproteins (LDL), fasting glucose, HbA_{1c}, high-sensitivity C-reactive protein (CRP), insulin [6], and Plasminogen-activator inhibitor-1 active (PAI-1 active), according to protocol described elsewhere [7].

Fasting blood samples were drawn between 7.00 and 7.30 a.m., aliquoted and stored at -80°C until analyses (with the exception of HbA_{1c} which was directly measured after the blood draw). Basic biological assays were performed in the biochemistry laboratory of the University Hospital of Clermont-Ferrand, France and comprised serum concentrations of triglycerides, high-density lipoprotein (HDL), low-density lipoproteins (LDL), fasting glucose, HbA_{1c}, and high sensitivity C-reactive protein (CRP). Insulin and Plasminogen-activator inhibitor-1 active (PAI-1 active) were assayed by ELISA with commercial kits (Millipore, Billerica, MA, USA). Sensitivity, intra, and inter-assay coefficient of variation were 3.0 ng.ml⁻¹, 2.6%, and 7.2% respectively for insulin, and 1.3 pg.ml⁻¹, 6.6% and 10% respectively for PAI-1 active. Insulin resistance was estimated by the calculation of the

homeostasis model assessment-insulin resistance (HOMA-IR) index (fasting plasma glucose \times fasting plasma insulin)/22.5 [6].

Detailed Limitations

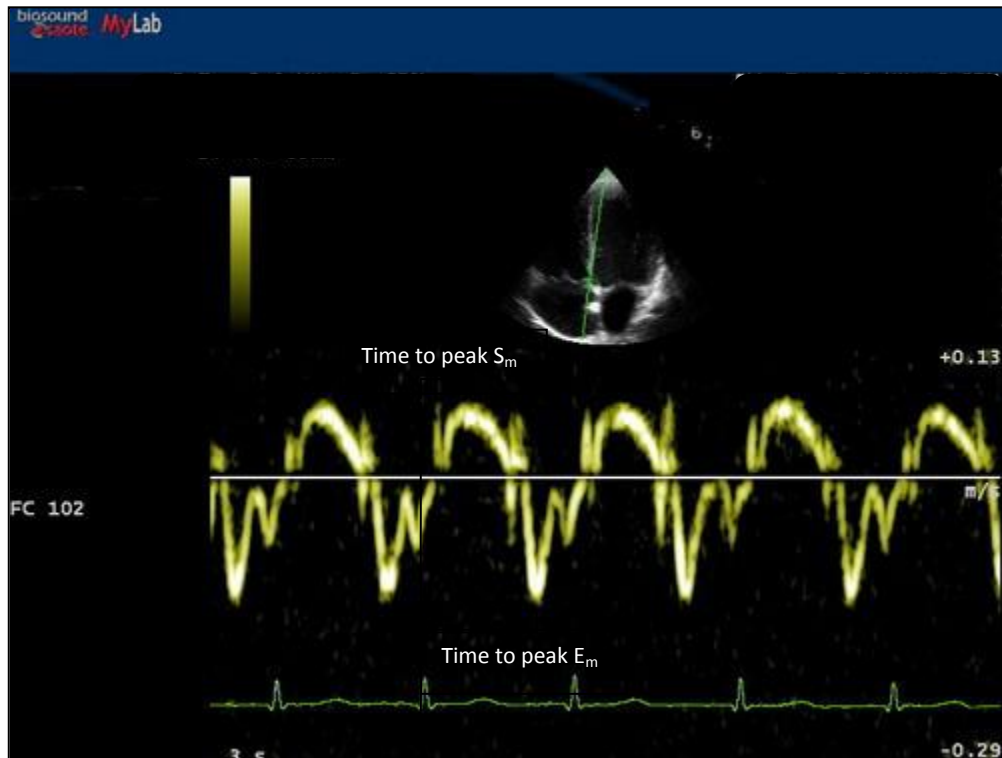
The present study had some limitations. Intra and inter-observer variability was slightly high for STE-dyssynchrony, but not dissimilar from other studies [5]. However, the accuracy of assessing LV dyssynchrony was increased by using several techniques (TDI, STE) and both maximum delay and SD. The percentage of poor quality cine-loops and images removal was similar to commonly reported values [5] and was related to the lack of echogenicity of this population. This limitation precluded accurate measurement of dyssynchrony in the more classical 12 to 18-segment model (e.g. 2 and 3-chamber views were poor). However, using 12 to 18-segments would have potentially contributed to increased, not decreased, maximum delay or SD. Accordingly, the existence of longitudinal dyssynchrony in MetS-NT2D and MetS-T2D cannot be contested, since its prevalence is more likely to have been underestimated using the 6-segment model. The cross-sectional design has limitations; however, proof of concept was important and achieved (with a possibility of future prospective observations of myocardial pathophysiology).

Supplemental Table S1. Significant correlates of systolic TDI and longitudinal STE-derived maximum delay dyssynchrony.

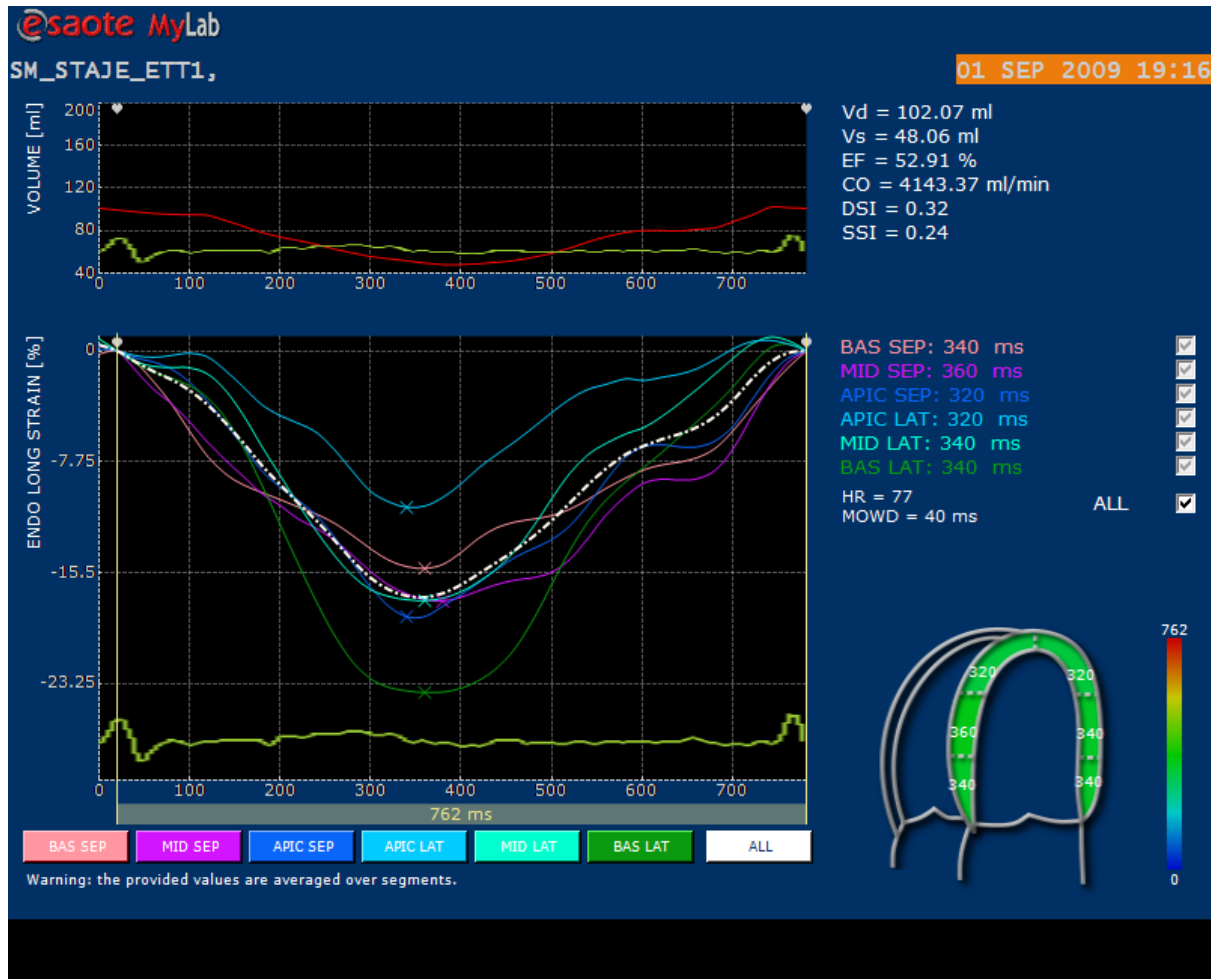
	Systolic TDI dyssynchrony		STE longitudinal dyssynchrony	
	Pearson's <i>r</i>	<i>P</i> -value	Pearson's <i>r</i>	<i>P</i> -value
LA diameter	0.35	P<0.001	0.23	P<0.05
LV mass index	0.30	P<0.001	0.37	P<0.001
Waist circumference	0.26	P<0.001	0.28	P<0.001
PAI-1 active	0.20	P<0.05	0.27	P<0.001
Systolic blood pressure	0.27	P<0.001	0.09	NS

TDI: Tissue Doppler imaging, STE: Speckle tracking echocardiography, LA: Left atrium, LV: Left ventricle, PAI-1: Plasminogen activator inhibitor-1.

Supplemental Figure S1. Example of measurement of time to peak systolic and diastolic myocardial velocity (TDI) at the septal annulus site, used in calculating LV-dyssynchrony. This was also calculated at the lateral, inferior and anterior annulus sites.

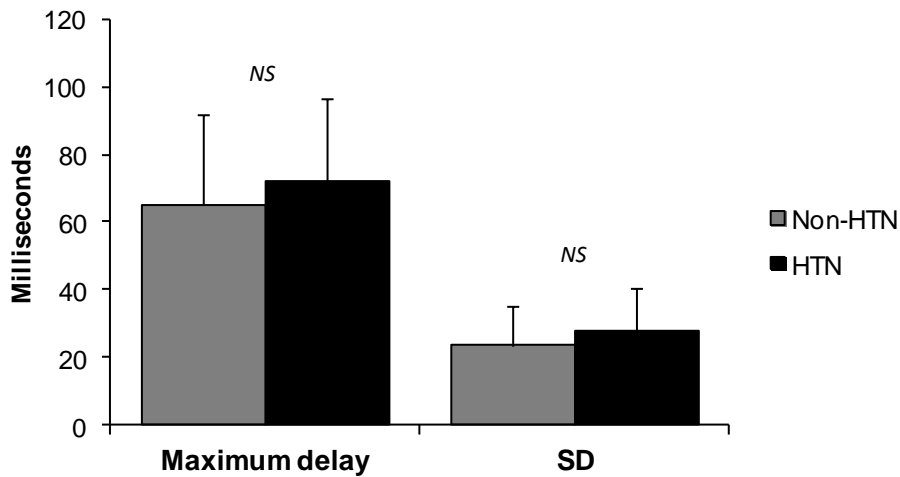


Supplemental Figure S2. Measurement of time to peak longitudinal strain derived from STE used in calculating LV-dyssynchrony. This was conducted in the basal-septal, mid-septal, apical-septal, apical-lateral, mid-lateral, and basal-lateral segments.

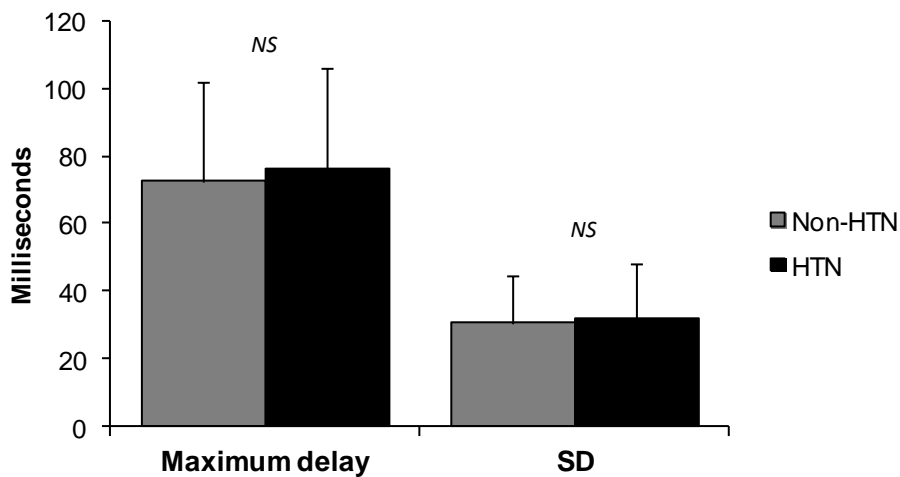


Supplemental Figure S3. Comparisons of STE LV-dyssynchrony in hypertensive and non-hypertensive individuals. HTN: Hypertension, STE: Speckle tracking echocardiography, & SD: Standard deviation.

STE longitudinal dyssynchrony



STE circumferential dyssynchrony



SUPPLEMENT SPECIFIC REFERENCE LIST

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4.3. STUDY 3

4.3.1. Preamble

The following is a presentation of the manuscript for Study 3 (the Aging Study) in the format that was submitted for publication, and is currently under review in the *Circulation: Cardiovascular Imaging Journal*. Briefly, this third study aimed to form a comprehensive profile of the healthy aging heart in males. It explored STE-derived myocardial function, dyssynchrony, epicardial fat, and routine biology across the lifespan, as well as the associations amongst these different parameters.

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Increased Myocardial Dysfunction and Dyssynchrony Across the Lifespan in Healthy Males

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ABSTRACT

Background: Evaluation of sensitive myocardial mechanics with speckle tracking echocardiography (STE) across the lifespan may reveal early indicators of cardiovascular disease (CVD) risk. Left ventricular (LV) myocardial dyssynchrony; a subclinical risk-factor of CVD, is of particular clinical interest in healthy aging people. However, the evolution of STE-derived LV-dyssynchrony across the lifespan, and its influence on myocardial dysfunction remains unclear.

Methods and Results: Forty-five healthy males aged 19-94 years were assigned to young (<40 years), middle (40-65 years), and older (>65) aged categories. Basic clinical and comprehensive echocardiographic cross-sectional profiles (conventional, tissue-Doppler imaging and STE) were established. LV-dyssynchrony was calculated from the maximum-delay of time-to-peak velocity/strain in the four LV-annulus sites (tissue-Doppler imaging), and six LV-segments (STE longitudinal and circumferential axes). An age-associated decrease in longitudinal strain, accompanied by increased circumferential apical strain and LV twist were confirmed. Moreover, independent of blood biology; significant stepwise increases were observed between age categories for epicardial fat (young: $0.25\text{mm}\pm 0.09$, middle: $0.39\text{mm}\pm 0.10$, older $0.57\text{mm}\pm 0.24$; $p<0.01$), longitudinal STE-dyssynchrony (young: $42\text{ms}\pm 7.7$, middle: $58.8\text{ms}\pm 18.9$, older $88.6\text{ms}\pm 18.2$; $p<0.05$), and circumferential-basal STE-dyssynchrony (young: $50.2\text{ms}\pm 20.5$, middle: $75.9\text{ms}\pm 20.6$, older $97.9\text{ms}\pm 20.2$; $p<0.05$). These variables collectively explained 37% and 31% ($p<0.01$) of longitudinal strain and LV twist, respectively.

Conclusions: We established a comprehensive profile of the aging-heart in healthy males. Of primary importance, we showed increased epicardial fat, and both longitudinal and circumferential LV-dyssynchrony across the lifespan. These three factors may be key underlying contributors to myocardial dysfunction. This advanced understanding of the healthy aging-heart may assist in the early identification of CVD.

KEY WORDS: Speckle-tracking echocardiography, myocardial dyssynchrony, aging

INTRODUCTION

Increased life expectancy and decreased birth rates contribute to global population aging,^{1, 2} with cardiovascular disease (CVD) remaining the primary contributor to mortality.³ A better understanding of the healthy aging-heart may support the early prevention of CVD. Evidence suggests that normal aging is associated with left ventricular (LV) hypertrophy and decreased diastolic LV function, assessed with conventional and tissue Doppler imaging (TDI) echocardiography.⁴⁻⁶ The use of speckle tracking echocardiography (STE) may permit more sensitive analyses of the aging-heart, through the angle-independent assessment of myocardial deformation (myocardial mechanics) in the longitudinal and circumferential axes, as well as twist mechanics.⁷ Previous studies of LV myocardial mechanics across the lifespan have found an age-related decrease in longitudinal deformation, as well as compensatory increases in LV twist,⁸⁻¹² while the evolution of circumferential deformation remains inconclusive. However, the underlying mechanisms behind these changes in healthy aging populations are poorly understood. 1) The accumulation of myocardial lipid content is a major underlying contributor to LV dysfunction in populations with metabolic disorders.¹³ Epicardial adipose tissue (EAT), measured by echocardiography, is consistently correlated with myocardial lipid content.¹⁴ However, the influence of EAT (and by extension; myocardial lipids) on LV myocardial mechanics in healthy aging people is unknown and warrants further attention. 2) Non-uniform contraction of LV myocardial walls due to declining electromechanical synchronicity (LV-dyssynchrony) may result in myocardial inefficiency,¹⁵ and could also be an underlying contributor to these age-related impairments in myocardial mechanics. Previous tagged-magnetic resonance imaging studies have found increasing circumferential,¹⁶ longitudinal and radial¹⁷ LV-dyssynchrony with age. Nonetheless, no studies have examined the association of both longitudinal and

circumferential LV-dyssynchrony with STE-derived indices of myocardial mechanics, such as LV longitudinal and circumferential strain as well as twist, in a healthy aging population. The aim of this cross-sectional study was therefore to establish a comprehensive profile of the healthy aging-heart using conventional, TDI and STE echocardiography. We also aimed to explore the associations amongst myocardial mechanics, LV dyssynchrony, and myocardial fat, independently of blood-biology, in this aging population.

METHODS

Population and design

The study was approved by the Human Research Ethics Committee of the Australian Catholic University, Melbourne, Australia. Volunteers were recruited through advertisements, and all participants provided informed consent. Inclusion criteria were males aged over 18 years with no maximum age limit, no cardiovascular disease, diabetes mellitus, dyslipidemia, or hypertension, no routine medication, and practising less than 10 hours per week of moderate to intense physical activity. We recruited males from three age categories: younger than 40 years (Y); 40 to 65 years (M); and older than 65 years (O).

Clinical and Biochemical parameters

Stature, body mass, and body mass index (BMI) were assessed. Blood pressure was measured with a digital sphygmomanometer (Carescape-V100, Dinamap, GE technology, USA) after 15 minutes lying in the supine position. Physical activity was quantified using the International Physical Activity Questionnaire modified for elderly populations.¹⁸

Fasted serum concentrations of glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoproteins (LDL), and high-sensitivity C-reactive protein (CRP) were measured at a commercial pathology laboratory.

Conventional echocardiography

Participants underwent transthoracic echocardiogram and electrocardiogram examination by the same experienced operator, following standard recommendations.¹⁹ Images and cine-loops were obtained using a GE equipment pack (Vivid-i, General Electric, Milwaukee, WI, USA) and a 3.5-MHz probe. Images were digitally recorded for subsequent offline analysis with dedicated software (EchoPAC PC, Version 5, GE Healthcare). Each variable was measured on an average of three consecutive cardiac cycles. M-Mode measurements were obtained in the parasternal long-axis view. Left atrial (LA) and LV dimensions were measured at end-diastole and end-systole. LV mass was calculated by the Devereux formula, and indexed for height,²⁰ and LV ejection fraction from the modified Quinones equation.²¹ Pulsed-Doppler LV transmitral blood velocity, including early (E) and atrial (A) waves, were measured in the apical four-chamber view. Isovolumic relaxation time (IVRT) was measured by pulsed Doppler in the apical five-chamber view, along with aortic ejection velocity. Pulsed-TDI measures of myocardial systolic (S_m), early diastolic (E_m), and late diastolic (A_m) velocities were assessed at the mitral annulus of the LV, in apical four (septal and lateral) and two (anterior and inferior) chamber views. The E/E_m ratio, recorded from the mitral annulus lateral wall, was used as an index of LV filling pressure. The tricuspid annular plane systolic excursion (TAPSE) and TDI S_m velocity were also assessed on the lateral (free) wall of the right ventricle. Epicardial adipose tissue thickness was measured on the free wall of the right ventricle from the parasternal long-axis view.²²

Speckle tracking echocardiography

The STE mode of two-dimensional strain imaging was used to measure more sensitive myocardial mechanics. Offline analyses were performed in the circumferential axis at the base, papillary-muscle level, and apex of the LV (parasternal short-axis), as well as in the longitudinal axis (apical four-chamber view), using software described above. In the respective B-mode two-dimensional grey-scale cine-loops, a region of interest was manually placed along the endocardial border, and automatically covered myocardial thickness to the epicardial border. Six segments were subsequently analysed in each view. Cine-loops without adequate endocardial border definition, frame rate (>60 Hz), and image tracking were excluded from analyses. The cine-loops were animated, and LV longitudinal strain, strain rate (SR), and time to peak strain/SR were obtained in the apical four-chamber view. Circumferential strain, SR, time to peak strain/SR, and rotation were obtained from the short-axis view at the LV base and apex. LV twist was calculated as the instantaneous difference between apical and basal rotation. To adjust all STE variables for inter-participant differences in heart rate, the time sequence was normalized to the percentage of systolic duration (i.e. 100% at aortic valve closure) using a toolbox generated in our laboratory (Scilab 4.1).²³ Inter and intra-observer reproducibility were estimated in three views from 10 randomly selected participants, and the coefficient of variations were 5.4% and 5.1%, respectively for strain, and 10.1% and 7.9%, respectively for twist.

Myocardial dyssynchrony

TDI LV-dyssynchrony was measured by recording time taken from the start of QRS complex to peak S_m (systolic dyssynchrony) or peak E_m (diastolic dyssynchrony) at the annulus of the septal and lateral walls (apical four-chamber view), and inferior and anterior walls (apical two-chamber view). For STE LV-dyssynchrony, time taken from the start of QRS to peak

strain in the six LV segments was measured in both longitudinal (apical 4-chamber) and circumferential (parasternal short-axis basal and apical levels) axes. LV-dyssynchrony was evaluated using the maximum delay technique in the four (TDI) or six (STE) segments. This modality of measuring LV-dyssynchrony is more sensitive than the Yu index or opposing wall delay.²⁴ Clinical diagnosis was made if maximum delay was greater than 65 ms²⁵ and 100 ms²⁶ for TDI and STE, respectively. For STE dyssynchrony, inter and intra-observer reproducibility (coefficient of variation) were 9.3% and 10.2%, respectively.

Statistical analyses

It was predicted that a minimum of 13 participants in each population sub-group (Young, Middle, and Older-aged) would allow detection of a significant difference if the mean of the major cardiac variables was between 1 and 1.25 standard deviations higher than the mean value for the control group (80% power; $p < 0.05$).²⁶ After verifying Gaussian distribution, clinical, biological, and echocardiographic data were presented as means \pm standard deviation. One-way analysis of variance (ANOVA) with Tukey's post-hoc tests were used to assess differences between Y, M, and O groups. In order to preclude outliers, we excluded data with values beyond plus or minus two standard deviations of the group mean. Pearson's r correlation analyses were performed between age, cardiac variables, and blood biology. Univariate analysis of co-variance (ANCOVA) was performed to test the strength of the relationships between LV-dyssynchrony and age after the effects of glucose, total cholesterol and CRP were controlled. A stepwise linear regression model assessed the predictive role of LV-dyssynchrony on major indices of myocardial mechanics (LV twist and longitudinal strain). Analyses were performed using SPSS Version 16.0 for windows (SPSS Inc), with statistical significance set at $p < 0.05$.

RESULTS

Participants

Participant recruitment is described in figure 1. Forty-five healthy males took part in the study. Fifteen participants per group were assessed using conventional, TDI, and longitudinal STE echocardiography. One (Y), two (M) and two (O) participants were excluded for circumferential STE analysis due to insufficient image quality.

Clinical and Biochemical parameters

Mean ages of participants were 25.7 ± 6.1 (Y), 49.3 ± 8.8 (M) and 77.7 ± 9.8 (O). Participant age ranged from 19 to 94 years. Age groups did not differ for body mass, BMI, systolic and diastolic blood pressure, high sensitivity CRP, triglycerides, HDL, LDL, total cholesterol, and glucose. Stature was lower in O compared with M and Y groups ($p < 0.05$) (Table 1).

Conventional echocardiographic and TDI parameters

Regarding *LV remodelling* (Table 2); only interventricular septum thickness and relative wall thickness (RWT) were greater in O than Y ($p < 0.05$), but not different from M. RWT was also greater in M than Y ($p < 0.05$).

For *functional LV parameters*; no group differences existed in systolic function (LVEF and S_m). However, diastolic function was impaired with age. E/A ratio was lower in O than both M and Y ($p < 0.001$), while no difference existed between M and Y. E_m was lower in both O and M compared with Y ($p < 0.001$), but no difference existed between O and M. Deceleration time of E and LV filling pressure (E/E_m) were greater in O than Y ($p < 0.05$), but not different

from M. Isovolumic relaxation time (IVRT) was prolonged in both O and M compared with Y ($p < 0.05$), but no difference existed between O and M.

For *RV analyses* (Table 2); no inter-group differences existed for tricuspid annular plane systolic excursion TAPSE. However, S_m was lower in both O and M compared with Y ($p < 0.05$), but no difference existed between O and M. EAT thickness was greater in O than both M and Y ($p < 0.001$), while M was also greater than Y ($p < 0.001$).

Speckle tracking echocardiographic parameters

For *longitudinal* strain and diastolic SR (Table 3), O was lower than M and Y, respectively ($p < 0.001$ and $p < 0.05$), while no differences were noticed between M and Y. Longitudinal systolic SR was not affected by age. Furthermore, while no differences existed between the groups for basal *circumferential* characteristics; apical circumferential strain as well as systolic SR were greater in O and M than Y ($p < 0.05$), but no difference existed between O and M. Apical circumferential diastolic SR was not affected by age (Table 3). Further analyses of circumferential mechanics showed no differences across the lifespan for basal rotation. However, apical rotation was greater in O and M than Y ($p < 0.05$). Subsequent LV twist and untwist rate was greater in O than Y ($p < 0.05$), but not different from M.

Myocardial dyssynchrony parameters

Measures of myocardial dyssynchrony across the lifespan are presented in Figure 2. Both *TDI-derived* systolic and diastolic maximum delay was greater in O than Y ($p < 0.05$). No differences between O and M, or M and Y existed. *STE-derived longitudinal* maximum delay was greater in O than both M and Y ($p < 0.001$), while also greater in M than Y ($p < 0.05$). Additionally, *STE-derived circumferential* maximum delay at the base was greater in O than

M and Y respectively ($p<0.05$), while also greater in M than Y ($p<0.05$). No inter-group differences existed for STE-derived circumferential maximum delay at the apex.

Relationships amongst cardiac variables, age, and blood biology

Age was significantly correlated with the following cardiac variables: longitudinal STE dyssynchrony ($r=0.79$), TDI E_m ($r=0.78$), circumferential-basal STE dyssynchrony ($r=0.77$), E/A ratio ($r=0.75$), EAT ($r=0.73$), LV twist ($r=0.68$), and longitudinal strain ($r=0.62$) (all $p<0.01$). Age was also significantly correlated with CRP ($r=0.40$) and triglycerides ($r=0.35$) (both $p<0.05$). Furthermore, some cardiac variables also correlated strongly with blood biology, including; apical circumferential rotation and TDI A_m with total cholesterol ($r=0.47$ and $r=0.46$, respectively), apical circumferential systolic SR with CRP ($r=0.42$), TDI E_m with fasting glucose ($r=0.42$), and EAT with triglycerides ($r=0.42$) and CRP ($r=0.40$) (all $p<0.05$). After controlling for total cholesterol, fasting glucose, and CRP; age was still significantly correlated with longitudinal STE LV-dyssynchrony (*adjusted* $r=0.72$; $p<0.001$).

Relationships between LV-dyssynchrony, EAT and indices of myocardial mechanics

Longitudinal LV-dyssynchrony was associated with longitudinal strain ($r=0.51$; $p<0.01$) while circumferential STE LV-dyssynchrony at the base was associated with LV twist ($r=0.52$ $p<0.05$). Circumferential STE LV-dyssynchrony at the apex was not associated with any indices of myocardial mechanics. EAT was also associated with longitudinal STE LV-dyssynchrony ($r=0.65$), TDI E_m ($r=0.60$), longitudinal strain ($r=0.50$), circumferential STE LV-dyssynchrony at the base ($r=0.44$), and LV twist ($r=0.44$) (all $p<0.01$). A stepwise linear regression model revealed that the combination of EAT, longitudinal STE LV-dyssynchrony, and circumferential STE LV-dyssynchrony at the base explained 37% of the variability in

longitudinal strain, and 31% of the variability in LV twist ($R^2=0.37$ and 0.32 , respectively; $p<0.05$).

DISCUSSION

We formed a comprehensive profile of the aging-heart, which enabled several major findings to emerge: First, we confirmed an age-associated decline in global diastolic function and longitudinal deformation, accompanied by compensatory increases in circumferential apical strain and LV twist. Additionally, for the first time with echocardiography, we showed increasing EAT, longitudinal and circumferential-basal LV-dyssynchrony across the lifespan, independent of the effects of blood biology. Moreover, these three factors emerged as significant correlates and predictors of key myocardial mechanics parameters (longitudinal strain and LV twist).

Rationale for elderly male population

Previous studies of LV myocardial function and dyssynchrony in aging people have not observed populations reaching older than 89 years of age.^{8, 9, 11, 12, 16, 17, 27} In the present study, our participants were aged up to 94 years. We demonstrated a continued increase in myocardial dysfunction and dyssynchrony into the late stages of the lifespan. Furthermore, prior studies of the aging-heart observed mixed gender populations. We present for the first time a study of males only. Evidence suggests myocardial tissue in females is more preserved than in males throughout aging.²⁸ We demonstrated that restriction to a male-only population minimised standard deviations and permitted detection of significant differences between groups, with fewer individuals.²⁷

Aging and conventional echocardiographic parameters

The present study confirms that healthy aging is associated with LV remodelling and declining global diastolic function, despite preserved LV ejection fraction.^{4-6, 29} The fundamental mechanisms behind these changes may be due to age-associated increases in arterial blood pressure and subsequently elevated afterload.³⁰ However, although E/E_m ratio (a measure of LV filling pressure) was elevated in older participants, both systolic and diastolic arterial blood pressures were similar across the lifespan. Analysis of cardiac function at the more regional (myocardial) level was therefore justified.

Aging and myocardial mechanics

We confirmed decreasing TDI-derived diastolic myocardial velocity with age.³¹⁻³⁴ On the other hand, we found preserved TDI systolic velocity across the lifespan, agreeing with some previous studies,^{31, 35, 36} but contradicting others that found an age-related decline.^{32, 37, 38} The exclusion of hypertensive individuals may explain this discrepancy since blood pressure strongly influences systolic function.³⁹ Furthermore, longitudinal deformation (strain and both systolic and diastolic SR) was impaired with advancing age in our healthy males, supporting previous studies.^{11, 40, 41} Most likely in response to the impaired longitudinal deformation, we observed compensatory increases in apical circumferential deformation and LV twist with age. This would help explain the observed preservation of LV ejection fraction, as also seen in others similar studies.^{13,14,36}

Aging and myocardial lipid accumulation

Another major novel finding from the present study was the age-associated increase in EAT. Echocardiography-derived EAT is consistently and strongly associated with myocardial lipid content.^{14, 42} Previous autopsy findings have demonstrated that increasing levels of

myocardial lipids is part of the normal aging process.⁴³ As lipid infiltration into the myocardium increases, myocardial function (such as longitudinal strain) is impaired.³³ In the present study, we found that rising levels of EAT was associated with declining systolic and diastolic indices of LV myocardial mechanics. While the exact mechanisms through which EAT impacts myocardial mechanics are still speculative, early findings suggest that lipid accumulation in the epicardium causes cardiomyocyte enlargement and thus decreased oxygen delivery. In turn, the lipid accumulation and hypoxia triggers pro-inflammatory and pro-atherogenic cytokines in the myocardium, cardiomyocyte excitation-contraction coupling abnormalities from altered calcium handling, and increased levels of oxidative stress from reactive oxygen species.^{13, 44, 45} Through a series of cellular pathways, these reactive oxygen species expedite interstitial and peri-vascular fibrosis in the aging heart,⁴⁶ further impairing myocardial mechanics.

Aging and LV-dyssynchrony

Elevated levels of fibrosis and lipid content in the remodelled aging-myocardium may also slow signal conduction,⁴⁶ thus leading to myocardial LV-dyssynchrony. In the present study, we observed increasing longitudinal and circumferential LV-dyssynchrony at the base across the lifespan independent of the effects of cholesterol, glucose, and CRP; while circumferential LV-dyssynchrony at the apex remained unchanged. Interestingly, we found age-related increases in apical circumferential strain and rotation, but no change in basal circumferential strain or rotation. This is in accord with previous observations of the aging-heart.¹⁰ Thus, the potential preservation of LV pump function may be predominantly governed by apical LV function; which resists dyssynchronous activity and increases deformation and rotation, leading to the aforementioned compensatory increase in LV twist.

Associations between LV dyssynchrony, myocardial fat, and myocardial mechanics

LV-dyssynchrony and EAT may thus be underlying factors responsible for altered myocardial mechanics across the lifespan. Only two MRI studies have observed increasing myocardial dyssynchrony in healthy aging people,^{16, 17} while no studies have previously examined EAT across the lifespan in healthy males. We found the combination of EAT, longitudinal, and circumferential LV-dyssynchrony at the base explained approximately one-third of the variability in longitudinal strain and LV twist, respectively. LV-dyssynchrony and EAT may therefore be valuable early indicators of LV dysfunction in healthy aging populations.

Clinical significance

The description of age-related dyssynchrony may permit clinicians to accept a certain ‘normal’ level of dyssynchrony in the healthy aging-heart. In the present study, half of the healthy elderly participants reached the cut-off values for both longitudinal and circumferential STE dyssynchrony,^{25, 26} despite our confirmation of preserved global systolic function across the lifespan.³⁶ Further research may be necessary to identify age-sensitive threshold values for myocardial dyssynchrony.

Study limitations

The cross-sectional design has limitations. However, a longitudinal follow-up across the lifespan seems unfeasible. While the number of participants in our study may appear limited, our sample size was estimated to reach significance between groups. The discarding of some poor quality cine-loops was inherent to echocardiography, with a standard percentage of rejection,²³ despite infrequent reporting in the literature.^{8, 27} A lack of echogenicity in our population precluded the capacity to confidently assess radial deformation. Intra and inter-

observer variability was slightly high but not dissimilar to other studies reporting dyssynchrony.⁴⁷ However, the accuracy of assessing LV dyssynchrony was increased by the use of several techniques (TDI, STE). Biological assessments were limited to the most clinically important parameters. Thus, understanding the aging-heart may warrant further more comprehensive investigations.

CONCLUSION

We established a comprehensive profile of the aging-heart: demonstrating an age-associated decline in diastolic function and longitudinal deformation, with a compensatory increase in apical circumferential deformation, rotation and LV twist. For the first time with echocardiography, we showed increasing EAT, longitudinal, and circumferential-basal LV-dyssynchrony across the lifespan, independent of the effects of blood biology. We found these variables together significantly explained the variability in longitudinal deformation and LV twist, possibly through pro-inflammatory and pro-fibrotic pathways in the myocardium. Understanding the healthy aging-heart and its typical evolution may assist in the early distinction and prevention of cardiovascular diseases in healthy aging people.

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DISCLOSURES

None

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Figure 1. Participant recruitment flow chart.

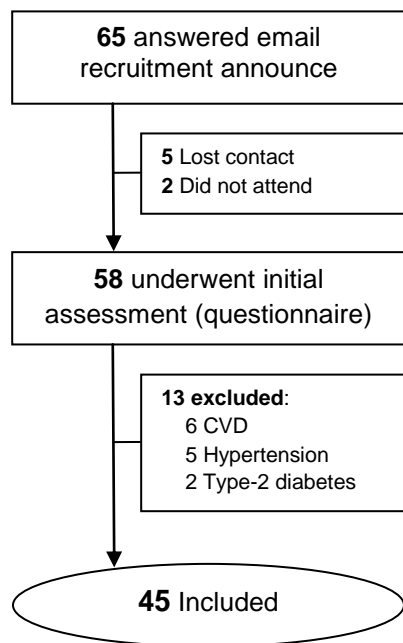


Figure 2. Data are means \pm standard deviations. Left ventricular dyssynchrony derived from STE or TDI across the lifespan in healthy males. STE: Speckle tracking echocardiography. TDI: Tissue Doppler imaging. L: Longitudinal. C: Circumferential (basal level). *: $p < 0.05$ vs. Young; ***: $p < 0.001$ vs. Young; #: $p < 0.05$ vs. Middle; ###: $p < 0.001$ vs. Middle.

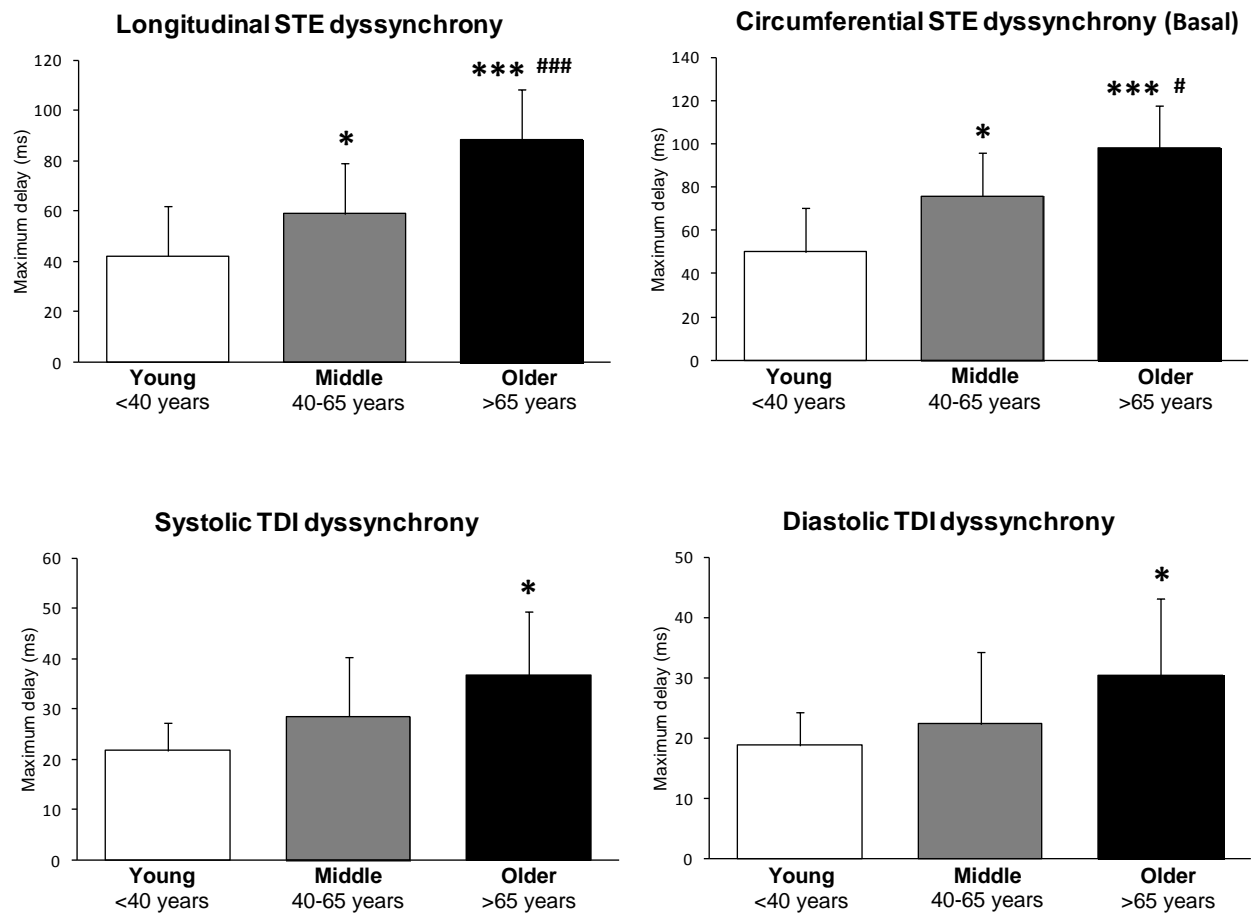


Table 1. Clinical and biological parameters of healthy males.

	Young	Middle	Older
	<40 years old	40-65 years old	>65 years old
<i>n</i>	15	15	15
Age (years)	25.7 ± 6.1	49.3 ± 8.8***	77.7 ± 9.8***###
Stature (cm)	180.5 ± 5.4	177.9 ± 4.9	172.6 ± 2.3*#
Body mass (kg)	80.2 ± 8.9	83.1 ± 11.7	76.7 ± 9.3
BMI (kg.m ⁻²)	24.6 ± 3.0	25.9 ± 3.5	25.7 ± 3.0
Systolic BP (mmHg)	124.5 ± 8.3	125.1 ± 9.8	122.4 ± 11.4
Diastolic BP (mmHg)	73.6 ± 8.5	79.6 ± 6.9	73.1 ± 9.0
Fasting glucose (mmol.L ⁻¹)	5.1 ± 0.3	5.3 ± 0.4	5.2 ± 1.0
HDL (mmol.L ⁻¹)	1.4 ± 0.2	1.5 ± 0.5	1.3 ± 0.2
LDL (mmol.L ⁻¹)	2.6 ± 1.1	3.0 ± 0.9	3.1 ± 0.9
Total cholesterol (mmol.L ⁻¹)	4.4 ± 1.2	4.9 ± 1.0	5.1 ± 1.0
Triglycerides (mmol.L ⁻¹)	0.8 ± 0.4	1.0 ± 0.6	1.3 ± 0.6
High sensitivity CRP (mg.L ⁻¹)	0.7 ± 0.4	1.0 ± 0.7	1.8 ± 1.7

Data are means ± standard deviations. BMI: Body mass index, BP: Blood pressure, HDL: High density lipoprotein, LDL: Low density lipoprotein, CRP: C-reactive protein. *: $p < 0.05$ vs. Young; ***: $p < 0.001$ vs. Young; #: $p < 0.05$ vs. Middle; ###: $p < 0.001$ vs. Middle.

Table 2. Conventional and tissue Doppler imaging echocardiography parameters of healthy males.

	Young <40 years old	Middle 40-65 years old	Older >65 years old
<i>n</i>	15	15	15
LV M-Mode			
HR (beats.min ⁻¹)	56.8 ± 8.1	59.1 ± 6.0	60.6 ± 14.1
LAD diameter (mm)	35.7 ± 4.6	35.6 ± 3.9	36.3 ± 5.5
LVED diameter (mm)	52.9 ± 3.7	50.0 ± 3.5	50.3 ± 3.5
LVES diameter (mm)	34.9 ± 4.0	32.1 ± 5.3	33.7 ± 5.6
IVSD diameter (mm)	10.5 ± 0.5	11.5 ± 1.4	11.8 ± 1.2*
PWD diameter (mm)	10.6 ± 0.9	11.3 ± 1.3	11.1 ± 1.1
RWT (mm)	0.40 ± 0.1	0.45 ± 0.1*	0.46 ± 0.1*
LV mass (g)	221.6 ± 36.3	216.5 ± 38.4	229.4 ± 42.5
LV mass index (g.m ^{-2.7})	44.9 ± 6.4	45.7 ± 7.7	52.9 ± 9.3*#
LV ejection fraction (%)	63.5 ± 5.2	64.2 ± 6.5	63.1 ± 6.9
LV Pulsed-Doppler			
E velocity (cm.s ⁻¹)	75.1 ± 13.1	67.5 ± 9.3	62.6 ± 14.7*
A velocity (cm.s ⁻¹)	40.4 ± 6.6	55.9 ± 11.7*	66.8 ± 25.8***
E/A	1.9 ± 0.5	1.3 ± 0.4***	1.0 ± 0.3***
E deceleration time (ms)	163.9 ± 22.4	168.1 ± 16.5	191.9 ± 38.8*
IVRT (ms)	94.3 ± 11.7	110.8 ± 16.4*	115.6 ± 25.0*
LV Pulsed-TDI			
E _m (cm.s ⁻¹)	14.2 ± 2.2	10.3 ± 2.8***	8.4 ± 2.2***
A _m (cm.s ⁻¹)	6.6 ± 0.8	8.0 ± 1.7	9.0 ± 2.4*
S _m (cm.s ⁻¹)	8.0 ± 0.7	7.4 ± 1.5	7.5 ± 1.3
E/E _m	4.9 ± 0.8	6.1 ± 1.7	7.4 ± 2.7*
Right ventricle			
EAT (mm)	0.25 ± 0.09	0.39 ± 0.10*	0.57 ± 0.24***#
RV S _m (cm.s ⁻¹)	13.6 ± 1.7	11.2 ± 1.5*	11.7 ± 2.2*
TAPSE (mm)	22.8 ± 1.8	20.6 ± 1.4	21.6 ± 3.4

Data are means ± standard deviations. LV: Left ventricular, HR: Heart rate, LAD: Left atrial diastolic, LVED: Left ventricular end diastolic, LVES: Left ventricular end systolic, IVSD: Interventricular septum diastolic, PWD: Posterior wall diastolic, RWT: Relative wall thickness, IVRT: Isovolumic relaxation time, EAT: Epicardial adipose tissue, RV: Right ventricle, TAPSE: Tricuspid annular plane systolic excursion. *: $p < 0.05$ vs. Young; ***: $p < 0.001$ vs. Young; #: $p < 0.05$ vs. Middle; ###: $p < 0.001$ vs. Middle.

Table 3. Left ventricular speckle tracking echocardiography parameters of healthy males.

	Young	Middle	Older
	<40 years old	40-65 years old	>65 years old
<i>n</i>	14	13	13
Longitudinal axis			
Strain (%)	20.6 ± 2.4	19.3 ± 2.9	15.7 ± 3.4***###
Diastolic SR (%.sec ⁻¹)	1.5 ± 0.3	1.3 ± 0.3	1.1 ± 0.2***#
Systolic SR (%.sec ⁻¹)	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.2
Base circumferential			
Strain (%)	16.5 ± 3.7	16.8 ± 5.9	15.8 ± 2.6
Diastolic SR (%.sec ⁻¹)	1.5 ± 0.3	1.6 ± 0.4	1.2 ± 0.3
Systolic SR (%.sec ⁻¹)	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.3
Apex circumferential			
Strain (%)	21.4 ± 2.7	26.0 ± 5.8*	26.8 ± 3.0*
Diastolic SR (%.sec ⁻¹)	1.8 ± 0.3	2.2 ± 0.9	2.0 ± 0.4
Systolic SR (%.sec ⁻¹)	1.2 ± 0.2	1.5 ± 0.3*	1.7 ± 0.3*
Rotational mechanics			
Apical rotation (°)	4.1 ± 2.1	6.4 ± 2.2*	6.1 ± 2.1*
Basal rotation (°)	4.6 ± 1.6	5.5 ± 2.5	6.4 ± 3.1
Peak twist (°)	6.6 ± 2.2	8.1 ± 3.6	11.6 ± 2.1*
Peak untwist-rate (°.sec ⁻¹)	-65.0 ± 19.5	-82.9 ± 16.0	-92.4 ± 22.1*

Data are means ± standard deviations. SR: Strain rate. *: $p < 0.05$ vs. Young; ***: $p < 0.001$ vs. Young; #: $p < 0.05$ vs. Middle; ###: $p < 0.001$ vs. Middle.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

5.1. REVIEW OF OBJECTIVES

To assist in the logical flow of the General discussion, the following is a brief résumé of the objectives for each Study:

5.1.1. Study 1

- 1) To characterize LV myocardial longitudinal, circumferential, and twist mechanics in adults with MetS compared with healthy matched controls, and elucidate the effects of multiple risk-factors on myocardial function using STE.
- 2) To explore associations between STE-based myocardial function indices and obesity-related clinical and biological parameters.

5.1.2. Study 2

- 1) To compare longitudinal and circumferential LV-dyssynchrony amongst MetS individuals with and without type-2 diabetes and healthy controls, using STE.
- 2) To explore associations between LV-dyssynchrony and other obesity-related clinical and biological parameters.

5.1.3. Study 3

- 1) To form a comprehensive profile of the aging-heart (systolic and diastolic velocities, deformation, twist, and dyssynchrony) in a single-sex aging-population, using TDI and STE echocardiography.
- 2) To explore the associations between myocardial mechanics, LV dyssynchrony, and epicardial adipose tissue, while controlling for the effects of blood biology (inflammation, glycemia, and dyslipidemia).

5.2. PRIMARY NOVEL FINDINGS

The following section now presents the primary novel findings from each of the three studies in this thesis.

5.2.1. Study 1

- The results extended earlier research via observed evidence of STE-derived myocardial dysfunction in a high-risk MetS population. STE permitted the detection of more subtle changes than conventional and TDI echocardiography alone (systolic impairments as well as diastolic).
- Impaired myocardial function was observed in the longitudinal axis, but not the circumferential axis or twist/untwist mechanics.
- The results also showed evidence of increasing longitudinal impairment as risk factors accumulated.
- Abdominal obesity, glucose intolerance, inflammation, and systolic intra LV-dyssynchrony explained a large proportion (48%) of the variability in longitudinal myocardial dysfunction.

5.2.2. Study 2

- TDI systolic and STE longitudinal dyssynchrony indices were already impaired in the non-diabetic MetS individuals compared with controls. No change in circumferential dyssynchrony was observed.
- Significant inter-relationships between cardiac morphology, abdominal-obesity, inflammatory markers, and LV dyssynchrony were observed, independent of diabetic status.

- Clinically diagnosed systolic TDI and longitudinal STE dyssynchrony were present even in MetS individuals with normal diastolic function. This highlights the clinical value of assessing LV-dyssynchrony, since it may be an earlier indicator of declining cardiac function than diastolic dysfunction.

5.2.3. Study 3

- The results confirmed existing studies reporting age-associated decline in diastolic function and longitudinal strain, accompanied by compensatory increases in twist/untwist mechanics and apical rotation/strain in healthy males.
- The results also extended these findings via observations of increasing epicardial adipose tissue, as well as increased myocardial dyssynchrony in the longitudinal (TDI and STE) and circumferential-basal (STE) axes across the lifespan in healthy males. Interestingly, circumferential-apical dyssynchrony did not increase with age, suggesting the compensatory preservation of LV function occurs primarily in the apex of the LV.
- The association between age and LV dyssynchrony occurred independent of markers of glycemic control, inflammation, and dyslipidemia.
- Longitudinal and circumferential LV-dyssynchrony together with epicardial adipose tissue explained approximately a third of the variability in LV twist and longitudinal strain. LV-dyssynchrony may be an important underlying mechanism in the pathophysiology of myocardial dysfunction.

5.3. LINKING OF THE STUDIES

The overall aim of this thesis was to examine myocardial mechanics using the sensitive STE technique in MetS and aging populations. The thesis was composed of two parts: Part 1, which incorporated two studies observing the MetS population, and part 2, which incorporated one study observing the healthy aging population. Despite the differing populations, there are several common observations between part 1 and part 2 regarding the evolution of myocardial mechanics. This information allows for improved understanding of the typical myocardial changes that occur throughout normal aging, and in response to metabolic pathology, thus unifying the theme of the present thesis.

5.3.1. Left ventricular remodelling

Hypertrophic remodelling of the LV (**Figure 19**) was evident in both the MetS and healthy aging populations, with increasing left ventricular mass, left ventricular mass index, and parietal wall thicknesses. These findings were in accord with the majority of the literature [2, 132]. While LV remodelling does not reflect myocardial mechanics per se, the morphological adaptation of the myocardium may underlie the mechanical changes seen in both MetS and aging individuals.

As discussed earlier, LV remodelling is likely due to several factors. First, as a muscular tissue, the myocardium is responsive to imposed stress, and thus adapts accordingly. Imposed stress to the myocardium is likely from the increased ventricular afterload, caused by deteriorating vascular compliance and increasing systolic pressure [129]. Second, cellular changes in the myocardium may also be responsible for this remodelling. In aging and MetS individuals, the accumulation of fibrosis in the myocardium is well documented [105, 133], with known contributions to the concentric remodelling of the LV. However, the mechanisms

through which fibrosis accumulates may differ slightly between MetS and aging populations. The normal process of aging is associated with a significant decrease in the number of myocardial cells, due to apoptosis [131]. During aging, these necrotic cells are replaced by fibrotic tissue, and the neighbouring cells enlarge to compensate. On the other hand, LV remodelling from metabolic dysfunction such as MetS and diabetes may be more related to abdominal obesity and pro-inflammation [105]. The obesity-related increase in inflammation and myocardial triglyceride content may impair myocardial cell function [194], and ultimately lead to fibrosis and LV remodelling. Interestingly, this accumulation of fat in the myocardium may also be relevant in healthy aging individuals. In Study 3, the novel finding of an age-related increase in epicardial fat was observed, as well as significant interrelationships between epicardial fat and indices of myocardial mechanics and dyssynchrony. Epicardial fat is considered a surrogate measure of myocardial triglyceride content [195], which is known to be a predictor of myocardial dysfunction via lipotoxicity of myocytes [136]. Thus, the remodelling of the LV may be central to the altered patterns of myocardial contractility and relaxation observed in both the MetS and aging populations.

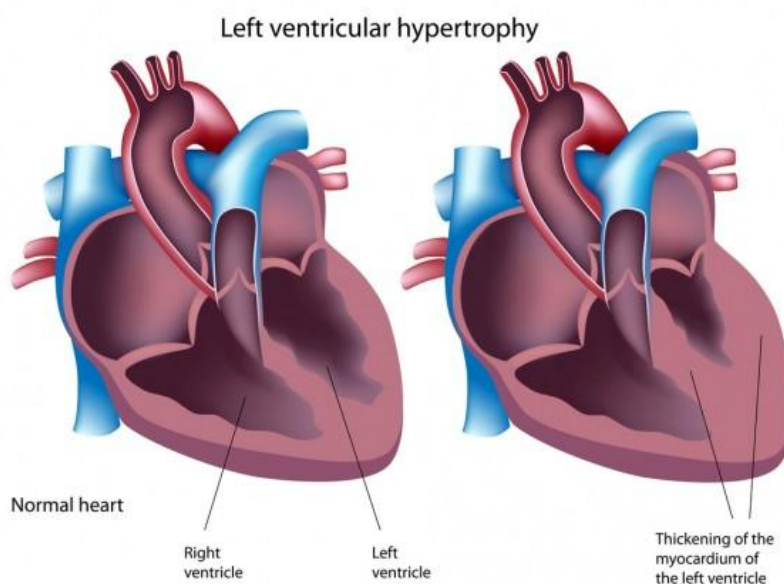


Figure 19. LV hypertrophic remodelling (http://en.wikipedia.org/wiki/Left_ventricular_hypertrophy).

5.3.2. Systolic and diastolic interaction

The evolution of myocardial contractility and relaxation across the lifespan was similar to that in MetS and diabetes pathophysiology. On a global scale, LV ejection fraction; indicative of systolic function, was preserved in healthy aging and MetS patients. Conversely, diastolic relaxation and filling capacity appeared more impaired with increasing age, and in MetS individuals with and without diabetes. Observations of shared diastolic dysfunction were in agreement with the majority of the literature [2, 132]. TDI analyses confirmed the pattern of preserved systolic function independent of impaired diastolic function both in healthy aging and MetS individuals.

On a regional scale however, more subtle changes in myocardial mechanics were detected using STE. Diastolic dysfunction, as measured by impaired longitudinal diastolic SR was confirmed in both populations. Moreover, systolic dysfunction, as measured by impaired strain and SR in the longitudinal axis was found in MetS individuals and with advancing age. This finding reflects the limitation of conventional and TDI echocardiography in detecting subtle changes in longitudinal systolic function, evident in both a MetS (independent of diabetic status) and healthy aging population, asymptomatic of CVD. The capacity to detect subtle changes may reflect an invaluable medium for early detection of CVD in using widely available, non-invasive measures. An additional advantage of STE is its ability to also measure circumferential LV function. Since LV contraction is a result of both longitudinal and circumferential mechanics, due to the helical arrangement of cardiomyocytes, an understanding of myocardial mechanics is incomplete without the evaluation of both axes.

5.3.3. Interaction between longitudinal and circumferential axes

The results strengthen the evidence base in existing literature that preservation and even compensatory increases observed in circumferential function typically accompany declining longitudinal function. Despite the detection of impaired longitudinal strain and both systolic and diastolic SR in MetS and aging individuals, circumferential mechanics did not deteriorate in either population. More specifically, in the MetS individuals, both basal and apical circumferential strain, SR, rotation, and resultant LV twist remained unchanged, while LV untwist rate was prolonged. In the healthy aging population, basal strain, SR, and rotation remained unchanged with advancing age. Conversely, apical strain, SR, and rotation increased significantly with age. Subsequently, LV twist and untwist also increased with age, in accordance with previous findings [166, 172]. These myocardial deformation findings corresponded with increased STE-derived longitudinal LV-dyssynchrony in MetS individuals, and increased longitudinal and circumferential-basal (but not apical) STE-derived LV-dyssynchrony in healthy older males.

Despite the inherent difference between MetS and healthy aging individuals in circumferential mechanics (with the exception of LV untwist), both populations shared characteristic adaptations in the longitudinal axis (Table 15). Decreased longitudinal strain and increased longitudinal LV-dyssynchrony was present in both MetS and healthy aging individuals, indicating a likely impairment of sub-endocardial function. While this impairment was potentially offset by the increased circumferential function in aging individuals, the lack of change in circumferential function in the MetS individuals may reflect a less advanced stage of myocardial pathology. Nonetheless, global systolic function was preserved in both populations, as discussed above, even in the very elderly group.

A more mechanistic explanation of this finding may lie in the myofiber spatial-organization within the LV, as discussed in chapter one. Briefly, sub-endocardial fibers running parallel to the LV long-axis and governing longitudinal function are more vulnerable to ischemia, fibrosis, and conduction delay (from the LV remodelling discussed above) due to their

inferior location [196]. This leads to earlier impairment of longitudinal function. Meanwhile, mid to superior myocardial/epicardial fibres, (more obliquely orientated and thus predominantly governing circumferential mechanics), may be preserved or even increased. MetS and aging populations may share the underlying mechanisms potentially responsible for declining sub-endocardial function, with aging reflecting a more progressed stage of these interactions.

Table 15. Shared and contrasting myocardial adaptations in MetS and Aging populations.

	MetS	Aging
Conventional and TDI echocardiography		
LV Mass	↑	↑
TDI E_m	↓	↓
TDI S_m	No change	No change
TDI dyssynchrony (E_m)	No change	↑
TDI dyssynchrony (S_m)	↑	↑
Speckle tracking echocardiography		
L strain	↓	↓
C-apical strain	No change	↑
C-apical rotation	No change	↑
C-basal strain	No change	No change
C-basal rotation	No change	No change
LV twist	No change	↑
LV untwist		↑
L dyssynchrony	↑	↑
C-apical dyssynchrony	No change	No change
C-basal dyssynchrony	No change	↑
EAT	NA	↑

LV: Left ventricular, TDI: Tissue Doppler imaging, L: Longitudinal, C: Circumferential, EAT: Epicardial adipose tissue.

5.3.4. Underlying factors of deteriorating longitudinal function

In both the MetS and aging populations, predictive associations were found between indices of longitudinal myocardial function and inflammatory blood biology markers such as CRP. The source of these pro-inflammatory markers may be the dysfunctional adipocytes in the MetS population [47], but the association is less clear in the healthy aging population, with limited but equivocal links between aging and rising systemic inflammation [146].

Additionally, systolic LV-dyssynchrony may also contribute to observed variability in myocardial mechanics across the lifespan and in MetS individuals. Results from Studies 1 and 2 support this, revealing TDI-derived systolic LV-dyssynchrony as an independent predictor of longitudinal strain. In Study 3 of the present thesis, STE-derived longitudinal and circumferential LV-dyssynchrony, combined with epicardial adipose tissue, explained approximately one-third of the variability in longitudinal strain and LV twist, independent of the effects of blood biology. These findings highlight at least a partial role of key factors influencing myocardial mechanics in a characteristic way, via impairment of sub-endocardial function.

The mechanisms of this impairment are thus far speculative, but several hypotheses previously discussed suggest impaired sub-endocardial function may occur via the deleterious effects of pro-inflammatory adipokines and epicardial/myocardial fat on related systemic and cellular function. More specifically: 1) macro and micro-circulatory structure and function, leading to reduced coronary myocardial blood flow and sub-endocardial hypoperfusion, 2) cell apoptosis and subsequent accretion of myocardial tissue fibrosis, 3) calcium-handling, leading to excitation-contraction coupling abnormalities, and 4) myocardial lipotoxicity [43, 48, 133, 136, 197]. Thus, a logical sequence emerges, linking inflammation, myocardial fibrosis, myocardial/epicardial fat, LV-dyssynchrony, and myocardial dysfunction in both MetS and aging populations.

Lastly, in both populations, a significant proportion (approximately 20%) of MetS individuals with normal LV diastolic function still manifested clinical LV-dyssynchrony. LV-dyssynchrony

may thus be a more sensitive and clinically valuable indicator of LV dysfunction than the more classic global diastolic parameters. Further studies are needed to elucidate the evolution of these sensitive indicators alongside other indices of myocardial mechanics, to better prevent the progression of CVD in high-risk but hitherto asymptomatic populations.

5.4. FUTURE DIRECTIONS

5.4.1. Prospective follow-up studies and exercise

The three studies provided important cross-sectional analyses, but would be strengthened with future investigations allowing prospective follow-up of the evolution of myocardial mechanics. For part 1 (Studies 1 and 2), this was essentially addressed with the RESOLVE trial over a period of 12 months. Complete echocardiographic and biological profiles were observed in the MetS participants following three-weeks, three-months, six-months, and 12-months of lifestyle intervention. Results from these observations are currently being analysed, and may present interesting findings on the potential reversal or delay of adverse MetS-related changes in myocardial mechanics following an intervention.

Previous studies have not reached consensus on the ability for lifestyle interventions such as exercise to reverse or attenuate myocardial dysfunction [198-201], and may warrant further investigation with STE. Additionally, although ethically and practically challenging; monitoring sensitive indices of myocardial mechanics over time in patients with untreated (worsening) metabolic disorders may also contribute to an improved understanding of characteristic patterns. For part 2, longitudinal assessment of myocardial mechanics in healthy individuals over a long-term period of time (e.g. 10-20 years) would permit more accurate assessment of the aging myocardium. However, the feasibility of conducting such studies is poor, as it would require rigorous long-term design, and a very large sample size to permit the exclusion of individuals who develop confounding factors, such as hypertension, throughout the observation period. Retrospective databases may be better available from large cohort studies. Nonetheless, only one very large-scale ongoing study (Framingham study) has thus far explored CVD risk-factors in healthy individuals across time [202]. A cross-sectional sample from this study was recently assessed with STE, and strong age and gender

influences were found on myocardial mechanics and dyssynchrony indices. This study helped identify reference limits for myocardial strain and dyssynchrony in healthy individuals. Similarly designed studies observing specific STE-derived indices of myocardial mechanics in the same sample across time would be highly informative, and may identify additional age-sensitive values for indices of myocardial parameters.

Lastly, using exercise as a method to amplify signs of LV myocardial dysfunction may also be useful in MetS or aging populations. Previous findings have revealed the magnification of LV dysfunction under exercise-stress conditions in aging individuals [203]. Observing responses of sensitive myocardial mechanics to exercise-imposed stress conditions may advance the understanding of early indicators of LV dysfunction, and improve the characterisation of myocardial mechanics in populations with subclinical risk of CVD.

5.4.2. Age sensitive thresholds

The need for age-sensitive normative values in some indices of myocardial mechanics is warranted, to better distinguish healthy normal aging from pathological myocardial changes unrelated to age. While several studies have reported reference limits for some parameters, for example; TDI-derived myocardial velocities [204] and some STE-derived SD indices [202], a need still exists to develop this database further. In Study 3, approximately 50% of the healthy elderly participants reached the cut-off values (>100 ms maximum delay) for both longitudinal and circumferential STE dyssynchrony, based on common criteria [22, 205]. This was despite our confirmation of preserved global systolic function in these individuals. Further research in healthy aging individuals may therefore be necessary to address this clinical limitation, since these existing cut-off values were derived from younger cohorts.

5.4.3. Exploring underlying mechanisms

An understanding of the underlying mechanisms influencing sub-endocardial function is needed, to allow even earlier detection of CVD pathophysiology. Growing evidence suggests fibrosis and myocardial triglyceride content are at the core of cellular changes in the sub-endocardium in aging and early MetS populations [105, 133, 136, 158, 194, 197]. The postulation is that fibrosis and myocardial triglyceride content in-turn affect myocardial mechanics. While several studies have found associations between fibrosis/myocardial triglycerides and declining cardiac function in high-risk asymptomatic populations [105, 136, 206], the literature remains equivocal, and warrants further investigation using accessible and sensitive techniques. Additionally, it would also be of interest to identify potential associations between myocardial fibrosis and myocardial triglyceride content or epicardial fat, to see whether these cellular changes behave in unison.

A better understanding of these underlying components would enable earlier identification of risk-factors contributing to myocardial dysfunction in healthy aging populations, or individuals with metabolic disorders, before clinical manifestation of CVD symptoms. Additionally, elucidating the impact of biological parameters such as hyperglycemia and pro-inflammatory markers on myocardial fibrosis and triglyceride accumulation would permit more targeted intervention, either by pharmacological or lifestyle means. A multi-systemic approach to detection and synergies appears to be the most logical direction for future research.

5.4.4. Right ventricular mechanics

While the majority of the literature focuses on myocardial mechanics in the LV, there is emerging interest in the evaluation of right ventricular (RV) mechanics using STE [207]. In clinical settings, longitudinal tissue shortening is commonly assessed with the M-mode

measure TAPSE (tricuspid annular plane systolic excursion). Longitudinal and circumferential strain measurements of the RV may enable more sensitive and angle-independent measurement of tissue deformation. Early evidence has shown the value of RV deformation imaging in risk-stratification and monitoring of CVD-risk populations [208-212]. However, further studies of RV mechanics in healthy aging or MetS individuals may advance the understanding of myocardial evolution in these populations, and allow identification of population-specific references.

5.4.5. Summary

The emerging theme from this thesis is therefore the value of early detection and the role of ultrasonography in the understanding of trends in myocardial mechanics, in populations at high risk, but asymptomatic of CVD. The three studies presented in this thesis have collectively profiled the characteristic evolution of myocardial function and structure under different conditions (metabolic dysfunction or aging). Common patterns of the evolving myocardium among the two populations advance the understanding of the progression of CVD, and thus allow more effective prevention/intervention.

CHAPTER 6

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CHAPTER 7

APPENDICES

7.1. APPENDIX A: RESEARCH OUTPUT

-July 2011: Poster presentation at the Avignon University Doctorate Day Seminar (Study 1)

-May 2012: Poster presentation at the European Congress of Obesity (Study 2)

-May 2013: Manuscript accepted for publication in Obesity Journal (Study 1- see *Appendix B*)

-October 2013: Manuscript accepted for publication in Canadian Journal of Cardiology (Study 2- see *Appendix C*)

-November 2013: Manuscript currently under review in Circulation: Cardiovascular Imaging (Study 3)

7.2. APPENDIX B: PROOF OF MANUSCRIPT ACCEPTANCE (STUDY 1)

Paul.Poirier@criucpq.ulaval.ca [via](mailto:Paul.Poirier@criucpq.ulaval.ca) manuscriptcentral.com



25-May-2013

Dear Mr. Crendal:

We are pleased to accept your manuscript entitled "MYOCARDIAL DEFORMATION AND TWIST MECHANICS IN ADULTS WITH METABOLIC SYNDROME: IMPACT OF CUMULATIVE METABOLIC BURDEN" in its current form for publication in Obesity.

We now have a new publisher (Wiley) who will be communicating with you regarding required forms, page proofs, and invoicing. Please note you will incur page charges for your manuscript. You will incur additional charges if you are printing color figures, have supplemental files or opt for Open Access publication.

*** NOTE ***

Please complete and send the attached Copyright Transfer Agreement (CTA) to Wiley. You can either fax it or email it. Wiley cannot process your accepted article without this form. Send the CTA to:

Wiley Obesity Production Manager
Fax: [717 738-9478](tel:7177389478) or
Fax: [717 738-9479](tel:7177389479) or
Email: jrnprod.OBY@cenvio.com

Once we've sent your article to Wiley and you've submitted the CTA form to Wiley, your article will soon be available online via Accepted Articles at the Obesity website on Wiley Online Library at: [http://onlinelibrary.wiley.com/journal/10.1002/\(ISSN\)1930-739X/accepted](http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1930-739X/accepted)

Enjoy a discount on page charges if the First or Corresponding author is a member of The Obesity Society. The rate is \$65/page for Society Members, and \$95/page for Non-Members. Check out the Society website at <http://www.obesity.org> to see all the benefits of membership.

Thank you for your fine contribution. On behalf of the Editors of Obesity, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Paul Poirier
Associate Editor, Obesity
Paul.Poirier@criucpq.ulaval.ca, Paul.Poirier@criucpq.ulaval.ca

7.3. APPENDIX C: PROOF OF MANUSCRIPT ACCEPTANCE (STUDY 2)

Stanley Nattel <stanley.nattel@icm-mhi.org>



30th October, 2013

Ms. Ref. No.: CJC-D-13-00965R2

Title: Left ventricular myocardial dyssynchrony is already present in non-diabetic patients with metabolic syndrome
Canadian Journal of Cardiology

Dear Mr. Edward Crendal,

I am pleased to inform you that your paper "Left ventricular myocardial dyssynchrony is already present in non-diabetic patients with metabolic syndrome" has been accepted for publication in Canadian Journal of Cardiology.

We would like to publish this in print in the March 2014 Focus Issue on Heart Failure (electronic publication will most likely be in 2013)- please let us know whether this is acceptable to you.

You should be receiving proofs from our publisher, Elsevier, within the next 4-8 weeks.

Thank you for submitting your lovely work to Canadian Journal of Cardiology.

Yours sincerely,

Stanley Nattel, MD
Editor-in-Chief
Canadian Journal of Cardiology

7.4. APPENDIX D: RECRUITMENT & PROTOCOL FOR RESOLVE TRIAL

Effect of Physical Activity and Diet on the Treatment of Metabolic Syndrome

Verified January 2011 by University Hospital, Clermont-Ferrand

First Received on June 9, 2009. Last Updated on January 18, 2011

Sponsor:	University Hospital, Clermont-Ferrand
Collaborator:	Fondation Coeur et Artères
Information provided by:	University Hospital, Clermont-Ferrand
ClinicalTrials.gov Identifier:	NCT00917917

► Purpose

Metabolic syndrome has been defined as a group of associated risk factors for cardio-vascular diseases and diabetes. It is usually treated with an association of restrictive diet, physical exercise and drugs. Nevertheless the type of exercise associated to reduction in cardio-vascular risks is not yet fully defined. Long term effects of such hygienic-diet programs are of great importance since it is well-known that compliance to such treatment are of short duration, namely when subjects return in routine life. Metabolic syndrome volunteer subjects (n=90), aged 50 to 70 yrs will be randomly assigned to 3 groups of investigation. One group will perform mostly resistance activity, a second mainly endurance activity and the third one will be composed of subjects not exercising a lot. All subjects will have the same restrictive diet (500-700 kcal/d) After the initial training (3 weeks), they will return home with

diet and physical program advises (personal compliance). They will be followed for one year (at 3, 6 and 12 months). Such a design may allow to find out the type of activity and power that are the best to reduce metabolic syndrome parameters and cardio-vascular risk factors.

The primary outcome variable is the reduction in abdominal circumference, which is the main criterion of MS.

<u>Condition</u>	<u>Intervention</u>
Metabolic Syndrome	Behavioral: Type of physical activity (resistance, endurance) Behavioral: Restrictive diet

Study Type: Interventional

Study Design: Allocation: Randomized
Endpoint Classification: Efficacy Study
Intervention Model: Parallel Assignment
Masking: Open Label
Primary Purpose: Prevention

Official Title: Role of Combined Intervention of Physical Activity and Nutrition in Metabolic Syndrome Treatment on Cardio-vascular Risk and Muscular- Skeletal Functions in Human Subject. Analysis of Patient's Compliance in Patient's Follow-up.

Resource links provided by NLM:

[MedlinePlus](#) related topics: [Exercise and Physical Fitness](#) [Metabolic Syndrome](#)

[U.S. FDA Resources](#)

Further study details as provided by University Hospital, Clermont-Ferrand:

Primary Outcome Measures:

- Decreased abdominal fat mass, measured after one year. Such measured will be done repetitively at Day 0, 21 and at 3, 6 and 12 months using abdominal circumference and DXA Decreased cardio-vascular risks [Time Frame: at Day 0, 21 and at 3, 6 and 12 months using abdominal circumference and DXA] [Designated as safety issue: Yes]

Secondary Outcome Measures:

- All the following measures will be done at day 0, 21 and at month 3, 6 and 12. Level of physical activities of each types Physical performances, Food intake and equilibrium Other Metabolic Syndrome inc [Time Frame: at day 0, 21 and at month 3, 6 and 12.] [Designated as safety issue: Yes]

Estimated Enrolment: 120

Study Start Date: May 2009

Estimated Study Completion Date: October 2012

Estimated Primary Completion Date: October 2011 (Final data collection date for primary outcome measure)

Intervention Details:

Behavioral: Type of physical activity (resistance, endurance)

To determine which type of physical activity is the best to reduce metabolic syndrome parameters especially abdominal circumference.

Group 1 will perform mostly resistance activities, group 2 mostly endurance activities, and Group 3 performing both activities at low level, serves as a control group for physical activities.

Behavioral: Restrictive diet

All subjects will have the same restrictive diet (500- 700 kcal/d).

Detailed Description:

The protocol is designed to determine which type of physical activity is the best to reduce metabolic syndrome parameters especially abdominal circumference.

90 Metabolic syndrome (MS) Patients will be recruited by advertising and checked for MS criteria. They will have a VO₂max test in order to be sure they can perform physical activities safely. After being checked, patients will have an eight day period to think about participation and to ask questions before signing consent. They have to feel 3 questionnaires, one about regular physical activities, one about food intakes

and a psychology one to measure reluctance to the program. After written informed consent obtained, patients will be randomly assigned to one of 3 groups of physical activity Group 1 will perform mostly resistance activities, group 2 mostly endurance activities, and group 3 performing both activities at low level, serves as a control group for physical activities and also to determine the importance of food reduction and food equilibrium in the treatment.

All subjects will have the same restrictive diet (500- 700 kcal/d). They will be followed for one year (at 3, 6 and 12 month), continuing at home the same program (diet and exercise training).

30 healthy subjects will be recruited for cross-sectional comparison (They will not follow any intervention, but will have the same investigation, only once).

Measured parameters Before and after the 3-week program and at 3, 6 and 12 months, the following measurements will be made Level of physical activities quantifying heart rate in each activity during the training 3 weeks and estimated thereafter on the same parameter.

Physical capacities With the 6 minute walking test, Food intake and equilibrium measured by full week records before training, during training and monthly thereafter. They will be quantified by a trained dietician using the reference French Cidal tables.

Metabolic syndrome factors Body composition including weight, height, abdominal circumference, total and torcular lean and total and abdominal fat mass measured by DXA Cardiac diastolic and systolic functions by means of standard, Tissue Doppler imaging and 2D-strain echocardiogram Vascular structure function in conduit and resistance arteries and microvascular reactivity Biological parameters with glycemic control : insulinemia and glazed haemoglobin Inflammatory syndrome and related cytokines: CRP, 1GPA, IL-6, TNF-, Il-12 and IL-10 blood protein: Albumine and transthyretin Appetite hormone: Leptine, adiponectin, Gremlin and CCK osteocalcin, BASP and CTx

Statical analysis Subject numbers were calculated from the results of a pilot. A statistical significance ($p=0,05$) may be reached with 22 subjects in group 1 and 2 for a difference of 0,6 kg of abdominal fat mass.

For cross-sectional comparison, healthy and MS subjects will be compared by unpaired Student test. The 3 groups of patients will be compared using a repeated measure ANOVA. If positive, a post-hoc test for mean comparison will be performed.

A correlation matrix will analyse relationships between studied parameters. A principal component analysis will allow to determine the reciprocal weight of positive explicating factors.

The study is done applying French and international regulations

► Eligibility

Ages Eligible for Study: 50 Years to 70 Years
Genders Eligible for Study: Both
Accepts Healthy Volunteers: Yes

Criteria

Inclusion Criteria:

- 50-70 years old from both sexes
- with metabolic syndrome
- affiliated to a social security system
- able to practice maximal physical exercises based on VO2 max
- able to sign inform consent

Exclusion Criteria:

- recent (6 month) major health conditions and patients with recurrent health problems (1 per year)
- patients who are not capable to perform VO2 max without abnormalities
- dyserection treated patients
- patients with insufficient comprehensive ability to feel questionnaire and/or to change habits

► Contacts and Locations

Please refer to this study by its ClinicalTrials.gov identifier: NCT00917917

Contacts

Contact: Patrick Lacarin 04.73.75.11.95 placarin@chu-clermontferrand.fr

Locations

France

CHU Clermont-Ferrand
Clermont-Ferrand, France, 63003
Contact: Patrick Lacarin 04.73.75.11.95 placarin@chu-clermontferrand.fr **Recruiting**

Sponsors and Collaborators

University Hospital, Clermont-Ferrand
Fondation Coeur et Artères

Investigators

Principal Investigator: Bruno Lesourd, MD University Hospital, Clermont-Ferrand

► More Information

Responsible Party: Patrick Lacarin, CHU Clermont-Ferrand
ClinicalTrials.gov Identifier: [NCT00917917](https://clinicaltrials.gov/ct2/show/study/NCT00917917)
Other Study ID Numbers: CHU-0053
Study First Received: June 9, 2009
Last Updated: January 18, 2011
Health Authority: France: Ministry of Health

Keywords provided by University Hospital, Clermont-Ferrand:

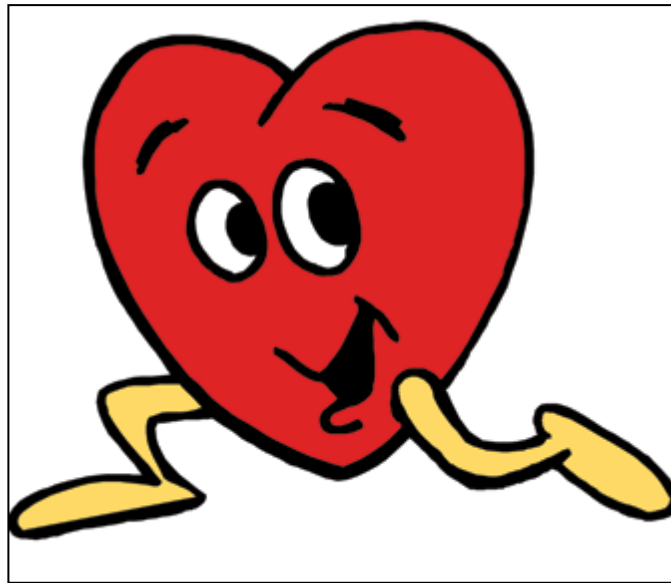
Metabolic syndrome
exercise training
abdominal fat mass
endothelial function
diastolic function

Additional relevant MeSH terms:

Metabolic Syndrome X
Insulin Resistance
Hyperinsulinism
Glucose Metabolism Disorders
Metabolic Diseases

ClinicalTrials.gov processed this record on August 01, 2012

RESEARCH PARTICIPANTS NEEDED FOR UNIVERSITY HEART STUDY!



Are you a male aged over 65 years of age?

Are you free from heart disease, diabetes and hypertension,
with a waist circumference that is less than 102cm?

**Are you interested in knowing more about
what your heart looks like and how it works?**

**Please register your interest with
Frances Russell**

Memo



Subject:

Invitation to participate in Exercise Science PhD project

Message:

Dear Residents,

You are warmly invited to participate in an Exercise Science PhD project, which centres on the effect of ageing on healthy hearts.

To participate, you need to be a male aged between 18 and 85 years old, independently-mobile, non-smoking, free from high blood pressure, diabetes, and with a waist circumference that is less than 102 cm.

We'd also prefer you not to be an ultra-endurance athlete (e.g. a marathon runner, triathlete, elite long-distance cyclist) participating in more than 7 hours per week of high-intensity exercise!

If you fit this category, then you would be most welcome in our research! You will need to attend just **one** 60 minute testing session (at a time convenient for you), where we will perform a few basic body-size measures (such as weight and height), two short questionnaires (about 5-10 minutes each), a finger prick and 5ml intravenous blood sample (for a duration of approximately 10 minutes), and a reasonably short (40 minutes) echocardiography (heart ultrasound) analysis.

Testing will take place in the Therapy & Lifestyle Centre at the RSL LifeCare ANZAC Village, Narrabeen, and you will be given an individualised report of your results when the project is finalised.

It's your chance to help us better understand how cardiovascular profiles change with age in healthy males!

If you are interested in participating or need more information, please email or call Eddy Crendal (PhD student) on edwardcrendal@gmail.com or **0414 449 284** or call **Frances Russell on 9739 2283**.

Hope to hear from you soon!

Regards,

Edward Crendal

7.6. APPENDIX F: ETHICAL APPROVAL FOR RESOLVE TRIAL

COMITE DE PROTECTION DES PERSONNES SUD-EST I

Président : M. Philippe RUSCH - Vice-Présidente : Mme le Dr Pascale VASSAL - Secrétaire : Mme le Dr Hélène BIEDERMANN

Date du comité	11/05/2009	Référence	JV-TV/2009-125
Projet relatif à	Recherche biomédicale		
Réception datée du	07/05/2009		
Demande d'avis concernant un	Projet initial		
Dans le cadre d'une	Nouvelle soumission d'un projet modifié		
Documents concernés	Protocole	Version N°3	17/05/2009
	Notice d'information	Version N°3	17/05/2009
	Formulaire de consentement	Version N°3	17/05/2009
	Document additionnel		
	Résumé de l'étude	Version N°3	17/05/2009
N°enregistrement EudraCT	2009-A00249-48	Réf. Promoteur	RBHP 2009 LESOURD
N°enregistrement CPP Sud-Est I	2009-12		
Titre du projet	Rôle d'une intervention associant l'exercice physique et la nutrition dans le traitement du syndrome métabolique, ses conséquences cardiovasculaires et ses effets sur le système musculo-squelettique, chez les sujets humains. Analyse de l'observance des patients lors du suivi thérapeutique		
Promoteur	CHU DE CLERMONT-FERRAND		
Représentant du promoteur			
Investigateur	M. le Pr Bruno LESOURD		
Avis du comité après examen, réexamen ou prise en compte des réserves mineures émises lors de la délibération initiale	Conformément à l'Article L 1123-7, le comité a adopté la délibération suivante : FAVORABLE		
Ont participé à la délibération	Membres du comité	Titulaires	Suppléants
	Qualifiés en recherche biomédicale	Pr D. GUYOTAT Dr P. FOURNEL	Dr C. BERGER
1er collègue	Compétents en biostatistique ou épidémiologie	M. P. RUSCH	
	Médecins généralistes	Dr I. CARRIERE	
	Pharmaciens		
2ème collègue	Infirmiers		
	Compétents en questions éthiques		
	Juristes	M. D. IMBERT-MAGAND	
	Psychologues	Mme A. GROSSELIN	
	Travailleurs sociaux		
Représentants d'associations agréées de malades et usagers du système de santé		M. B. OSDOIT	
		M. F. FAISAN	

A Saint-Etienne, le 20/05/09

Le Président - M. Philippe RUSCH

Siège Social : CHU de Saint-Etienne - Direction des Affaires Médicales et de la Recherche
Hôpital de la Charité - 42055 SAINT-ETIENNE Cedex 2
Téléphone : 04.77.12.70.09 - Télécopie : 04.77.12.70.15
email : cpp.sudest1@chu-st-etienne.fr

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7.7. APPENDIX G: ETHICAL APPROVAL FOR STUDY 3



Human Research Ethics Committee

Committee Approval Form

Principal Investigator/Supervisor: Geraldine Naughton Melbourne Campus

Co-Investigators: Melbourne Campus

Student Researcher: Edward Crendal Melbourne Campus

Ethics approval has been granted for the following project:

The Impact of Ageing on Global and Regional Cardiac Morphology and Function: A Comprehensive Analysis Using Doppler Echocardiography and 2-D Strain Imaging.

for the period: 3/11/2011-10/12/2012

Human Research Ethics Committee (HREC) Register Number: V2011 127

Special Condition/s of Approval

Prior to commencement of your research, the following permissions are required to be submitted to the ACU HREC:

RSL Lifecare ANZAC Village, Narrabeen, 2101 NSW (received)

The following **standard** conditions as stipulated in the *National Statement on Ethical Conduct in Research Involving Humans (2007)* apply:

- (i) that Principal Investigators / Supervisors provide, on the form supplied by the Human Research Ethics Committee, annual reports on matters such as:
 - security of records
 - compliance with approved consent procedures and documentation
 - compliance with special conditions, and


(ii) that researchers report to the HREC immediately any matter that might affect the ethical acceptability of the protocol, such as:

- proposed changes to the protocol
- unforeseen circumstances or events
- adverse effects on participants

The HREC will conduct an audit each year of all projects deemed to be of more than low risk. There will also be random audits of a sample of projects considered to be of negligible risk and low risk on all campuses each year.

Within one month of the conclusion of the project, researchers are required to complete a *Final Report Form* and submit it to the local Research Services Officer.

If the project continues for more than one year, researchers are required to complete an *Annual Progress Report Form* and submit it to the local Research Services Officer within one month of the anniversary date of the ethics approval.

Signed:  Date:03/11/2011.....
(Research Services Officer, Melbourne Campus)

7.8. APPENDIX H: INFORMATION LETTER FOR RESOLVE TRIAL

Notice d'information remise au volontaire atteint de syndrome métabolique

TITRE de l'ETUDE : Rôle d'une intervention associant l'exercice physique et la nutrition dans le traitement du syndrome métabolique, ses conséquences cardiovasculaires et ses effets sur le système musculo-squelettique, chez les sujets humains. Analyse de l'observance des patients lors du suivi thérapeutique.

Investigateur principal : Pr **Bruno Lesourd**, tel : 04 73 17 79 45

Co-investigateur : Pr **Martine Duclos** ;

Collaborateur scientifique : Dr **Robert Chapier** ; **Frédéric Dutheil**

Promoteur : **CHU de Clermont-Ferrand**

Lieux d'étude : CHU de Clermont-Ferrand

Thermes de Châtel-Guyon, BP 51, 63140 Châtel-Guyon

« Cette étude respecte les normes de Bonne Pratique Clinique définies par le Ministère de la Santé. Elle a été soumise à l'approbation du Comité de Protection des Personnes Sud Est I (comité réuni spécialement pour juger de la possibilité de réaliser ce travail sans danger pour les sujets concernés) qui a donné son approbation en date du 20/05/2009 ».

Madame, Monsieur,

Nous vous proposons de participer à un travail de recherche médicale sur le traitement du syndrome métabolique et en particulier sur l'activité physique la plus appropriée pour le traiter. Il est important que vous preniez connaissance de toutes les informations données dans ce formulaire avant d'accepter ou non de participer à cette étude.

Qu'est-ce que le syndrome métabolique et quelles sont ses conséquences ?

Le syndrome métabolique consiste en une augmentation importante de la graisse abdominale qui est due à leur dépôt autour des viscères abdominaux. Celui-ci est le plus souvent dû à une alimentation trop abondante [notamment en lipides (graisses) et en glucides (sucres)] associée à une activité physique trop faible pour "brûler" cet excès d'apports caloriques. Il entraîne une augmentation des risques pour la santé en particulier, un dépôt de graisses dans les artères qui conduit aux maladies

cardio-vasculaires. De plus, des troubles de la régulation de l'appétit sont associés faisant ainsi perdurer cette situation dangereuse pour la santé.

Quel est le but de l'étude ?

Le but principal de cette étude est d'identifier une méthode optimale du traitement du syndrome métabolique pour réduire les dépôts de cette graisse abdominale et donc réduire les risques de maladies cardio-vasculaires. Pour cela, nous allons tester différentes pratiques d'activités physiques (cf ci-dessous) chez différents participants au programme. Vous devrez tous réduire votre consommation alimentaire au niveau que nous déterminerons. Cette double action permettra de réajuster votre équilibre entre apport d'énergie (alimentation) et dépenses énergétiques (activité physique) pour vous éviter de stocker de la graisse abdominale et en perdre. Ce protocole s'adresse à des sujets de 50 à 70 ans atteints de syndrome métabolique afin de trouver les activités physiques les plus appropriées pour traiter ce syndrome métabolique.

Nous avons quelques objectifs secondaires :

- Mesurer l'augmentation de votre masse musculaire et de votre force musculaire acquise par cet exercice physique et voir comment celles-ci évoluent quand vous serez retourné chez vous.
- Vérifier que ce programme permet aussi de conserver un os de qualité. En effet la pratique d'une activité physique intense peut avoir des effets bénéfiques ou à l'inverse néfaste sur la qualité de l'os.
- Analyser les niveaux des hormones secrétées par cette graisse abdominale. En effet ces hormones influencent votre appétit et entraînent une inflammation qui favorise les dépôts de graisses dans les artères.
- Analyser les réticences personnelles que vous avez à ce programme afin de trouver les conseils les plus adaptés à chacun d'entre vous pour que vous puissiez plus facilement continuer ce programme une fois retourné chez vous.

Comment se déroulera l'étude ?

Cette étude se déroulera de la façon suivante : un bilan initial, puis une cure de trois semaines à Châtel-Guyon où vous serez hébergé gratuitement dans l'hôtel du Dr Chapier. Au cours de cette cure, nous vous enseignerons le bon équilibre alimentaire (qualité et quantité) et quelle activité physique (qualité et quantité) il faut pratiquer pour réduire votre graisse abdominale. Puis vous serez suivi mensuellement pendant un an.

① La **visite de préadmission** sera constituée d'un interrogatoire et d'un examen médical pour vérifier votre état de santé. Une épreuve d'effort maximale vous sera proposée afin de vérifier que la pratique de l'exercice physique est sans risque pour vous. Cette épreuve consiste à pédaler (60 tours par minute) sur une bicyclette ergométrique. L'intensité est progressivement augmentée par palier et s'arrête lorsque la vitesse imposée ne peut plus être soutenue. L'activité électrique du cœur est enregistrée tout au long de l'épreuve. Le sujet est équipé d'un embout buccal relié à un système informatique afin d'analyser cycle par cycle la consommation d'Oxygène (VO₂). Cette consommation à l'exercice maximal (VO₂max) est un excellent marqueur de votre niveau d'aptitude physique. Un médecin cardiologue est constamment présent lors de cette épreuve afin de contrôler votre activité cardiaque et d'arrêter l'épreuve si nécessaire.

② **La visite d'inclusion.** Après votre délai de réflexion d'au moins huit jours et après avoir posé toutes les questions que vous voulez, en présence si vous le désirez d'une personne de confiance, si vous désirez participer à cette étude, vous signerez votre accord de participation. Il vous sera fait un prélèvement sanguin à jeun (25,5 ml) pour réaliser un bilan biologique. Cet examen comprendra l'évaluation des critères du syndrome métabolique dont vous êtes atteints, et une évaluation des risques cardio-vasculaires qui vous menacent pour vérifier que vous répondez aux critères d'inclusion du protocole : critères biologiques du syndrome métabolique.

③ **La cure :** Vous bénéficierez d'une cure de 3 semaines à Châtel-Guyon, sous la responsabilité du Docteur Chapier. Celle-ci comportera plusieurs aspects :

Alimentation : Il s'agit de rééquilibrer votre alimentation pour qu'elle soit plus équilibrée et respecte les recommandations du programme national nutrition-santé. Vous suivrez un régime alimentaire avec une réduction de votre alimentation de 500 à 700 kcal/j nécessaire au traitement. Nous vous apprendrons à gérer vos rations alimentaires pour les adapter à votre activité physique.

Activité physique : 3 groupes seront constitués : un groupe dit "endurance", un groupe dit "résistance" et un groupe dit activité "physique modérée". Votre attribution à l'un de ces groupes se fera par tirage au sort. Les activités physiques en résistance (type musculation) et en endurance (type marche) seront au début de faible intensité, puis augmentés progressivement tout au long de la cure.

Cours de nutrition et de cuisine : ils concerneront notamment le choix des aliments ainsi que la quantité appropriée à ingérer en fonction de chacun et de l'activité physique pratiquée.

Autres : une psychologue verra avec vous les difficultés que vous rencontrez et vous aidera à les résoudre. Vous suivrez aussi le programme de soin de la cure de Châtel-Guyon.

④ **Suivi de 1 an :** Le programme que vous aurez suivi en cure en termes d'activité physique et d'alimentation se continuera une fois que vous serez retourné chez vous. Un suivi sera mis en place pour savoir comment ce qui vous a été enseigné pendant la cure est observé dans vos conditions de vie de tous les jours. Vous serez ainsi contacté tous les mois, par voie téléphonique et par écrit afin que nous puissions suivre votre adhésion au programme et afin de vous aider dans les difficultés que vous rencontrerez dans la gestion de votre alimentation et de vos activités physiques. Vous reviendrez deux jours tous les 3 mois à Châtel-Guyon pour refaire un bilan complet des paramètres que nous suivrons (cf ci-dessous).

Quelles explorations aurez-vous pendant ce protocole et comment cela se passera ?

L'ensemble des explorations suivantes sera fait en début (J0) et en fin de cure (21) puis vous reviendrez deux jours dans l'hôtel du Dr Chapier pour avoir les mêmes explorations à 3 mois (M3), 6 mois (M6) et 12 mois (M12). Ce sera alors la fin du programme pour vous.

✧ Nous mesurerons votre masse grasse (graisse) abdominale et aussi votre masse musculaire et votre masse grasse totale par Absorptiométrie rayons X sur DXA. Vous serez allongé pendant environ 20 minutes et l'appareil de mesure défilera au dessus de vous pour effectuer ses mesures. La DXA s'effectuera dans le service de radiologie du CHU de Clermont-Ferrand, vous y serez transporté gratuitement.

✧ Une **Prise de sang** de 25 ml nous permettra de suivre les modifications biologiques liés au syndrome métabolique : le bilan lipidique (cholestérol et HDL cholestérol, triglycérides), le bilan glucidique [glycémie, insulinémie et hémoglobine glyquée (HBA1c)], les hormones impliquées dans la régulation de l'appétit (adiponectine, ghréline, leptine, cholécystokinine), des protéines inflammatoires (CRP et orosomucoïde) ainsi que les cytokines pro et anti-inflammatoires qui contrôlent cette réponse inflammatoire (IL-6, TNF α , IL-12 et IL-10). Sera aussi mesuré la tolérance rénale à cette pathologie (créatinine afin de calculer la fonction rénale) et votre taux de protéines sériques (albumine et préalbumine) afin de vérifier que vous mangez assez de protéines pour bénéficier de toute l'efficacité de l'activité physique que vous pratiquez. Nous mesurerons aussi des marqueurs de construction et de destruction osseuse pour vérifier que le programme n'a aucun effet délétère mais plutôt bénéfique sur vos os.

Les volumes de sang prélevé sont de 25,5 ml à chaque temps du protocole (bilan biologique d'inclusion, J20, M3, M6, M12) soit un total de 127,5 ml pour l'ensemble de l'étude. A titre d'exemple un don du sang correspond à un prélèvement de 450 ml.

Ces échantillons sanguins ne servent qu'à mesurer les paramètres biologiques dont nous avons besoin. Ils seront utilisés au cours de l'étude et aucun échantillon ne sera conservé à la fin de celle-ci.

✧ **Métabolisme de base** : Il sera estimé à partir d'une équation (Harris et Benedict). Celle-ci nous permet de calculer votre dépense énergétique de repos, c'est-à-dire ce que vous dépensez quand vous êtes au repos. Le but est d'adapter votre alimentation à vos besoins : besoins de votre corps au repos + besoin liés à votre activité physique.

✧ Le **Bilan cardiovasculaire** : Il s'agit d'une phase importante de votre bilan de santé. En effet, les patients porteurs du SM présentent un risque cardiovasculaire aggravé avec des anomalies de fonctionnement du cœur et des artères (grosses et petites). Ces anomalies sont dues à des dépôts de cholestérol dans les gros vaisseaux et les petits vaisseaux, y compris dans les artères qui irriguent le cœur, sans aucun symptôme ressenti. Les moyens actuels d'exploration permettent de proposer un dépistage et un suivi de ces dépôts à l'origine de lésions. Celles-ci sont fréquentes et peuvent être prévenues grâce à un bon contrôle de l'équilibre glycémique (taux de sucre dans le sang), de la tension artérielle et du bilan lipidique qui sont les paramètres biologiques du syndrome métabolique. L'exercice peut corriger certaines de ces lésions déjà constituées.

Le bilan cardiovasculaire qui vous sera proposé repose sur l'utilisation de techniques récentes d'exploration, très performantes et déjà pratiquées sans risque chez de nombreux sujets atteints de maladies cardio-vasculaires. Vous ne devrez ni boire du café ni fumer 12 heures avant ce bilan.

Il comporte :

- Un examen **cardiologique**, d'une durée d'environ 30 minutes en position allongée. Celui-ci intègre un électrocardiogramme de repos et une échographie (par ultrasons) cardiaque. Ce sont des examens non invasifs et indolores, renseignant sur la morphologie et la fonction du cœur.

- Un examen **vasculaire** :

Nous analyserons l'état fonctionnel de la paroi des **grosses artères**. L'exploration dure environ 45-60 minutes en position allongée. Au repos, un écho-Doppler mesure le diamètre et la vitesse de circulation du sang dans les grosses artères du bras et de la jambe. On place ensuite un brassard à tension sur l'avant-bras (puis la cuisse) qui reste gonflé durant 5 minutes. Nous arrêterons de gonfler dès l'absence de flux sanguin afin de limiter la gêne (engourdissement du bras ou de la cuisse). Nous remesurerons diamètre et vitesse de circulation après dégonflage du brassard puis 3 à 5 minutes après une dose orale unique de dérivé nitré (molécule utilisée dans le traitement de l'hypertension qui provoque une dilatation de ces vaisseaux). Parfois sont rapportés de légers maux de tête. Ces effets secondaires sont toujours transitoires et extrêmement rares aux posologies utilisées ici. La comparaison des mesures après relâchement du garrot et après administration du dérivé nitré permet de différencier les anomalies potentielles des parois de ces artères.

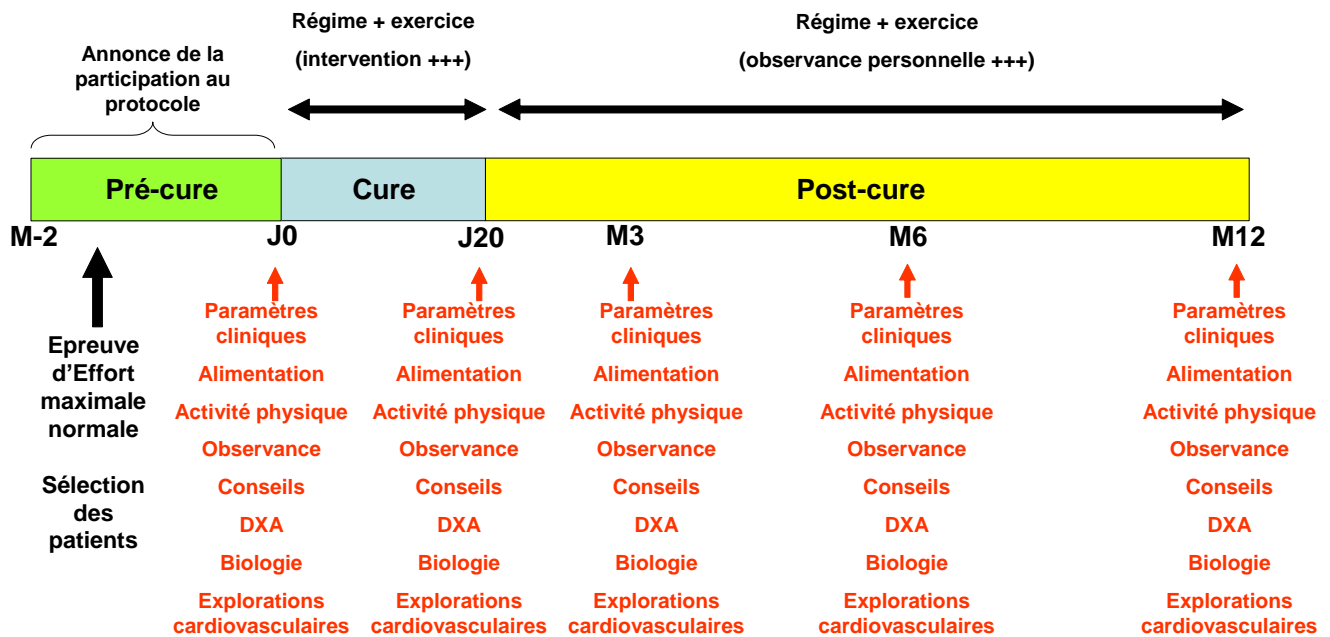
Nous analyserons également l'état fonctionnel de la paroi de **petites artères**. L'exploration dure environ 45-60 minutes en position allongée. Elle est réalisée au niveau de l'avant-bras par une technique de Laser Doppler couplé à l'iontophorèse. Il s'agit d'un examen non-invasif, non vulnérant et non douloureux (seuls de faibles picotements peuvent être parfois ressentis). Des agents vasodilatateurs seront dispensés au niveau de la peau grâce à l'application d'un courant de faible intensité (d'où l'appellation iontophorèse), ce qui permettra l'étude de la réactivité des petites artères qui irriguent la peau. La durée totale d'application sera de 2min20s pour l'acétylcholine, 3min pour le sodium nitroprusside et 4min pour l'insuline. L'iontophorèse a déjà été utilisée dans d'autres études chez des sujets ayant un syndrome métabolique de votre âge et aucun effet indésirable n'a jamais été rapporté. Si toutefois vous présentiez une sensation de picotement, nous arrêterions immédiatement l'application.

⊗ Le **Test de force maximale volontaire** permettra de mesurer le niveau maximal de force musculaire de votre quadriceps (muscle de la cuisse essentiel pour la marche). Vous serez assis sur un fauteuil-ergomètre équipé d'un capteur de force à la cheville. Il faudra pousser le plus fortement possible vers l'avant l'une des jambes sans déplacement durant 2 à 3 secondes. Ce test est sans risque particulier car les jambes restent fixes. Il sera réalisé dans la salle de remise en forme d'un centre thermal en présence d'un médecin et d'éducateurs sportifs.

⊗ **Test de marche de 6 minutes**. Il s'agit de marcher pendant 6 minutes sur un parcours déterminé, le plus vite possible. Ce test nous permet de mesurer la progression de vos capacités de marche et est un excellent indicateur de votre niveau de condition physique.

⊗ A chaque temps vous aurez un suivi médical et en entretien avec la psychologue. Les résultats de ces bilans vous seront donnés le jour même.

En résumé, voici le plan expérimental du protocole :



Modalités pratiques

La durée de votre participation sera de 13 mois.

La durée totale de l'étude sera de 36 mois. Il n'y aura pas d'indemnités.

Aspects éthiques et réglementaires

Les données recueillies seront traitées confidentiellement et identifiées par un numéro de code. Conformément aux dispositions de loi relative à l'informatique aux fichiers et aux libertés, vous disposez d'un droit d'accès et de rectification et d'un droit d'opposition à la transmission des données couvertes par le secret professionnel. Vos échantillons sanguins ne seront conservés que pendant la durée de l'étude, le temps pour nous de mesurer tous les paramètres biologiques.

Votre participation volontaire à cette étude peut faire progresser le corps médical dans la compréhension et dans le traitement du Syndrome Métabolique. Cependant, vous ne devez en attendre aucun bénéfice pour votre état de santé même s'il est probable qu'il y en est un.

Vous acceptez :

- que les données puissent faire l'objet d'un traitement informatisé anonyme,
- que vos données médicales ainsi que vos habitudes de vie soient transmises au Promoteur de la recherche (Pr Bruno Lesourd), à ses collaborateurs participant à la recherche et éventuellement à un représentant des autorités de santé,

- votre inscription dans le fichier national des personnes qui se prêtent à des recherches biomédicales (article L.1121-16 du Code de la santé publique).

Vous pouvez également accéder directement ou par l'intermédiaire d'un médecin de votre choix à l'ensemble de vos données médicales (article L 1111-7 du Code de la Santé Publique) et les résultats globaux du projet vous seront communiqués à l'issue de la recherche.

Le promoteur a souscrit une assurance responsabilité civile contractée auprès de la compagnie SHAM (N° de police : 126016) afin de pouvoir vous dédommager si votre état de santé s'altérait suite à votre participation à cette étude et dans la mesure où il pourra être établi que ces dommages sont la conséquence directe de l'étude.

Vous ne signerez votre consentement écrit que si, après lecture de cette note, délai de réflexion d'au moins 8 jours et discussion avec le médecin, vous vous sentez, à priori, disposé à participer à l'étude. Si vous décidez de participer, vous signerez le consentement écrit indiquant que vous avez lu et compris cette information et que votre participation a été librement décidée. Toutefois si vous changez d'avis pour quelques raisons que ce soit, vous restez libre de quitter l'étude à tout moment, sans préjudice légal ou médical.

Vous avez un délai d'au moins 8 jours pour donner votre réponse. Nous restons à votre entière disposition pour répondre à toutes vos questions. Vous pouvez vous faire accompagner par une personne de confiance pour poser toutes les questions que vous avez sur cette étude.

Si vous acceptez de participer à cette étude, nous vous demandons de bien vouloir signer les deux exemplaires de l'avis d'acceptation ci-joint :

- **un exemplaire** vous étant destiné, à conserver avec le formulaire d'information,
- **un exemplaire à remettre au médecin.**

Formulaire de Consentement pour les patients atteints de Syndrome Métabolique

TITRE de l'ETUDE : Rôle d'une intervention associant l'exercice physique et la nutrition dans le traitement du syndrome métabolique, ses conséquences cardiovasculaires et ses effets sur le système musculo-squelettique, chez les sujets humains. Analyse de l'observance des patients lors du suivi thérapeutique.

Investigateur principal : Pr **Bruno Lesourd**, tél : 04 73 17 79 45; *Co-investigateur* : Pr **Martine Duclos**

Collaborateurs scientifiques : Dr **Robert Chapier** ; **Frédéric Dutheil**

Promoteur : **CHU de Clermont-Ferrand**

Lieux d'étude : CHU de Clermont-Ferrand

Thermes de Châtel-Guyon, BP 51, 63140 Châtel-Guyon

Je soussigné(e) (nom, prénom)

Né(e) le

Domicilié(e) à

Certifie être affilié (ou bénéficiaire) d'un régime de Sécurité Sociale N° SS :
.....

Déclare :

- Que le Docteur (nom, prénom) m'a proposé de participer à l'étude sus-nommée,
- Qu'il m'a expliqué en détail le protocole dont le titre est en début de page,
- Qu'il m'a notamment fait connaître :

- L'objectif du projet qui est de traiter le syndrome métabolique et donc de réduire les risques cardio-vasculaire qui y sont associés.

- La méthode consiste à associer une réduction importante et une rééquilibration de mon alimentation à une augmentation de mon activité physique. Ce programme débutera par une cure de 3 semaines à Châtel-Guyon où ce que je dois faire doit m'être appris. Elle continuera pendant 1 an, de retour chez moi où je devrais appliquer au mieux ce qui m'est enseigné pendant cette cure. J'ai bien compris que j'appartiendrai ou non, de façon aléatoire, à un groupe qui aura une activité physique différente.

- J'ai compris que régulièrement, en début (J0) et en fin de cure (J21) puis à 3 (M3), 6 (M6) et 12 (M12) après j'aurai les examens suivants : mesures de ma composition corporelle par DXA, de mes risques cardio-vasculaires avec et notamment un bilan cardiologique et vasculaire, de ma force musculaire maximum et de mes capacités à marcher 6 minutes, ainsi que des prélèvements sanguins qui permettront de mesurer les effets de ce traitement.

- L'avis favorable du Comité de Protection des Personnes Sud Est I a été émis en date du .../.../..... .

- Mon droit de refuser de participer et de retirer mon consentement à tout moment sans avoir de justification à donner et sans que mes rapports avec l'équipe médicale ne soient modifiés.

- Que je ne serai pas autorisé à participer à d'autres études cliniques pendant les 13 mois de ma participation à ce programme.

Les informations relatives à l'étude, recueillies par l'investigateur, sont traitées confidentiellement. J'accepte :

- que ces données puissent faire l'objet d'un traitement informatisé anonyme (*Loi informatique et liberté, Article 40*). J'ai bien noté que le droit d'accès prévu par la loi "Informatique et Liberté (*Article 40*)" s'exerce à tout moment auprès du Dr ou de son représentant. Je pourrai exercer un droit de rectification auprès de lui.

- mon inscription dans le Fichier National des personnes qui se prêtent à des recherches biomédicales (*Art. 1121-16 du Code de la Santé Publique*).

Après avoir discuté librement et obtenu réponse à toutes mes questions, j'accepte en toute connaissance de cause de participer à cette étude.

Fait à, le.....

Signature du participant précédée de la mention "lu et compris"

Signature du médecin investigateur ayant recueilli le consentement et personnes à contacter en cas d'explications complémentaires ou de retrait du consentement

Docteur Robert CHAPIER, 04 73 86 09 65

Mr Frédéric DUTHEIL interne et doctorant, 06 88 22 48

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LE FORMULAIRE DE CONSENTEMENT EST UN DOCUMENT MEDICO-LEGAL IMPORTANT. L'investigateur doit le conserver pendant 15 ans au moins.

7.9. APPENDIX I: INFORMATION LETTER FOR STUDY 3



INFORMATION LETTER TO PARTICIPANTS

TITLE OF PROJECT: The Effect of Ageing on Hearts

STUDENT RESEARCHER: Mr Edward Crendal

RESEARCH ASSISTANT: Audrey Legrand

SUPERVISING INVESTIGATOR: Professor Geraldine Naughton

CO-INVESTIGATOR: Professor Tracey McDonald

ENROLMENT PROGRAM: PhD (Exercise Physiology)

Dear Sir,

You are warmly invited to participate in a PhD research project, aiming to better understand the ageing process of healthy human hearts.

In this research, we would like to take images of your heart using non-invasive ultrasound techniques, in the hope of developing a clearer understanding of how the heart changes through the lifespan. At the end of the project we hope to have better knowledge of how healthy hearts age in males.

The risks associated with this research project are minimal. One small sample of blood (5 ml, or one teaspoon) will be collected from a vein in your arm by a qualified phlebotomist just once. Ultrasound (echocardiography) testing will be completely pain free, with the only requirement for you being that you will need to lie comfortably and reasonably still for up to 40 minutes.

To take part in the project, you will need to be a male, aged between 18 and 90 years. You also need to be independently-mobile, a non-smoker, and free from cardiovascular disease. Additionally, to

avoid confusing the results, you must also NOT be participating in elite ultra-endurance sports such as marathon racing (running, swimming OR cycling) and triathlons (with training exceeding more than seven hours per week).

For this research project, you will be asked to attend ONE echocardiography session, which should last no longer than 60 minutes, and will include the following:

- (a) Measurement of height, body weight, waist circumference, and blood pressure, (approximately 10 minutes)
- (b) Very brief medical and exercise questionnaires
- (c) Echocardiography scan; an ultrasound probe will be manipulated over your chest to gather images of your heart, while you lie on your left side (approximately 40 minutes)

A second very brief session will be required to obtain the blood sample

This process will occur at the Therapy & Lifestyle Centre (a central multi- health facility within the village) at the RSL LifeCare ANZAC Village, Narrabeen. We will conduct your testing session at a time convenient for you.

There are several potential benefits of this research. Cardiovascular disease in Australia contributes significantly to deaths in males of all ages; more so than any other disease! Often, cardiovascular disease progresses “silently”, meaning that you may not be aware of it. This research is about investigating early signs of cardiac changes in ageing, but otherwise healthy, hearts.

As a participant, you have our guarantee of full confidentiality, and protection of your identity. Any data and personal information gathered from you will be stored in a secure place, and coded so that participant recognition is not possible by others other than the researchers named on this project.

At all times, you have the right to withdraw from this research project.

You also have the right to refuse consent altogether, without having to justify your decision, and this will not affect any association you have with the Australian catholic University (or for Sydney-based participants, the RSL Lifecare ANZAC Village).

Any questions regarding this project should be directed to the Supervising Investigator or the Student

Researcher:

<p>Professor Geraldine Naughton 03 9953 3034 School of Exercise Science St Patrick’s Campus, Fitzroy, VICTORIA Australian Catholic University</p>	<p>Mr Edward Crendal 0414 449 284 School of Exercise Science St Patrick’s Campus, Fitzroy, VICTORIA Australian Catholic University</p>
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This study has been approved by the Human Research Ethics Committee of the Australian Catholic University. In the event that you have any complaint or concern, or if you have any query that the Investigator (or Supervisor and Student Researcher) has not been able to satisfy, you may write to the Chair of the Human Research Ethics Committee care of the nearest branch of the Research Services Office.

VIC: Chair, HREC

C/- Research Services
Australian Catholic University
Melbourne Campus
Locked Bag 4115
FITZROY VIC 3065
Tel: 03 9953 3158
Fax: 03 9953 3315

QLD: Chair, HREC

C/- Research Services
Australian Catholic University
Brisbane Campus
PO Box 456
Virginia QLD 4014
Tel: 07 3623 7429
Fax: 07 3623 7328

NSW and ACT: Chair, HREC

C/- Research Services
Australian Catholic University
North Sydney Campus
PO Box 968
NORTH SYDNEY NSW 2059
Tel: 02 9739 2105
Fax: 02 9739 2870

Any complaint or concern will be treated in confidence and fully investigated. The participant will be informed of the outcome.

If you agree to participate in this project, please sign both copies of the Consent Form, retain one copy for your records, and return the other copy to the Supervising Investigator or Student Researcher at the soonest convenient time.

We look forward to hearing from you soon!

Kind regards,

Professor Geraldine Naughton

(Supervising Investigator)

Mr Edward Crendal

(PhD Researcher)

CONSENT FORM

Copy for Researcher

TITLE OF PROJECT: The Effect of Ageing on Healthy Hearts

STUDENT RESEARCHER: Mr Edward Crendal

SUPERVISING INVESTIGATOR: Professor Geraldine Naughton

CO-INVESTIGATOR: Professor Tracey McDonald

ENROLMENT PROGRAM: PhD (Exercise Physiology)

I (*the participant*) have read and understood the information provided in the Information Letter to Participants.

Any questions I have asked have been answered to my satisfaction. I agree to participate in this 60 minute testing session, including a full echocardiography exam, blood sampling, and completing two short questionnaires, realising that I can withdraw my consent at any time without adverse consequences. I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT:

SIGNATURE:

DATE

.....

SIGNATURE OF SUPERVISING INVESTIGATOR:

DATE:.....

SIGNATURE OF STUDENT RESEARCHER:

DATE:.....

CONSENT FORM

Copy for Participant

TITLE OF PROJECT: The Effect of Ageing on Healthy Hearts

STUDENT RESEARCHER: Mr Edward Crendal

SUPERVISING INVESTIGATOR: Professor Geraldine Naughton

CO-INVESTIGATOR: Professor Tracey McDonald

ENROLMENT PROGRAM: PhD (Exercise Physiology)

I (*the participant*) have read and understood the information provided in the Information Letter to Participants.

Any questions I have asked have been answered to my satisfaction. I agree to participate in this 60 minute testing session, including a full echocardiography exam, blood sampling, and completing two short questionnaires, realising that I can withdraw my consent at any time without adverse consequences. I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT:

SIGNATURE:

DATE

.....

SIGNATURE OF SUPERVISING INVESTIGATOR:

DATE:.....

SIGNATURE OF STUDENT RESEARCHER:

DATE:.....

7.10. APPENDIX J: DATA COLLECTION FORMS FOR RESOLVE TRIAL

NB: Due to the large page number (91) of data collection forms for the RESOLVE Trial, these are not included in this Thesis.

They may be obtained upon request from the primary investigator of the trial:

Fred Dutheil: fred_dutheil@yahoo.fr

7.11. APPENDIX K: DATA COLLECTION FORMS FOR STUDY 3

PARTICIPANT GENERAL INFORMATION



Date of exam: *Time:*

Name:

Date of birth:

Blood Pressure (mmHg): **Resting HR (bpm):**

Body Mass (kg): **Waist Circumference (cm):**

Height (cm):

PARTICIPANT MEDICAL BACKGROUND QUESTIONNAIRE



Name: _____

Please tick the appropriate box in the following 7 questions regarding your medical background:

QUESTION:	YES	NO
1. Do you have any PERSONAL history of Heart disease?		
i.e. Coronary Heart Disease		
Stroke		
Cardiac arrhythmias		
Heart Failure		
Other		
Do you take medication for the above?		
Do you have any FAMILY history of Heart disease?		
2. Do you have any PERSONAL history of Dyslipidemia?		
i.e. Elevated Cholesterol		
Elevated LDL		
Elevated Triglycerides		
Decreased HDL		
Do you take medication for the above?		
Do you have any FAMILY history of Dyslipidemia?		
3. Do you have any PERSONAL history of Diabetes?		
Type-1 Diabetes Mellitus		
Type-2 Diabetes Mellitus		
Do you take medication for the above?		
Do you have any FAMILY history of Diabetes?		
4. Do you have any PERSONAL history of Hypertension?		
i.e. High blood pressure (typically > 140/90 mmHg)		
Do you take medication for the above?		
Do you have any FAMILY history of Hypertension?		
5. Do you have any other health condition?		
Please list:		
6. Do you participate in any Ultra-endurance exercise?		
e.g. Marathon racing		

International Physical Activity Questionnaire for over 65s (IPAQ-E)

(To be completed with the Researcher)

1. The first question involves the time you spend sitting down for work, studies, in your home or your free/leisure time. E.g. at your work desk, at a friend's place or on the TV couch .

How much time on average did you spend sitting during the day, based on the past 7 days?

Hours_____ Minutes_____

2. During the past 7 days have you walked for at least 10 minutes without stopping? This includes walking at work, as part of different chores and any walking activity, in leisure time.

How much time on average did you spend walking during the day, based on the past 7 days?

Hours_____ Minutes_____

3. During the past 7 days have you performed somewhat strenuous physical activity such as raking leaves, washing windows, riding a bike, swimming or other physical exercise at a moderate pace? Only answer to the activities where the exercise was performed for at least 10 min continuously. Please do not include walking.

How many days did you spend doing moderate intensity (somewhat harder breathing than normal) physical activity, based on the past 7 days?

Days_____ (Approximately) Hours_____ Minutes_____ per day

4. During the past 7 days have you performed highly strenuous physical activity (resulting in higher breathing rate than normal) such as heavy lifting, heavy building or gardening work, wood chopping, aerobics, running and bike riding at a faster pace? Only answer to the activities where the exercise was performed for at least 10 min continuously.

How many days did you spend doing high intensity physical activity, based on the past 7 days?

Days_____ (Approximately) Hours_____ Minutes_____ per day

ECHO EXAM

Name: _____

PLAV	MODE	IMAGES	COMMENT
Aorta end-diastole internal diameter (at annulus)	B mode (2D)	5 x 1 cycle	
LA end-systole maximal internal diameter	M mode	2 images	
LV septal wall thickness, posterior wall thickness, internal diameter (at end-diastole & end-systole)	M mode	2 images	
EAT echo free space superficial to RV	B mode	5 x 1 cycle	
PSAV			
LV Base – mitral valve level	B mode (2D)	5 x 1 cycle	
LV Mid – papillary muscles level	B mode (2D)	5 x 1 cycle	
LV Apex – apical level	B mode (2D)	5 x 1 cycle	
A4CV			
LV transmitral blood flow	PW doppler	2 images	
LV septal wall tissue motion	PW + TDI	2 images	
LV lateral wall tissue motion	PW + TDI	2 images	
LV ejection fraction & speckle tracking	B mode (2D)	5 x 1 cycle	
RV TAPSE	M mode	2 images	
RV free wall tissue motion	PW + TDI	2 images	
A5CV			
LV aortic outflow	PW doppler	3 images	
IVRT - Isovolumic relaxation time	PW doppler	2 images	
A2CV			
LV inferior wall tissue motion	PW + TDI	2 images	
LV anterior wall tissue motion	PW + TDI	2 images	

-END-