## DOCTOR OF PHILOSOPHY

# Converging Biological Effects of Physical Activity on Cardiovascular Health in Ageing

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# Converging Biological Effects of Physical Activity on Cardiovascular Health in Ageing

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in Medicine

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2. Gao F, Kovalik JP, Zhao X, Chow VJ, Chew H, Teo LL, Tan RS, Leng S, Ewe SH, Tan HC, Tan TY, Lee LS, Ching J, Keng BM, Zhong L, Koh WP and **Koh AS**. Exacerbation of cardiovascular ageing by diabetes mellitus and its associations with acyl-carnitines. *Aging (Albany NY)*. 2021;13:14785-14805.

3. Kovalik JP, Zhao X, Gao F, Leng S, Chow V, Chew H, Teo LLY, Tan RS, Ewe SH, Tan HC, Wee HN, Lee LS, Ching J, Keng BMH, Koh WP, Zhong L and **Koh AS**. Amino acid differences between diabetic older adults and non-diabetic older adults and their associations with cardiovascular function. *J Mol Cell Cardiol*. 2021;158:63-71. doi: 10.1016/j.yjmcc.2021.05.009.:63-71.

4. **Koh AS**, Gao F, Tan RS, Zhong L, Leng S, Zhao X, Fridianto KT, Ching J, Lee SY, Keng BMH, Yeo TJ, Tan SY, Tan HC, Lim CT, Koh WP and Kovalik JP. Metabolomic correlates of aerobic capacity among elderly adults. *Clin Cardiol*. 2018;41:1300-1307.

5. Tan YH, Lim JP, Lim WS, Gao F, Teo LLY, Ewe SH, Keng BMH, Tan RS, Koh WP and **Koh AS**. Obesity in Older Adults and Associations with Cardiovascular Structure and Function. Obes Facts. 2022;15:336-343.

6. Loh R, Yeo SY, Tan RS, Gao F and **Koh AS**. Explainable machine learning predictions to support personalised cardiology strategies. *Eur Heart J Digit Health*. 2022;3:49-55.

**DECLARATION**

I hereby declare that the publications that support this thesis are original peer reviewed research derived from primary data. All references cited within this thesis were selected by me. I am the sole author of this thesis, unless otherwise stated.

I also declare that the work described in this thesis was carried out by me directly in my capacity as principal investigator, clinician scientist and consultant cardiologist at the National Heart Centre Singapore, Singapore. All the publications were designed, collected, and analysed by me or my team of co-authors as indicated in the publications.

The work took place in the Department of Cardiology, National Heart Centre Singapore. I have received competitive and intra-mural research grants including national grants from National Medical Research Council of Singapore, to perform the research work over the years. Other funders of the work are indicated in the publications.

This PhD by publication was performed under the supervision of Professor Chim Lang and Professor Anna Maria Choy from the School of Medicine, University of Dundee.

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As a practicing cardiologist, clinician scientist and a mother to two school going children (my son is 11 years old while my daughter is 6 years old), this PhD is especially important and meaningful. This thesis is a product of unconditional support that I had received from my family including my husband and my parents who have been cheering me on as I worked towards the completion of this thesis over the past year.

While the publications were led by me as a principal investigator of the research, I had received strong backing and guidance from numerous mentors in my career over the years, notably Professor Koh Woon Puay who mentored me on my research track when I first started in 2012 at the Duke-National University of Singapore, and Professor Terrance Chua, from National Heart Centre Singapore, who supported my lifelong career ambition to be a clinician scientist.

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**ABSTRACT**

***Background***

Physical activity has been traditionally used to maintain health, promote total well-being, and prescribed as part of holistic approach to disease treatments such as cardiovascular disease. In ageing adults, physical activity has purported benefits ranging from improvements in aerobic capacity and skeletal muscle function. However, ageing is frequently accompanied by other changes in the body systems such as loss of skeletal muscle mass, frailty, cardiorespiratory functions and dysmetabolism, which produces heterogenous effects of physical activity on aged adults. These heterogeneities often influence targets used to measure the effectiveness of exercise on health outcomes. For example, body mass index (BMI) is frequently used as a target of exercise regimens. However, BMI in aged adults may be reduced due to loss of skeletal muscle mass as part of sarcopenic processes in ageing, rendering BMI less accurate as a conventional outcome for exercise. Lipids profiles alone are also insufficient to address the impact of ageing on health outcomes, given that ageing of the cardiovascular system for instance, may march on, independent of lipid levels in the blood. Therefore, there is a lack of a converging marker that can be used to measure/assess the effect of lifestyle habits such as physical activity, commonly recommended as a longevity tool, which is suitable for aged adults. In the field of cardiovascular ageing, there is a critical need to identify suitable biomarkers that can be used as exercise targets are necessary to measure the effects of exercise on aged adults. Since ageing is a life course phenomenon, dynamic lifestyle factors such as physical activity, alcohol use and food intake can alter the course of physical ageing. Given that these dynamic factors all converge upon the human metabolome, metabolomics might provide a comprehensive and integrated picture of these lifelong environmental exposures, alongside exercise as a frequently practised intervention to alter the course of ageing.

***Objective***

The principal aim of the proposed research is to examine how metabolomics may be used as measurable biomarkers that represents the convergence of all physiological processes that occur with ageing, which accounts for the effect of exercise on the sum of these processes.

***Research Questions***

1. Would metabolomics biomarkers identified from blood samples of older adults be associated with cardiovascular ageing, as defined by changes in cardiac structure and function?
2. Would metabolomics biomarkers identified in (1) differentiate between physical activity levels, i.e., high versus low physical activity practices, among older adults with cardiovascular ageing?
3. Is there a better measure of cardiovascular health outcome, compared to traditional markers such as body mass index?
4. Recognising the need to incorporate multiple biological inputs, would an expansive machine learning (ML) approach help rank key factors that determine healthy cardiovascular health in ageing?

***Methodology***

To answer these questions, we will use data from a cohort study of older adults recruited from community population. The Cardiac Ageing Study (CAS) is a community-based study of middle aged to older adults (mean age 72±4 years) examined in 2014-2017 who did not have clinical cardiovascular disease (CVD) at baseline. In CAS, we characterised CV structure and function using novel cardiovascular imaging techniques. We found that these imaging markers defined individuals with worse structural and functional alterations that likely represent *cardiovascular ageing*. In conjunction with physical activity, skeletal muscle mass, dietary capture and circulating metabolites in this population, this cohort will provide the data to answer these research questions. Furthermore, apart from cross-sectional analytical approaches, we will include biomarker samples obtained at time points over longitudinal follow-up to chart changes in CV

longevity over time. In such an endeavour that involves multiple biological inputs, an expansive machine learning (ML) approach will additionally help identify key factors that determine healthy cardiovascular longevity. We will use machine learning techniques to analyse these multiple inputs. The automatic feature detection of machine learning will efficiently detect the association between the combination of metabolomics features, exercises and cardiac health.

**Prior publications that support this thesis:**

- **Koh AS**, Gao F, Leng S, Kovalik JP, Zhao X, Tan RS, Fridianto KT, Ching J, Chua SJ, Yuan JM, Koh WP and Zhong L. Dissecting Clinical and Metabolomics Associations of Left Atrial Phasic Function by Cardiac Magnetic Resonance Feature Tracking. *Sci Rep*. 2018;8:8138-26456.

***This paper integrates clinical and metabolomics signals for left atrial phasic function in older adults. We found that left atrial function alterations were a marker of cardiovascular ageing in older adults and medium and long chain acylcarnitines including amino acids such as serine, citrulline and valine were associated with phases of left atrial function. By integrating these clinical and metabolomics signals of left atrial function, metabolite signals may be useful for advancing mechanistic understanding of LA disease in future studies.***

- Gao F, Kovalik JP, Zhao X, Chow VJ, Chew H, Teo LL, Tan RS, Leng S, Ewe SH, Tan HC, Tan TY, Lee LS, Ching J, Keng BM, Zhong L, Koh WP and **Koh AS**. Exacerbation of cardiovascular ageing by diabetes mellitus and its associations with acyl-carnitines. *Aging (Albany NY)*. 2021;13:14785-14805.

***This paper highlights the work we did to define relationships between acylcarnitines and cardiovascular function in ageing. We found that distinct alterations in fuel oxidation pathways in short chain and long chain acyl-carnitines, di-carboxyl and hydroxylated acyl-carnitines. These links between fuel oxidation pathways in older adults were associated with impairments in myocardial relaxation and worse left atrial function, likely reflecting early disturbances in diastolic function.***

- Kovalik JP, Zhao X, Gao F, Leng S, Chow V, Chew H, Teo LLY, Tan RS, Ewe SH, Tan HC, Wee HN, Lee LS, Ching J, Keng BMH, Koh WP, Zhong L and **Koh AS**. Amino acid differences between diabetic older adults and non-diabetic older adults and their associations with cardiovascular function. *J Mol Cell Cardiol*. 2021;158:63-71. doi: 10.1016/j.yjmcc.2021.05.009.:63-71.

***This paper highlights the work we did to define relationships between amino acids and cardiovascular function in ageing. We found correlations between metabolites in the one-carbon and nitrogen handling pathways and ageing heart functions. These findings point to a potential role for changes in nitrogen handling in the pathogenesis of heart failure in older subjects.***

- **Koh AS**, Gao F, Tan RS, Zhong L, Leng S, Zhao X, Fridianto KT, Ching J, Lee SY, Keng BMH, Yeo TJ, Tan SY, Tan HC, Lim CT, Koh WP and Kovalik JP. Metabolomic correlates of aerobic capacity among elderly adults. *Clin Cardiol*. 2018;41:1300-1307.

***Combining echo-based and CMR-based imaging techniques to characterise cardiac ageing, this paper investigated metabolomics markers in relation to aerobic capacity. We found that low physical activity, associated with deleterious changes in cardiovascular structure and function, was distinguished by a metabolomic signature of wide-spectrum acylcarnitines and several amino acids. Combined cardiac and metabolomics phenotyping may be useful for tracking future interventions related to physical activity among community cohorts.***

- YH Tan, JP Lim, WS Lim, F Gao, LLY Teo, SH Ewe, BMH Keng, RS Tan, WP Koh, **Koh AS**. Obesity in Older Adults and Associations with Cardiovascular Structure and Function. *Obesity Facts*, 2022.

***This paper evaluated body mass index versus percentage fat mass in determining cardiovascular structure and function in older adults. Waist circumference, rather than body mass index, identified***

***higher prevalence of obesity. Across body mass index categories, waist circumference identified more adverse measurements in myocardial relaxation, aerobic capacity and left atrial structure.***

- Loh DR, Yeo SY, Tan RS, Gao F, **Koh AS**. Explainable Machine-Learning Predictions To Support Personalised Cardiology Strategies. *European Heart Journal - Digital Health*. 2022;3:49-55.

***This paper tested a method in Artificial Intelligence, known as Explainable Machine Learning, to identify personalised factors related to cardiovascular health state among older adults. Our work showed that machine learning could converge heterogenous features, including metabolomics and physical activity and demonstrate its effects on cardiovascular health.***

**Innovations and Importance of this Proposal:**

*This approach attempts to conglomerate the complexities of ageing.* Some ageing studies have been cross-sectional and thus are somewhat limited in their ability to detect causal associations between biochemical pathways and the effects of exercise on ageing. Pre-specified cohorts that study ageing and exercise, independently of traditional risk factors are necessary. Furthermore, analysis of community cohorts that include biomarker samples obtained at multiple time points is necessary to provide future reference targets for community cohorts.

*Now is the right time for this idea.* Ageing is a global problem. By 2030, approximately 20% of the world population will be aged 65 years or older. There is growing awareness and practice of using exercise as a lifestyle intervention to reduce ill-health associated with ageing. Yet, there is hardly any measurable biomarker that can quantify the effect of exercise on the individual older adult at a *personalised* level. Without robust methods of measuring the effect of exercise on ageing, exercise advice is prescribed blindly, indiscriminately while ignoring innate differences between individuals and their corresponding responses to exercise. Metabolomic profiling is an important systems biology tool that measures large numbers of metabolites with diverse chemical properties in a quantitatively rigorous and reproducible fashion. In contrast to other 'omics' platforms, such as genomics, transcriptomics and proteomics, metabolomics measures the net composition of genomic, transcriptomic, and proteomic variability providing an integrated profile of an individual's biological status. Thus, the metabolome provides a comprehensive picture of the immediate effects of exercise on the body, potentially preceding end-organ effects, exerting maximal preventative effect and personalised feedback to the user.

# INTRODUCTION

## I. Cardiovascular Ageing

### *Scale of the problem*

By 2030, approximately 20% of the world population will be aged 65 years or older[1]. Furthermore, the cost to treat cardiovascular disease will triple by that time[1, 2]. As a leading cause of death in older adults, understanding risk factors that lead toward cardiovascular disease in older adults is important[3]. Occurring in tandem with chronological ageing, cardiovascular ageing refers to cardiovascular structural and functional alterations which lead to the development of cardiovascular disease.

Typical age-related changes such as increased stiffness occur in the central arteries, resulting in loss of elastic fibres and arterial stiffness[4]. Arterial stiffness increases afterload which influences ventriculo-arterial coupling and affects ventricular relaxation[5]. With increased stiffness and reduced left ventricular relaxation, the left ventricle develops diastolic dysfunction, while preserving normal systolic function[6]. Diastolic dysfunction contributes to heart failure in ageing and is associated with increased mortality. Diastolic dysfunction also correlates with exercise capacity in older patients with heart failure[7].

While the underlying pathophysiological mechanisms behind diastolic dysfunction are complex, impairments in mitochondrial oxidation and fuel metabolism pathways have delineated diastolic failure from systolic failure [8, 9]. These pathways were similarly observed among ageing, asymptomatic older adults with impairments in myocardial relaxation and mitochondrial fuel metabolism [10, 11]. Thus, metabolomics may be useful to delineate metabolic changes in ageing-related ventricular stiffness and may reveal mechanistic insights before the onset of clinical diastolic failure, common to ageing.

## II. Physical Activity For The Preservation Of Cardiovascular Health

***Physical activity to achieve cardiovascular disease-free health span and maintenance of cardiovascular health***

Regardless of extensions in life expectancy, ageing predisposes people to developing cardiovascular disease which reduces health span, i.e., preserved healthy lifespan. Ageing increases biological vulnerability to cardiovascular events resulting in increased vulnerability with downward spirals into frailty. Data from centenarians suggest that cardiovascular disease-free health-span ('cardiovascular longevity') is possible[12]. Centenarians had more favourable biomarker profiles than non-centenarians for almost one decade prior to death[13]. Older adults who participate in higher amounts of physical activity have lower mortality risks and healthier cardiovascular longevity[14].

The role of physical activity as a primary prevention strategy against incident cardiovascular disease and secondary prevention strategy towards maintaining cardiovascular health is well established[15-18]. Physical activity impacts traditional risk factors such as hypertension, dyslipidaemia, and diabetes mellitus[19-21]. Specific to ageing, physical activity is the major modifiable lifestyle factor that may mitigate age-related deteriorations in cardiovascular health, in conjunction with age-related derangements muscle health such as sarcopenia and physical frailty[22].

Given the importance of physical activity, better understanding of the underlying biological and physiological processes stimulated by physical activity is warranted. Uncovering the mechanisms for the beneficial effects of physical activity will improve our basic understanding of disease pathophysiology, highlight new potential pathways for intervention and identify biomarkers to help guide exercise prescriptions. Current evidence points to the importance of fuel metabolism and mitochondrial oxidation pathways for physical activity effects on cardiovascular health[23]. Metabolomics, defined as the study of chemical processes involving metabolites within the human biological system, can serve as a useful tool to

guide further investigative work in these areas. Since ageing is a life course phenomenon, dynamic lifestyle factors such as physical activity, alcohol use and food intake can alter the course of physical ageing. Given that these dynamic factors all converge upon the human metabolome, metabolomics might provide a comprehensive and integrated picture of these lifelong environmental exposures, alongside exercise as a frequently practised intervention to alter the course of ageing.

## OBJECTIVE AND RESEARCH HYPOTHESES

### Objective

The principal aim of the proposed research is to examine how metabolomics may be used as measurable biomarkers that represents the convergence of all physiological processes that occur with ageing, which accounts for the effect of physical activity on the sum of these processes.

### Research Questions

1. Would metabolomics biomarkers identified from blood samples of older adults be associated with cardiovascular ageing, as defined by changes in cardiac structure and function?

Alterations in fuel-metabolism related markers in humans with ageing have been reported. The mechanism through which ageing-related changes such as arterial stiffness drives metabolic changes may similarly correlate with changes in cardiac structure and function. Identification of metabolomic profiles that is associated with changes in cardiac structure and function in older adults may reveal key metabolic pathways that lead to cardiovascular disease in ageing and allow for early identification of those at risk for progression towards clinical disease.

2. Would metabolomics biomarkers identified in (1) differentiate between physical activity levels, i.e., high versus low physical activity practices, among older adults with cardiovascular ageing?

Trials have shown that exercise leads to differences in metabolomic profiles. Physical activity increases energy demand across multiple tissues and stimulates acute and chronic changes in metabolic pathways. These changes can be detected through metabolomics analysis of serum. For instance, in the first 24 hours after a bout of exercise lactate, pyruvate, TCA cycle intermediates, fatty acids, acylcarnitines, and ketone

bodies all typically increase whereas bile acids decrease[24]. We therefore hypothesise that in conjunction with cardiovascular ageing phenotypes, metabolomics biomarkers would differentiate older adults with high versus low physical activity practices.

3. Is there a better measure of cardiovascular health outcome, compared to traditional markers such as body mass index?

While physical activity is frequently advocated to older adults, using body mass index (BMI) as a target of exercise outcome, may not be appropriate among older adults who may have weight changes due to sarcopenia. This is because sarcopenia results in reductions in body weight, leading to lower BMI values. Despite strong correlations between BMI and cardiovascular health, BMI may not be a suitable anthropometric target for older adults. We test the hypothesis by evaluating the impact of using BMI versus waist circumference in the study of cardiovascular function in older adults.

4. Recognising the need to incorporate multiple biological inputs, would an expansive machine learning (ML) approach help rank key factors that determine healthy cardiovascular health in ageing?

The large dimensionality of the multiple biological variables makes it challenging to analyse data directly. Some dimension reduction tools such as the Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA) are linear methods which are not appropriate for non-linear data. In this project, the extraction of features will be done through a combination of machine learning methods which generate a set of outputs from a set of inputs. These outputs will be used to evaluate top ranked factors that include

physical activity variables, metabolites and clinical variables that determine cardiovascular health of older adults.

## Research Question #1:

## Would metabolomics biomarkers identified from blood samples of older adults be associated with cardiovascular ageing, as defined by changes in cardiac structure and function?

**PUBLICATION #1**

**Dissecting Clinical and Metabolomics Associations of Left Atrial Phasic Function by Cardiac Magnetic Resonance Feature Tracking**

**Koh AS, Gao F, Leng S, Kovalik JP, Zhao X, Tan RS, Fridianto KT, Ching J, Chua SJ, Yuan JM, Koh WP and Zhong L.**

***Sci Rep*. 2018;8:8138-26456[25].**

*"Among community cohorts, associations between clinical and metabolite factors and complex left atrial (LA) phasic function assessed by cardiac magnetic resonance (CMR) feature tracking (FT) are unknown. Longitudinal LA strain comprising reservoir strain (εs), conduit strain (εe) and booster strain (εa) and their corresponding peak strain rates (SRs, SRe, SRa) will be measured using CMR FT. Targeted mass spectrometry will measure 83 circulating metabolites in serum. Sparse Principal Component Analysis will be used for data reduction. Among community adults (n = 128, 41% female) (mean age: 70.5 ± 11.6 years), age was significantly associated with εs (β = −0.30, p < 0.0001), εe (β = −0.3, p < 0.0001), SRs (β = −0.02, p < 0.0001), SRe (β = 0.04, p < 0.0001) and SRe/SRa (β = −0.01, p = 0.012). In contrast, heart rate was significantly associated with εa (β = 0.1, p = 0.001) and SRa (β = −0.02, p < 0.0001). Serine was significantly associated with εs (β = 10.1, p = 0.015), SRs (β = 0.5, p = 0.033) and Sra (β = −0.9, p = 0.016). Citrulline was associated with εs (β = −4.0, p = 0.016), εa (β = −3.4, p = 0.002) and SRa (β = 0.4, p = 0.019). Valine was associated with ratio of SRe:SRa (β = −0.4, p = 0.039). Medium and long chain dicarboxyl carnitines were associated with εs (β = −0.6, p= 0.038). Phases of LA function were differentially associated with clinical and metabolite factors. Metabolite signals may be used to advance mechanistic understanding of LA disease in future studies."*

SCIENTIFIC REPORTS

OPEN

# Dissecting Clinical and Metabolomics Associations of Left Atrial Phasic Function by Cardiac Magnetic Resonance Feature Tracking

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Angela S. Koh[1,2], Fei Gao[1,2], Shuang Leng[1], Jean-Paul Kovalik[2,3], Xiaodan Zhao[1], Ru San Tan[1,2], Kevin Timothy Fridianto[2], Jianhong Ching[2], Serene JM Chua[1], Jian-Min Yuan[5,6], Woon-Puay Koh[2,4] & Liang Zhong[1,2]

**Among community cohorts, associations between clinical and metabolite factors and complex left atrial (LA) phasic function assessed by cardiac magnetic resonance (CMR) feature tracking (FT) are unknown. Longitudinal LA strain comprising reservoir strain (εs), conduit strain (εe) and booster strain (εa) and their corresponding peak strain rates (SRs, SRe, SRa) were measured using CMR FT. Targeted mass spectrometry measured 83 circulating metabolites in serum. Sparse Principal Component Analysis was used for data reduction. Among community adults (n = 128, 41% female) (mean age: 70.5 ± 11.6 years), age was significantly associated with εs (β = −0.30, p < 0.0001), εe (β = −0.3, p < 0.0001), SRs (β = −0.02, p < 0.0001), SRe (β = 0.04, p < 0.0001) and SRe/SRa (β = −0.01, p = 0.012). In contrast, heart rate was significantly associated with εa (β = 0.1, p = 0.001) and SRa (β = −0.02, p < 0.0001). Serine was significantly associated with εs (β = 10.1, p = 0.015), SRs (β = 0.5, p = 0.033) and SRa (β = −0.9, p = 0.016). Citrulline was associated with εs (β = −4.0, p = 0.016), εa (β = −3.4, p = 0.002) and SRa (β = 0.4, p = 0.019). Valine was associated with ratio of SRe:SRa (β = −0.4, p = 0.039). Medium and long chain dicarboxyl carnitines were associated with εs (β = −0.6, p = 0.038). Phases of LA function were differentially associated with clinical and metabolite factors. Metabolite signals may be used to advance mechanistic understanding of LA disease in future studies.**

Historically, evaluation of left atrial (LA) function such as left atrial strain, strain rate and LA active or passive emptying fractions was performed using two-dimensional echocardiography techniques employing tissue Doppler imaging or speckle tracking methods[1,2]. More recently, studies have used cardiac magnetic resonance (CMR) feature tracking to characterize LA function since it allows more comprehensive assessment of complex LA phasic behaviour[3–5]. This technique has been used to assess left atrial phasic behaviour in clinical cohorts for risk stratification[6] as well as for prognostication of incident cardiovascular events[7]. These developments suggest that left atrial phasic function investigations, in contrast to gross changes in left atrial size commonly used for risk stratification and prognostication, may be valuable for studying development of cardiovascular disease. Alterations in left atrial size are commonly associated with CVD risk factors such as hypertension and diabetes mellitus. However, there is little data on factors that influence left atrial phasic behaviour, particularly using community-based cohorts prior to development of overt cardiovascular disease. It is therefore unknown if underlying risk factors influence left atrial phasic behaviour prior to disease. Furthermore, while it has been reported that left atrial function may be significantly altered with aging[1], no studies have included elderly adults primarily

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in their analysis[4]. The fact that cardiovascular disease (CVD) is a leading cause of death in older adults[8], underscores the importance of gaining a better understanding of the impact of age (also an independent risk factor of CVD) on left atrial function as assessed by CMR feature tracking (FT).

Mounting data suggest that changes in fuel metabolism are associated with clinical CVD in both the general population[9–12] as well as in elderly subjects[13]. In a study of patients with established cardiovascular disease, disturbances in the dicarboxyl/hydroxyl acyl-carnitine pathway were able predict incident cardiovascular events[12]. Furthermore, a distinct signature comprising of medium and long chain dicarboxyl and hydroxyl acyl-carnitines, likely reflecting changes in cellular fatty acid oxidation, was independently associated with arterial stiffness among aged adults without clinical cardiovascular disease[14]. Therefore, identification of distinct associations between metabolic perturbations with phases of left atrial function may therefore advance mechanistic understanding of how disordered metabolism drives left atrial diseases with aging, providing translatable knowledge for future therapeutics and/or preventative treatments.

In this study, we hypothesized that left atrial phasic function is related to clinical factors. We further hypothesize that changes in circulating metabolite profiles could be related to alterations in left atrial phasic function, independent of clinical factors. Therefore, we aimed to study the association between individual components of LA phasic function and clinical factors. Secondly, we aimed to characterize the relationship between metabolic profile of these subjects and components of left atrial function.

## Methods

The subjects were recruited from the Cardiac Aging Study (CAS), a prospective study initiated in 2014 that examines characteristics and determinants of cardiovascular function in elderly adults[14].

The current analysis is a cross-sectional analysis between left atrial function and metabolomics profiling obtained from subjects recruited from the CAS study. Subjects who had self-reported history of physician-diagnosed cardiovascular disease (such as coronary heart disease, atrial fibrillation and stroke) or cancer were excluded. A total of 128 participants were studied in this analysis. The SingHealth Centralised Institutional Review Board had approved the study protocol. Informed consent was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

All participants were examined and interviewed on one study visit by trained study coordinators. Participants completed a standardized questionnaire that included medical history and coronary risk factors. Hypertension was defined by current use of antihypertensive drugs or physician-diagnosed hypertension. Diabetes mellitus was defined by current use of anti-diabetic agents or physician-diagnosed diabetes mellitus. Dyslipidemia was defined by current use of lipid-lowering agents or physician-diagnosed dyslipidemia. Smoking history was defined as ever smokers (former or current smoking) or never smokers. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Sinus rhythm status was ascertained by resting electrocardiogram. Clinical data were obtained on the same day as assessment of cardiac magnetic resonance (CMR) imaging and serum collection.

**CMR protocol and analysis.** Cine CMR scans were performed using balanced fast field echo sequence (BFFE). All subjects were imaged on a 3 T magnetic resonance imaging system (Ingenia, Philips Healthcare, The Netherlands) with a dStream Torso coil (maximal number of channels 32). BFFE end-expiratory breath hold cine images were acquired in multi-planar long-axis views (2-, 3-, and 4-chamber views). Typical parameters were as follows: TR/TE 3/1 ms; flip angle, 45°; in-plane spatial resolution, 1.0 mm × 1.0 mm to 1.5 mm × 1.5 mm; slice thickness, 8 mm; pixel bandwidth, 1797 Hz; field of view, 300 mm; frame rate, 30 or 40 per cardiac cycle. We developed an in-house semi-automatic algorithm to track the distance (L) between the left atrioventricular junction and a user-defined point at the mid posterior LA wall on standard CMR 2- and 4-chamber views. Both 2- and 4-chamber views were used to generate the average strain and strain rate results. Longitudinal strain ($\varepsilon$) at any time point ($t$) in the cardiac cycle from end-diastole (time 0) was calculated as: $\varepsilon(t) = (L(t) - L_0)/L_0$. LA reservoir strain ($\varepsilon_s$), conduit strain ($\varepsilon_e$) and booster strain ($\varepsilon_a$) were calculated at $t$ equals left ventricular end-systole, diastasis and pre-LA systole, respectively, and their corresponding peak strain rates (SR) derived (Fig. 1). Strain and strain rate parameters from both 2- and 4-chamber views were averaged to obtain mean results for analysis. Using data from 20 randomly selected subjects, intra- and inter-observer comparability was assessed using Bland-Altman plot (Supplementary Fig. S1a,b). Two independent observers analyzed all cases in the evaluation of inter-observer variability (SL & XDZ), while intra-observer variability was assessed from a repeated analysis by the first observer (SL) after 7 days (Supplementary Fig. S1a,b). This technique has been validated against volumetric measurements and the strain results obtained from commercial software. Details about the technique can be found in the Supplementary Table S1 and Supplementary Fig. A.

**Central Hemodynamics.** We measured central blood pressure parameters such as central systolic blood pressure, central diastolic blood pressure, central mean arterial pressure and central pulse pressure noninvasively using applanation tonometry (SphygmoCor system, AtCor Medical, Sydney, Australia). All measurements were performed in the daytime, in a quiet environment, at stable room temperature. Participants were studied in the supine position.

Antecubital venous blood samples (20–30 ml) were taken from consenting participants in the morning; fasting was not required before blood collection. After collection, the blood samples were immediately placed on ice for transportation and were processed within 6 h to obtain serum samples, which were subsequently stored at −80 °C.

Serum metabolomic profiling analysis was performed in the Duke-NUS Metabolomics Facility as previously described[15]. Thawed serum samples (100 µl) were spiked with 20 µl deuterium-labelled amino acid/acyl-carnitine mixture and diluted with 800 µl methanol. After centrifugation of the mixture at 17,000 g for 5 mins at 20 °C, the supernatant fraction was collected and divided into two parts: one (100 µl) for acylcarnitine analysis and one

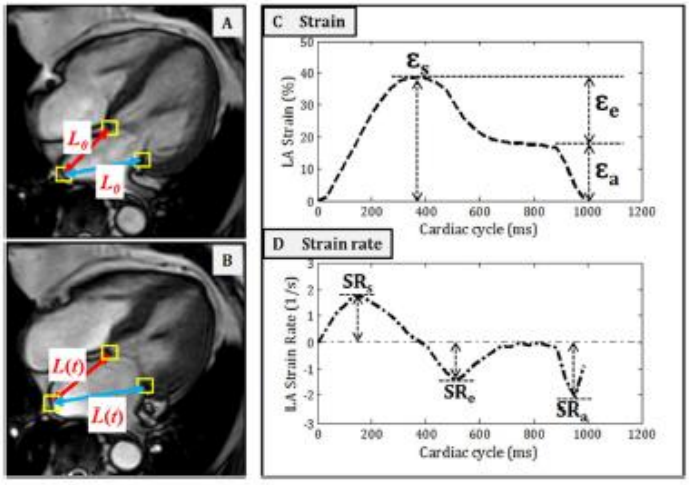


**Figure 1.** (**A**,**B**) semi-automatic algorithm to track the distance (L) between the left atrioventricular junction and a user-defined point at the mid posterior left atrial (LA) wall on standard CMR 2- and 4-chamber views. (**C**,**D**) Longitudinal strain (ε) at any time point (t) in the cardiac cycle from end-diastole (time 0) was calculated as: $\varepsilon(t) = (L(t) - L_0)/L_0$. LA reservoir strain ($\varepsilon_s$), conduit strain ($\varepsilon_e$) and booster strain ($\varepsilon_a$) were calculated at *t* equals left ventricular end-systole, diastasis and pre-LA systole, respectively, and their corresponding peak strain rates (SR) derived.

(10 µl) for amino acid analysis. A pooled quality control (QC) sample was prepared by mixing equal amounts (10 µl) of each extracted serum sample. Amino acids were separated using a C8 column (Rapid Resolution HT, 4.5 × 50 mm, 1.8 µm, Zorbax SB-C8) on a Agilent 1290 Infinity LC system (Agilent Technologies, CA, USA) coupled with quadrupole-ion trap mass spectrometer (QTRAP 5500, AB Sciex, DC, USA). Mobile phase A (10/90 Water/Acetonitrile) and Mobile phase B (90/10 Water/ Acetonitrile), both containing 10 mM of Ammonium formate, were used for chromatography separation. Acylcarnitine measurements were made using flow injection tandem mass spectrometry on the Agilent 6430 Triple Quadrupole LC/MS system (Agilent Technologies, CA, USA). The sample analysis was carried out at 0.4 ml/min of 80/20 Methanol/water as mobile phase, and injection of 4 µL of sample. Data acquisition and analysis were performed on Agilent MassHunter Workstation B.06.00 Software. Free and total L-carnitine analysis was carried out as previously described[16]. Data acquisition and analysis were performed on an Agilent MassHunter Workstation B.06.00 Software.

**Statistical methodology.** Clinical characteristics are presented as mean and standard deviation (SD) for continuous data and frequency and percentage for categorical data. We analysed 83 metabolites comprising 65 acyl-carnitine metabolites, 16 amino acid metabolites and 2 carnitine metabolites. Metabolites with >25% of values below the lower limit of quantification were excluded from analysis (only C10:2 was excluded, hence a total of 83 metabolites were analyzed in the final sample). We normalized the distributions of all metabolites by a logarithmic transformation.

The association between clinical risk factors and LA function was assessed in 2 steps. First, simple linear regression with LA function as dependent variable was used individually. Further all clinical risk factors that show an association with $p < 0.05$ with LA function in univariate analysis were included in the multivariate linear regression respectively. In this analysis since central pulse pressure (PP) were highly correlated to central systolic blood pressure (SBP) (The Pearson correlation between central SBP and central PP is 0.81), we only included central SBP into the multivariable model if both central SBP and central PP are significant in the univariate analysis.

We identified amino acids associated with LA function, respectively, in 3 ways. Firstly, simple linear regression with LA function as a dependent variable was used respectively to determine the significance of the individual amino acids. Secondly, multivariate linear regression was conducted for each amino acids with $p < 0.05$ in univariate analysis adjusting for significant clinical risk factors identified. Thirdly, multivariate linear regression was conducted including all amino acids that show an association with $p < 0.05$ with LA function in the multivariate analysis adjusting for clinical confounders.

To identify metabolites correlations (65 acyl-carnitine metabolites and 2 carnitine metabolites) and reduce the dimensionality of correlated metabolites, we performed sparse principal component analysis (SPCA), which used a penalized matrix decomposition[17]. Comparing with the regular principal component analysis that suffers from the fact of a dense loading matrix from all variables, SPCA is capable of producing sparse loadings which makes it more biologically interpretable. Specifically, we set the orthogonality constraint[17] on each component

| Variable | Overall |
|---|---|
| Age (years) | 70.5 (11.6) |
| Female | 52 (40.6%) |
| Ever smoked | 26 (20.3%) |
| Body mass index (kg/m$^2$) | 23.4 (3.1) |
| Hypertension | 68 (53.1%) |
| Diabetes mellitus | 28 (21.9%) |
| Dyslipidemia | 64 (50.0%) |
| Heart rate (beats per minute) | 74 (13) |
| Central systolic blood pressure (mmHg) | 139 (18) |
| Central diastolic blood pressure (mmHg) | 76 (11) |
| Central mean arterial pressure (mmHg) | 102 (12) |
| Central pulse pressure (mmHg) | 62 (17) |
| **Left atrial function** | |
| Reservoir strain (εs) | 31.1 (7.8) |
| Conduit strain (εe) | 13.2 (5.5) |
| Booster strain (εa) | 16.6 (5.0) |
| Reservoir strain rate (SRs) | 1.6 (0.5) |
| Conduit strain rate (SRe) | −1.4 (0.7) |
| Booster strain rate (SRa) | −2.2 (0.7) |
| Ratio of SRe/SRa | 0.7 (0.5) |

**Table 1.** Baseline clinical characteristics of study participants (N = 128). Mean (SD) are presented for continuous variables. SRe/SRa = ratio of SRe over SRa.

and the number of components to be 10. We reported the description on each component and the proportion of variance-accounted.

To assess the association between the 10 SPCA factors and LA function, we first performed simple linear regression with LA function as dependent variable, respectively. Further, for each SPCA factor, we performed multivariable linear regression adjusting for significant clinical confounders identified.

All statistical analyses were performed using STATA 13 (College Station, Texas, USA), while the SPCA and correlation matrix heatmap showing pairwise Pearson correlations (r) between amino acids and LA function were performed by R. For all analysis, a two-tailed *P* value of $<0.05$ was considered significant.

## Results

A total of 128 participants (mean age $70.5 \pm 11.6$ years; 52 women) were included in this analysis. All completed cardiac magnetic resonance imaging and had blood sample acquired on the same day. The majority of participants had vascular risk factors of hypertension (53.1%) and dyslipidemia (50.0%) while some had diabetes mellitus (21.9%). The central systolic and diastolic blood pressures of the participants were $139 \pm 18$ mmHg and $76 \pm 11$ mmHg respectively. Baseline clinical characteristics of the study sample are presented in Table 1. Additional CMR measurements of the left ventricle and echocardiogram-derived measurements are shown in Supplementary Table S2.

We observed univariate associations between clinical variables and left atrial function. Age, central pulse pressure were significant for εs; age, ever smoking, hypertension, diabetes, dsylipidemia, central systolic blood pressure were significant for εe; heart rate was significant for εa; age, BMI, heart rate, central diastolic blood pressure, central pulse pressure were significant for SRs; age, ever smoking, hypertension, diabetes, dyslipidemia, central systolic blood pressure were significant for SRe; female gender, BMI, heart rate, central pulse pressure were significant for SRa; age, ever smoked, diabetes mellitus, dyslipidemia, central systolic blood pressure were significant for SRe/SRa.

Table 2 showed multivariate linear regression analysis between significant clinical variables and left atrial function, respectively. In multivariate analysis, age was significantly associated with εs ($\beta = -0.30$, $p < 0.0001$), εe ($\beta = -0.3$, $p < 0.0001$), SRs ($\beta = -0.02$, $p < 0.0001$), SRe ($\beta = 0.04$, $p < 0.0001$) and SRe/SRa ($\beta = -0.01$, $p = 0.012$). In contrast, only heart rate was significantly associated with εa ($\beta = 0.1$, $p = 0.001$) and SRa ($\beta = -0.02$, $p < 0.0001$). Except for diabetes mellitus that was associated with εe ($\beta = -2.0$, $p = 0.032$), hypertension, body mass index, dyslipidemia and gender were not associated with left atrial function.

We analysed 83 metabolites comprising 65 acyl-carnitine metabolites, 16 amino acid metabolites and 2 carnitine metabolites. The list of measured metabolites is presented in supplementary Tables S3 to S4.

Correlations for the 16 amino acids were assessed using the Pearson correlation analysis (Fig. 2). We observed serine was significantly correlated with all LA function except the ratio SRe/SRa (r ranges from −0.36 to 0.32; all $p < 0.05$) whilst arginine, histidine, ornithine, tryptophan and tyrosine were not correlated with any LA function. Table 3 shows multivariate analysis between individual amino acids and corresponding left atrial functions, adjusting for prior clinical covariates. Serine remains significantly associated with reservoir strain ($\beta = 10.1$; 95% CI 2.0, 18.2; $p = 0.015$), reservoir strain rate ($\beta = 0.5$; 95% CI 0.04, 1.0; $p = 0.033$) and booster strain rate ($\beta = -0.9$; 95% CI −1.7, −0.2; $p = 0.016$). Citrulline was associated with reservoir strain ($\beta = -4.0$; 95% CI −7.2,

| Clinical covariates | LA function | Coef. (95% CI) | P-value |
|---|---|---|---|
| Age (years) | εs | −0.3 (−0.4, −0.1) | **<0.0001** |
| | εe | −0.3 (−0.4, −0.2) | **<0.0001** |
| | SRs | −0.02 (−0.03, −0.01) | **<0.0001** |
| | SRe | 0.04 (0.04, 0.1) | **<0.0001** |
| | SRe/SRa | −0.01 (−0.02, −0.003) | **0.012** |
| Female | SRa | 0.2 (−0.04, 0.5) | 0.10 |
| Ever smoked | εe | −1.2 (−3.0, −0.5) | 0.16 |
| | SRe | 0.1 (−0.05, 0.3) | 0.14 |
| | SRe/SRa | −0.2 (−0.4, 0.04) | 0.10 |
| Body mass index ((kg/m$^2$) | SRs | −0.02 (−0.05, 0.001) | 0.063 |
| | SRa | 0.02 (−0.02, 0.1) | 0.31 |
| Hypertension | εe | 0.1 (−1.5, 1.7) | 0.91 |
| | SRs | −0.01 (−0.2, 0.2) | 0.89 |
| | SRe | 0.03 (−0.1, 0.2) | 0.71 |
| Diabetes mellitus | εe | −2.0 (−3.7, −0.2) | **0.032** |
| | SRe | 0.2 (−0.003, 0.4) | 0.054 |
| | SRe/SRa | −0.2 (−0.4, 0.1) | 0.14 |
| Dyslipidemia | εe | 0.2 (−1.3, 1.7) | 0.79 |
| | SRe | −0.04 (−0.2, 0.1) | 0.62 |
| | SRe/SRa | −0.1 (−0.3, 0.1) | 0.29 |
| Heart rate (beats per minute) | εa | 0.1 (0.1, 0.2) | **0.001** |
| | SRs | 0.006 (−0.001, 0.01) | 0.080 |
| | SRa | −0.02 (−0.03, −0.01) | **<0.0001** |
| Central systolic blood pressure (mmHg) | εe | −0.004 (−0.05, 0.04) | 0.85 |
| | SRe | 0.001 (−0.003, 0.01) | 0.61 |
| | SRe/SRa | −0.004 (−0.01, 0.001) | 0.12 |
| Central diastolic blood pressure (mmHg) | SRs | 0.01 (−0.0002, 0.01) | 0.058 |
| Central pulse pressure (mmHg) | εs | −0.03 (−0.1, 0.1) | 0.56 |
| | εe | −0.03 (−0.1, 0.05) | 0.44 |
| | SRs | 0.001 (−0.004, 0.01) | 0.69 |
| | SRa | 0.001 (−0.01, 0.01) | 0.88 |

**Table 2.** Multivariate analysis of clinical covariates associated with left atrial function. Variables were selected based on simple linear regression with $P < 0.05$ at univariate analysis. Univariable analysis results are described in the text. Multiple regression models performed. Outcome εs; Confounders Age, Central pulse pressure. Outcome εe; Confounders Age, Ever smoked, Hypertension, Diabetes mellitus, Dyslipidemia, Central systolic blood pressure. Outcome εa; Confounders Heart rate. Outcome SRs; Confounders Age, Body mass index, Heart rate, Central diastolic blood pressure, Central pulse pressure. Outcome SRe; Confounders Age, Ever smoked, Hypertension, Diabetes mellitus, Dyslipidemia, Central systolic blood pressure. Outcome SRa; Confounders Female, Body mass index, Heart rate, Central pulse pressure. Outcome SRe/SRa; Confounders Age, Ever smoked, Diabetes mellitus, Dyslipidemia, Central systolic blood pressure.

−0.7; p = 0.016), booster strain ($\beta = -3.4$; 95% CI −5.5, −1.2; $p = 0.002$) and booster strain rate ($\beta = 0.4$; 95% CI 0.1, 0.7; $p = 0.019$). Valine was associated with ratio of conduit strain rate to booster strain rate ($\beta = -0.4$; 95% CI −0.7, −0.02; $p = 0.039$).

Sparse principal component analysis identified 10 metabolite factors clustering in biologically related groupings (Table 4). Loading values for the 10 metabolite factors are illustrated in Supplementary Figure P. Univariate association between each of the 10 SPCA factors and left atrial function is shown in Supplementary Table S5. We noted only Factor 3 showed significant association with εs, εe, SRs, SRe and SRa in univariate analysis. However, after adjustments for significant clinical covariates Factor 3 (medium and long-chain dicarboxyl/hydroxyl acyl-carnitines) was only associated with reservoir strain (εs) ($\beta = -0.6$, $p = 0.038$) (Table 5).

## Discussion

Among community adults free of symptomatic cardiovascular disease, left atrial reservoir, conduit, and booster strain and strain rates were differentially associated with clinical variables and circulating metabolomics profiles. Age was independently associated with reservoir and conduit strain and strain rate, diabetes mellitus was associated with conduit strain, whereas heart rate was associated with booster strain and strain rate. Among the specific phases of left atrial function, serine was associated with reservoir function and booster strain rate, citrulline was associated with reservoir, booster and booster strain rate and valine was associated with ratio of conduit strain rate to booster strain rate. A combination of medium to long chain dicarboxyl acylcarnitines were associated with reservoir strain.

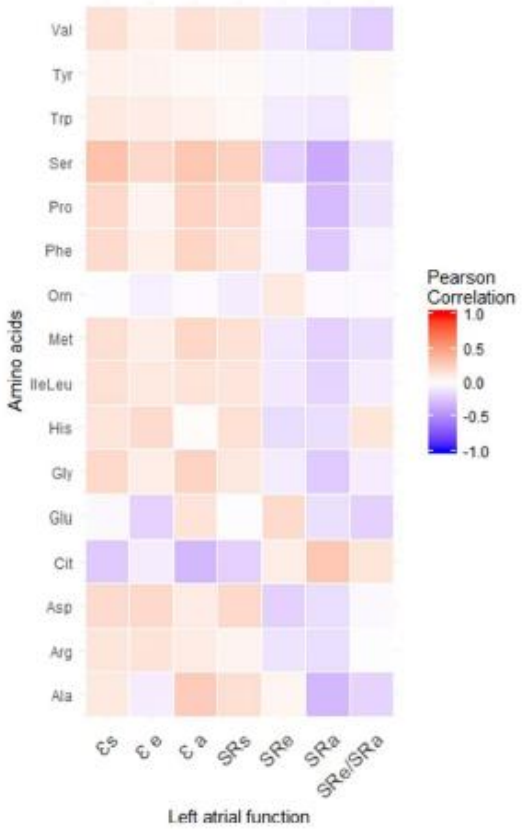


**Figure 2.** Heat map of correlations betweewas associated with ratio of conduitn amino acids and outcomes individually. The correlations increased from purple to red. Significant correlations are coloured while non-significant correlations are colourless).

**Clinical Implications.** This study advances the field of CMR feature tracking for future clinical applications. Previous studies have supported the concept that left atrial dilation and impairments in left atrial function may reflect the influence of concomitant risk factors[18] rather than age alone. In our study consisting of adults with risk factors, age is seen in independent associations with left atrial function even in the presence of risk factors, emphasizing that age plays an important role in assessment of left atrial function.

Using real-world data obtained from a community cohort of aged adults, our results suggest that age was independently associated with reservoir and conduit strain and strain rate. Our observations concur with data demonstrated by Evin *et al.*[4], who found similar decreases in left atrial reservoir and conduit phases with age, while also observing no association between booster strain and age. Our cohort included elderly subjects which were importantly lacking in the Evin *et al.*'s cohort. As such, our data nicely complement their findings, strengthening the observation that association between age and reservoir and conduit phases extends into older age groups. Taken together, future studies using reservoir and conduit strain and strain rates should therefore consider the critical role that age may play in influencing reservoir and conduit strain parameters. Age did not appear to influence booster functions.

We found that heart rate was independently associated with booster strain and booster strain rate. This is a novel finding not previously observed in similar asymptomatic cohorts, and deserves special attention, given the widespread use of medications that may influence heart rate in patients such as those with hypertension. Our observations about the association between heart rate and booster strain reflect similar concerns by a recent study that demonstrated impairments in booster strain associated with use of beta-blockers, among a hypertensive cohort[19]. While assessment for medication use such as beta-blockers might have sharpened our current observation regarding heart rate, which we did not have information for, our findings highlight the importance for future therapeutic trials to consider the impact of interventions that may have influence heart rate, particularly for improving booster function. Furthermore, booster strain and booster strain rate may be viewed as diagnostic

| Amino acids | LA function | Coef (95% CI) | *P*-value |
|---|---|---|---|
| Ala | εa | 0.2 (−4.3, 4.7) | 0.93 |
| | SRa | −0.2 (−0.8, 0.5) | 0.57 |
| | SRe/SRa | −0.3 (−0.7, 0.04) | 0.081 |
| Arg | — | — | — |
| Asp | — | — | — |
| Cit | εs | −4.0 (−7.2, −0.7) | **0.016** |
| | εa | −3.4 (−5.5, −1.2) | **0.002** |
| | SRa | 0.4 (0.1, 0.7) | **0.019** |
| Glu | — | — | — |
| Gly | εs | 1.5 (−5.5, 8.6) | 0.67 |
| | εa | 2.1 (−2.3, 6.6) | 0.35 |
| | SRa | −0.1 (−0.8, 0.6) | 0.76 |
| His | — | — | — |
| IleLeu | SRa | 0.1 (−0.5, 0.8) | 0.68 |
| Met | εa | 0.7 (−2.1, 3.5) | 0.63 |
| | SRa | −0.1 (−0.5, 0.4) | 0.78 |
| Orn | — | — | — |
| Phe | εs | 5.1 (−1.8, 12.0) | 0.14 |
| | εa | 3.8 (−0.9, 8.6) | 0.11 |
| | SRa | −0.6 (−1.3, 0.2) | 0.13 |
| Pro | εs | 0.2 (−5.7, 6.1) | 0.95 |
| | εa | 0.1 (−3.9, 4.2) | 0.95 |
| | SRs | 0.2 (−0.1, 0.6) | 0.20 |
| | SRa | −0.2 (−0.8, 0.4) | 0.56 |
| Ser | εs | 10.1 (2.0, 18.2) | **0.015** |
| | εa | 4.5 (−0.8, 9.8) | 0.098 |
| | SRs | 0.5 (0.04, 1.0) | **0.033** |
| | SRa | −0.9 (−1.7, −0.2) | **0.016** |
| Trp | — | — | — |
| Tyr | — | — | — |
| Val | SRe/SRa | −0.4 (−0.7, −0.02) | **0.039** |

**Table 3.** Multivariable model for association between individual amino acids and left atrial function. (1) The association of amino acids with LA function was first assessed using simple linear regression, individually. (2) For each amino acids with $p < 0.05$ in univariate analysis, multivariate linear regression was further conducted adjusting for clinical confounders. The adjustments are. Outcome εs; Confounder age. Outcome εe; Confounder Age, Diabetes mellitus. Outcome εa; Confounder Heart rate. Outcome SRs; Confounder Age. Outcome SRe; Confounder Age. Outcome SRa; Confounder Heart rate. Outcome SRe/SRa; Confounder Age. (3) Finally multivariate linear regression was conducted including all amino acids that show an association with $p < 0.05$ in the multivariable analysis (2).

markers of left atrial function that is independent of age (and risk factors), providing a unique opportunity for clinical applications to use them additionally as robust markers of disease progression less likely influenced by chronological aging. For instance, it has been shown that in the area of post-operative atrial fibrillation prediction, data from echocardiography have identified subclinical left atrial booster strain dysfunction in patients with severe aortic stenosis as a predictive marker of atrial fibrillation, regardless of global left ventricular function, left atrial volume index or aortic stenosis severity[20]. Subtle changes in booster strain function may be used as future marker of atrial fibrillation detection, which is particularly common among elderly populations[21].

**Mechanistic Underpinnings.** To the best of our knowledge, our study is the first to measure circulating metabolomics profiles in conjunction with left atrial function using the CMR feature tracking technique in a cohort without overt CVD (i.e., with preserved left ventricular function). This is in contrast to previous work that has studied metabolomics of the heart under conditions where the disease of interest involved impairments in left ventricular function[22,23]. The current work provides a unique opportunity to understand the association between atrial function and metabolomics profiles, which is otherwise difficult to demonstrate in disease states where the left ventricle is frequently abnormal. Our observations are further strengthened by adjustments for clinical variables, known to influence these phases of left atrial function.

Acyl-carnitines reflect upon mitochondrial fuel metabolism and changes in the pattern of individual acyl-carnitine species may reflect both global alterations in mitochondrial function as well as specific changes in patterns of fuel use. Alteration in long-chain acyl carnitines have previously been detected in symptomatic

| Factors | Description | Components | Percentage of variance accounted |
|---|---|---|---|
| 1 | Medium and long-chain carnitines | C8, C8-DC, C12:1, C12, C12-OH/C10-DC, C14:2, C14:1, C14, C16:3, C16:2, C16:1, C18:1 | 11 |
| 2 | Short chain dicarboxyl/hydroxyl carnitines | C3, C4, C5:1, C5, C4-OH, C6, C5OHC3DC, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C103, C81DC, C8-DC | 6.3 |
| 3 | Medium and long chain dicarboxyl/hydroxyl carnitines | C81OHC61DC, C122OHC102DC, C121OH, C142OH, C141OH, C163OHC143DC, C162OH C183OHC163DC, C182OHC162DC, C201, C20, C202OHC182DC, C201OHC181DC, C20OHC18DC, C221 | 7.2 |
| 4 | Long chain carnitines | C16, C183, C182, C181, C18, C204, C203, C202, C201, C202OHC182DC, C225, C224 C223 | 6.0 |
| 5 | Medium and long chain dicarboxyl/hydroxyl carnitines | C4OH, C8OHC6DC, C8DC, C12OHC10DC, C141OH, C14OHC12DC, C162OH, C161OHC141DC, C16OH, C181OHC161DC, C18OHC16DC, C20, C201OHC181DC, C20OHC18DC | 7.4 |
| 6 | Wide spectrum carnitines including odd short chain carnitines | C2, C3, C51, C5, C5OHC3DC, C101, C7DC, C121, C12, C14, C142OH, C163, C162OH, C16OH, C183, C182, C18, C183OHC163DC, C182OHC162DC, C204, C203, C202, C201, C203OHC183DC, C225, C223, C222, C22, Free Carnitine, Total Carnitine | 3.8 |
| 7 | Wide spectrum carnitines including ketone-derived carnitine | C2, C4OH, C6, C81, C5DC, C81OHC61DC, C103, C101, C10, C81DC, C122, C143, C142, C14, C142OH, C14OHC12DC, C162, C161, C16, C162OH, C161OHC141DC, C183, C182, C183OHC163DC, C18OHC16DC, C204, C202, C201OHC181DC, C224, C222, C22 | 4.5 |
| 8 | Wide spectrum carnitines including odd short chain carnitines | C3, C51, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C7DC, C81DC, C8DC, C122, C121, C12OHC10DC, C14OHC12DC, C16, C16OH, C183OHC163DC, C18OHC16DC, C204, C201, C20OHC18DC, C224, C223, C222, C221, Free Carnitine, Total Carnitine | 2.2 |
| 9 | Wide spectrum carnitines including ketone-derived carnitine | C2, C51, C4OH, C6, C5OHC3DC, C81OHC61DC, C101, C81DC, C12, C122OHC102DC, C121OH, C14, C142OH, C14OHC12DC, C162, C183, C182, C181, C183OHC163DC, C182OHC162DC, C181OHC161DC, C18OHC16DC, C204, C203, C201OHC181DC, C20OHC18DC, C225, Free Carnitine, Total Carnitine | 2.3 |
| 10 | Medium and long chain carnitines | C10, C143, C142, C14, C143OHC123DC, C142OH, C163, C16, C181, C18, C182OHC162DC, C204, C203, C201, C20, C221, C22 | 2.3 |

**Table 4.** Factors identified by sparse principal component analysis and the associated individual components, description and variance.

cohorts with clinical cardiovascular disease[9–12], including high-risk elderly with coronary artery disease or stroke[13]. The findings underscore important associations between mitochondrial pathways and cardiovascular disease. The dicarboxyl and hydroxyl acyl-carnitines are a specific class of acyl-carnitines generated via omega- and alpha-oxidation. Changes in the dicarboxyl- and hydroxyl-carnitines thus may reflect alterations in pathways spanning the endoplasmic reticulum (ER)[24], the peroxisome[25,26] and mitochondria. Our study identifies a unique association between a combination of medium and long chain dicarboxyl carnitines and reservoir function. This finding highlights potential links between ER, peroxisomal and mitochondrial function and left atrial reservoir function.

In addition, we observed novel patterns between left atrial function and circulating amino acids. Serine is a glucogenic amino acid which can also contribute to the biosynthesis of nucleotides as well as the ceramides, important signalling intermediates which have been linked to the development of cardiovascular disease[27]. Our novel data demonstrates an association between circulating levels of serine with left atrial function preceding clinically manifest atrial disease. Our hypothesis-generating data (1) supports the emerging recognition of serine-related molecules in atrial-related function[28–31]; (2) demonstrates it for the first time in a pre-disease cohort; and (3) suggests that circulating levels of serine are significantly associated with larger magnitude (i.e., beneficial) of left atrial reservoir strain and strain rate. Longitudinal studies in the future may use circulating profiles of serine to further investigate associations from a phase of pre-disease, atrial remodelling to clinical atrial dysfunction and disease.

We found that citrulline was associated with reservoir strain and booster strain rate. Citrulline contributes to the urea cycle, a mitochondrial-based pathway which has been reported to be involved in CVD[32]. Citrulline is also a major component of the nitric oxide pathway, which has been heavily implicated in the development of CVD[33] and may be important in atrial dysfunction[34]. Finally, valine was associated with ratio of conduit strain rate to booster strain rate. Valine is a branched-chain amino acid and has been consistently found to be associated with the development of insulin resistance and type 2 diabetes[35,36]. Changes in the branched-chain amino acids have also been linked to cardiovascular disease[11]. While the link between branched chain amino acids and disease is still unclear, evidence suggests that it contributes to altered mitochondrial function[35]. These results further

| | Unadjusted Coef (95% CI) | *P*-value | Adjusted Coef (95% CI) | *P*-value |
|---|---|---|---|---|
| εs | −0.8 (−1.4, −0.2) | **0.006** | **−0.6 (−1.1, −0.03)** | **0.038** |
| εe | −0.5 (−0.9, −0.1) | **0.017** | −0.2 (−0.5, 0.1) | 0.16 |
| εa | −0.3 (−0.7, 0.04) | 0.076 | −0.3 (−0.6, 0.1) | 0.18 |
| SRs | −0.04 (−0.1, −0.01) | **0.026** | −0.03 (−0.1, 0.01) | 0.14 |
| SRe | 0.1 (0.01, 0.1) | **0.014** | 0.02 (−0.01, 0.1) | 0.20 |
| SRa | 0.1 (0.002, 0.1) | **0.044** | 0.04 (−0.01, 0.1) | 0.13 |
| SRe/SRa | 0.01 (−0.03, 0.1) | 0.63 | 0.03 (−0.01, 0.1) | 0.19 |

**Table 5.** Linear regression on PCA factor 3 formed using acylcarnitine. εs, SRs, SRe, and SRe/SRa: age is adjusted; εa and SRa: heart rate is adjusted; εe: age and diabetes are adjusted;. SRe/SRa = ratio of SRe over SRa. Bold indicates significance at the 5% level.

highlight the potential importance of mitochondrial fuel metabolism changes in the pathogenesis of altered atrial function.

**Study limitations.** While our study was prospective, sample size was relatively small although we were able to identify statistically significant associations within the group. We acknowledge that samples obtained in a non-fasting state may potentially introduce analytic differences in post-absorptive states between the subjects studied. As a community-based driven study, we recognize challenges in getting subjects to fast while participating in these studies. Future studies comprising of fasting samples may provide additional insights as to the effect of fasting on similar analyses.

While we corrected for available clinical factors, we cannot exclude the possibility that additional factors that were not included could have influenced our findings. Our study design is cross-sectional and hence we cannot infer causal relationships. Future longitudinal follow-up in a larger cohort may provide greater power as well as further insights as to causality. However, our results highlight the clinical relevance of pursuing future clinical investigations using both a clinical imaging and a molecular approach as such a novel approach may help identify mechanisms involved in cardiovascular diseases in specific cohorts[37].

## Conclusion

The different phases of left atrial function as measured by CMR feature tracking were differentially associated with clinical and circulating metabolite factors. Our results emphasize the need to appreciate the impact of age and heart rate separately on phases of reservoir, conduit and booster functions. Furthermore, our results highlight potentially important upstream metabolic perturbations involving mitochondrial fuel metabolism which may result in changes to the ceramide pathway, urea cycle as well as nitric oxide metabolism.

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## Author Contributions

A.S.K. conceived the study and drafted the manuscript. F.G. performed the statistical analysis. L.S., J.P.K., X.D.Z., R.S.T., K.T.F., J.H.C., S.C. performed the study; J.M.Y., W.P.K. and L.Z. helped to revise the manuscript. All authors read and approved the final manuscript.

## Additional Information

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**Supplementary table S1: Intra and inter observer variability for strain and strain rate**

| | Intra-observer | | | Inter-observer | | |
|---|---|---|---|---|---|---|
| | r | Mean difference ± SD | COV (%) | r | Mean difference ± SD | COV (%) |
| $\mathbf{\varepsilon_s}$ | 0.995 | -0.11 ± 0.75 | 2.3 | 0.997 | -0.24 ± 0.69 | 2.2 |
| $\mathbf{\varepsilon_e}$ | 0.999 | 0.06 ± 0.25 | 1.6 | 0.991 | -0.22 ± 0.69 | 4.6 |
| $\mathbf{\varepsilon_a}$ | 0.993 | -0.06 ± 0.48 | 3.2 | 0.986 | 0.19 ± 0.72 | 4.9 |
| $\mathbf{SR_s}$ | 0.992 | -0.01 ± 0.07 | 3.9 | 0.987 | -0.03 ± 0.09 | 5.4 |
| $\mathbf{SR_e}$ | 0.996 | 0.01 ± 0.06 | 3.4 | 0.998 | -0.01 ± 0.06 | 3.4 |
| $\mathbf{SR_a}$ | 0.995 | -0.01 ± 0.07 | 3.6 | 0.997 | 0.04 ± 0.08 | 4.1 |

**Supplementary table S2: Other cardiac measurements of the cohort**

| **CMR measurements** | **Mean (SD)** |
|---|---|
| LV mass (g) | 75.0 (20.0) |
| LV mass index ($g/m^2$) | 46.6 (11.1) |
| LVEDV (ml) | 106.7 (27.9) |
| LVESV (ml) | 37.1 (17.3) |
| LV stroke volume (ml) | 69.3 (15.7) |
| LV ejection fraction (%) | 65.5 (7.3) |
| Echocardiogram-derived measurements | |
| Left atrial volume index ($ml/m^2$) | 23.6 (8.1) |
| MV E peak (ms) | 0.7 (0.2) |
| MV A peak (ms) | 0.8 (0.2) |
| E/A (ratio) | 0.9 (0.3) |
| Mitral deceleration time (ms) | 210.0 (37.0) |
| PASP (mmHg) | 27.6 (6.5) |
| PVS (cm/s) | 57.7 (11.5) |
| PVD (cm/s) | 47.5 (14.7) |
| PVA (cm/s) | 28.9 (5.2) |
| Septal Sm (m/s) | 0.1 (0.01) |
| Septal Em (m/s) | 0.1 (0.02) |
| Septal Am (m/s) | 0.1 (0.02) |
| Lateral Sm (m/s) | 0.1 (0.02) |
| Lateral Em (m/s) | 0.1 (0.02) |
| Lateral Am (m/s) | 0.1 (0.02) |

*Left ventricle (LV); end-diastolic volume (EDV); mitral valve (MV); peak blood velocity from Doppler echocardiography at early filling phase (E) and at atrial contraction phase (A); PASP (pulmonary artery systolic pressure); pulmonary vein blood velocity at systolic phase (PVS), diastolic phase (PVD) and atrial reversal phase (PVA); myocardial velocity from tissue Doppler imaging at systolic phase (Sm), early filling phase (Em) and atrial contraction phase (Am)*

**Supplementary table S3: List of measured metabolites**

| Short name | Name |
|---|---|
| **Ala** | Alanine |
| **Arg** | Arginine |
| **Asp** | Aspartic acid |
| **Cit** | Citrulline |
| **Glu/Gln** | Glutamate/Glutamine |
| **Gly** | Glycine |
| **His** | Histidine |
| **Ile/Leu** | Leucine/Isoleucine |
| **Met** | Methionine |
| **Orn** | Ornithine |
| **Phe** | Phenylalanine |
| **Pro** | Proline |
| **Ser** | Serine |
| **Trp** | Tryptophan |
| **Tyr** | Tyrosine |
| **Val** | Valine |
| **C2** | Acetyl carnitine |
| **C3** | Propionyl carnitine |
| **C4** | Butyryl carnitine or isobutryl carnitine |
| **C5:1** | Tiglyl carnitine or 3-methyl crotonyl carnitine |
| **C5** | Isovaleryl, 3-methylbutyryl carnitine , 2-Methylbutyryl, valeryl or pivaloyl carnitine |
| **C4-OH** | D-3-Hydroxy-butyryl carnitine, L-3-hydroxybutyryl carnitine |
| **C6** | Hexanoyl carnitine |
| **C5-OH/C3-DC** | 3-Hydroxy-isovaleryl carnitine or malonyl carnitine |
| **C4-DC/C6-OH** | Methylmalonyl carnitine or succinyl carnitine |
| **C8:1** | Octenoyl carnitine |
| **C8** | Octanoyl carnitine |
| **C5-DC** | Glutaryl carnitine, ethylmalonyl carnitine |
| **C8:1-OH/C6:1-DC** | 3-Hydroxy- octenoyl carnitine or hexenedioyl carnitine |
| **C8-OH/C6-DC** | 3-hydroxy octanoyl carnitine or adipoyl carnitine, 3-methylglutaryl carnitine |
| **C10:3** | Decatrienoyl carnitine |
| **C10:1** | Decenoyl carnitine |
| **C10** | Decanoyl carnitine |
| **C7-DC** | Pimeloyl carnitine, heptanedioyl carnitine |
| **C8:1-DC** | Octadecenedioyl carnitine |
| **C8-DC** | Suberoyl carnitine |
| **C12:2** | - |

| | |
|---|---|
| **C12:1** | Dodecenoyl carnitine |
| **C12** | Lauroyl carnitine |
| **C12:2-OH/C10:2-DC** | - |
| **C12:1-OH** | Hydroxydodecenoyl carnitine |
| **C12-OH/C10-DC** | 3-Hydroxy-dodecanoyl carnitine or sebacoyl carnitine |
| **C14:3** | - |
| **C14:2** | Tetradecadienoyl carnitine |
| **C14:1** | Tetradecenoyl carnitine |
| **C14** | Myristoyl carnitine |
| **C14:3-OH/C12:3-DC** | - |
| **C14:2-OH** | 3-Hydroxytetradecenoylcarnitine |
| **C14:1-OH** | 3-Hydroxy-tetradecenoyl carnitine |
| **C14-OH/C12-DC** | 3-Hydroxy-tetradecanoyl carnitine or dodecanedioyl carnitine |
| **C16:3** | - |
| **C16:2** | Hexadecadienoyl carnitine |
| **C16:1** | Palmitoleoyl carnitine |
| **C16** | Palmitoyl carnitine |
| **C16:3-OH/C14:3-DC** | - |
| **C16:2-OH** | 3-Hydroxyhexadecadienoyl carnitine |
| **C16:1-OH/C14:1-DC** | 3-Hydroxy-palmitoleoyl carnitine or cis-5-tetradecenedioyl carnitine |
| **C16-OH** | 3-Hydroxy-hexadecanoyl carnitine |
| **C18:3** | Linolenyl carnitine |
| **C18:2** | Linoleyl carnitine |
| **C18:1** | Oleyl carnitine |
| **C18** | Stearoyl carnitine |
| **C18:3-OH/C16:3-DC** | 3-Hydroxyl-linolenyl carnitine or |
| **C18:2-OH/C16:2-DC** | 3-Hydroxy-linoleyl carnitine or hexadecadienedioyl carnitine |
| **C18:1-OH/C16:1-DC** | 3-Hydroxy-octadecenoyl carnitine or hexadecanedioyl carnitine |
| **C18-OH/C16-DC** | 3-Hydroxy-octadecanoyl carnitine or hexadecanedioyl carnitine, thapsoyl carnitine |
| **C20:4** | Arachidonoyl carnitine |
| **C20:3** | Dihomogammalinolenyl carnitine |
| **C20:2** | - |
| **C20:1** | - |
| **C20** | Arachidoyl carnitine, eicosanoyl carnitine |
| **C20:3-OH/C18:3-DC** | - |
| **C20:2-OH/C18:2-DC** | - |
| **C20:1-OH/C18:1-DC** | Octadecenedioyl carnitine |
| **C20-OH/C18-DC** | 3-Hydroxy-eicosanoyl carnitine or octadecanedioyl carnitine |
| **C22:5** | - |
| **C22:4** | - |
| **C22:3** | - |

| | |
|---|---|
| **C22:2** | - |
| **C22:1** | - |
| **C22** | Docosanoyl carnitine, Behenoyl carnitine |
| **Free Carnitine** | |
| **Total Carnitine** | |

**Supplementary table S4: Summary of amino acids**

| **Metabolites** | **Mean (SD) µm** |
|---|---|
| **Ala** | 495.1 (136.6) |
| **Arg** | 116.4 (27.6) |
| **Asp** | 23.2 (6.2) |
| **Cit** | 34.4 (13.9) |
| **Glu** | 93.4 (23.5) |
| **Gly** | 233.5 (49.5) |
| **His** | 77.8 (22.6) |
| **IleLeu** | 151.5 (45.6) |
| **Met** | 27.2 (10.1) |
| **Orn** | 86.2 (28.3) |
| **Phe** | 77.3 (15.7) |
| **Pro** | 255.6 (72.2) |
| **Ser** | 122.4 (24.1) |
| **Trp** | 55.1 (14.1) |
| **Tyr** | 71.8 (21.2) |
| **Val** | 246.1 (61.5) |

**Supplementary table S5: Coefficient and 95% confidence generated using linear regression on 10 PCA formed using acylcarnitines with left atrial function**

| | Ɛs | Ɛe | Ɛa | SRs | SRe | SRa | SRe/SRa |
|---|---|---|---|---|---|---|---|
| **Factor 1** | 0.3 (-0.2, 0.8) | 0.3 (-0.1, 0.6) | 0.1 (-0.3, 0.4) | 0.02 (-0.01, 0.05) | -0.03 (-0.1, 0.02) | -0.01 (-0.1, 0.04) | 0.01 (-0.03, 0.04) |
| **Factor 2** | -0.2 (-0.9, 0.5) | -0.2 (-0.7, 0.3) | -0.03 (-0.5, 0.4) | 0.001 (-0.04, 0.05) | 0.04 (-0.03, 0.1) | 0.01 (-0.1, 0.1) | 0.003 (-0.05, 0.05) |
| **Factor 3** | **-0.9 (-1.5, -0.3)** | **-0.6 (-1.0, -0.1)** | -0.3 (-0.7, 0.04) | **-0.04 (-0.1, -0.01)** | **0.1 (0.02, 0.1)** | **0.1 (0.001, 0.1)** | 0.01 (-0.03, 0.05) |
| **Factor 4** | -0.2 (-0.8, 0.5) | -0.1 (-0.6, 0.3) | -0.1 (-0.5, 0.3) | 0.002 (-0.04, 0.04) | 0.01 (-0.04, 0.1) | 0.01 (-0.05, 0.07) | -0.001 (-0.04, 0.04) |
| **Factor 5** | -0.2 (-0.8, 0.4) | -0.3 (-0.7, 0.1) | 0.1 (-0.2, 0.5) | 0.003 (-0.03, 0.04) | 0.04 (-0.01, 0.1) | -0.02 (-0.1, 0.03) | -0.03 (-0.1, 0.01) |
| **Factor 6** | -0.2 (-1.1, 0.7) | -0.1 (-0.7, 0.5) | -0.02 (-0.6, 0.5) | -0.03 (-0.08, 0.03) | 0.02 (-0.1, 0.1) | -0.001 (-0.1, 0.1) | 0.001 (-0.1, 0.1) |
| **Factor 7** | 0.3 (-0.4, 1.1) | -0.03 (-0.6, 0.5) | 0.3 (-0.2, 0.8) | 0.02 (-0.03, 0.07) | 0.01 (-0.1, 0.1) | -0.05 (-0.1, 0.02) | -0.02 (-0.1, 0.04) |
| **Factor 8** | 0.5 (-0.6, 1.7) | 0.1 (-0.7, 0.9) | 0.2 (-0.6, 0.9) | 0.03 (-0.05, 0.1) | -0.03 (-0.1, 0.1) | -0.01 (-0.1, 0.1) | 0.003 (-0.1, 0.1) |
| **Factor 9** | -0.6 (-1.7, 0.6) | 0.03 (-0.8, 0.8) | -0.5 (-1.3, 0.2) | 0.003 (-0.07, 0.1) | -0.02 (-0.1, 0.1) | 0.1 (-0.04, 0.2) | 0.03 (-0.05, 0.1) |
| **Factor 10** | 0.8 (-0.1, 1.8) | 0.5 (-0.2, 1.2) | 0.4 (-0.2, 1.0) | 0.02 (-0.04, 0.08) | -0.1 (-0.1, 0.02) | -0.1 (-0.1, 0.04) | 0.04 (-0.03, 0.1) |

Bold indicates significance at the 5% level.

## COMMENTARY

### Cardiac structural and functional changes in ageing detected by cardiac imaging

Cardiovascular imaging has evolved tremendously over the last century. From detecting disease to picking up early preclinical stages of disease, modern imaging techniques have revolutionised the study of medicine[26]. In ageing, quantifying subtle functional cardiovascular changes through imaging is important in the setting of preclinical disease. In a large community study from MESA (Multi-Ethnic Study of Atherosclerosis), myocardial fibrosis in asymptomatic older adults was detected by T1 mapping on cardiac magnetic resonance imaging[27]. Endothelial dysfunction in the coronary circulation has also been measured in humans with microvascular dysfunction by positron emission tomography[28, 29]. Imaging tools such as phosphorus-31 spectroscopy for example, has depicted mitochondrial dysfunction in humans in relation to physical training[30]. Applying the use of imaging techniques to detect features of cardiac ageing when used in parallel with novel omics technologies will enhance discovery of biomarkers of cardiac ageing.

### Main findings of this study:

#### a) Left atrial function alterations as a marker of cardiovascular ageing in older adults

Age-related decreases in left ventricular relaxation increases left ventricular end-diastolic pressure, left atrial pressure and which results in increases in left atrial volume with time[31]. While left atrial volume may increase as a result of normal ageing[32], previous studies have attributed left atrial enlargement and left atrial function to the influence of concomitant risk factors[33], rather than due to the effect of age alone.

Among 128 participants (mean age 70.5 ± 11.6 years; 52 women) in this study, majority of participants had vascular risk factors of hypertension (53.1%) and dyslipidaemia (50.0%) while some had diabetes mellitus (21.9%). Despite the presence of risk factors, we found that age was independently associated with left

atrial function, emphasizing that age plays an important role in assessment of left atrial function. Importantly, the participants in our study were free of prevalent cardiovascular disease, highlighting that left atrial function alterations is a marker of cardiovascular ageing in older adults, observed even in the absence of cardiovascular disease[34].

Based on cardiac magnetic resonance imaging, an advanced method of cardiac imaging, left atrial function measurements were performed. Cine cardiac magnetic resonance was performed using balanced steady state free precession sequence. All participants were imaged on a 3T magnetic resonance imaging system (Ingenia, Philips Healthcare, The Netherlands) with a dStream Torso coil (maximal number of channels 32). BFFE end-expiratory breath hold cine images were acquired in multi-planar long-axis views (2-, 3-, and 4-chamber views) and a stack of parallel short-axis views to cover the left ventricle (LV) from base to apex. Typical parameters were as follows: TR/TE 3/1 ms; flip angle, 45°; in-plane spatial resolution, 1.0 mm x 1.0 mm to 1.5 mm x 1.5 mm; slice thickness, 8 mm; pixel bandwidth, 1797 Hz; field of view, 300 mm; frame rate, 30 or 40 per cardiac cycle. We developed an in-house semi-automatic algorithm to track the distance (L) between the left atrioventricular junction and a user-defined point at the mid posterior LA wall on standard CMR 2- and 4-chamber views[35, 36]. Both 2- and 4-chamber views were used to generate the average strain and strain rate results. Longitudinal strain ($\varepsilon$) at any time point ($t$) in the cardiac cycle from end-diastole (time 0) was calculated as: $\varepsilon(t) = (L(t) - L_0)/L_0$. LA reservoir strain ($\varepsilon_s$), conduit strain ($\varepsilon_e$) and booster strain ($\varepsilon_a$) were calculated at $t$ equals left ventricular end-systole, diastasis and pre-LA systole, respectively. To derive the peak strain rate (SR) indices, peak values of the first time derivative of the strain-time curve at systole, diastasis and LA contraction were measured. Strain and SR parameters from both 2- and 4-chamber views were averaged to obtain mean results for analysis (Figure 1).

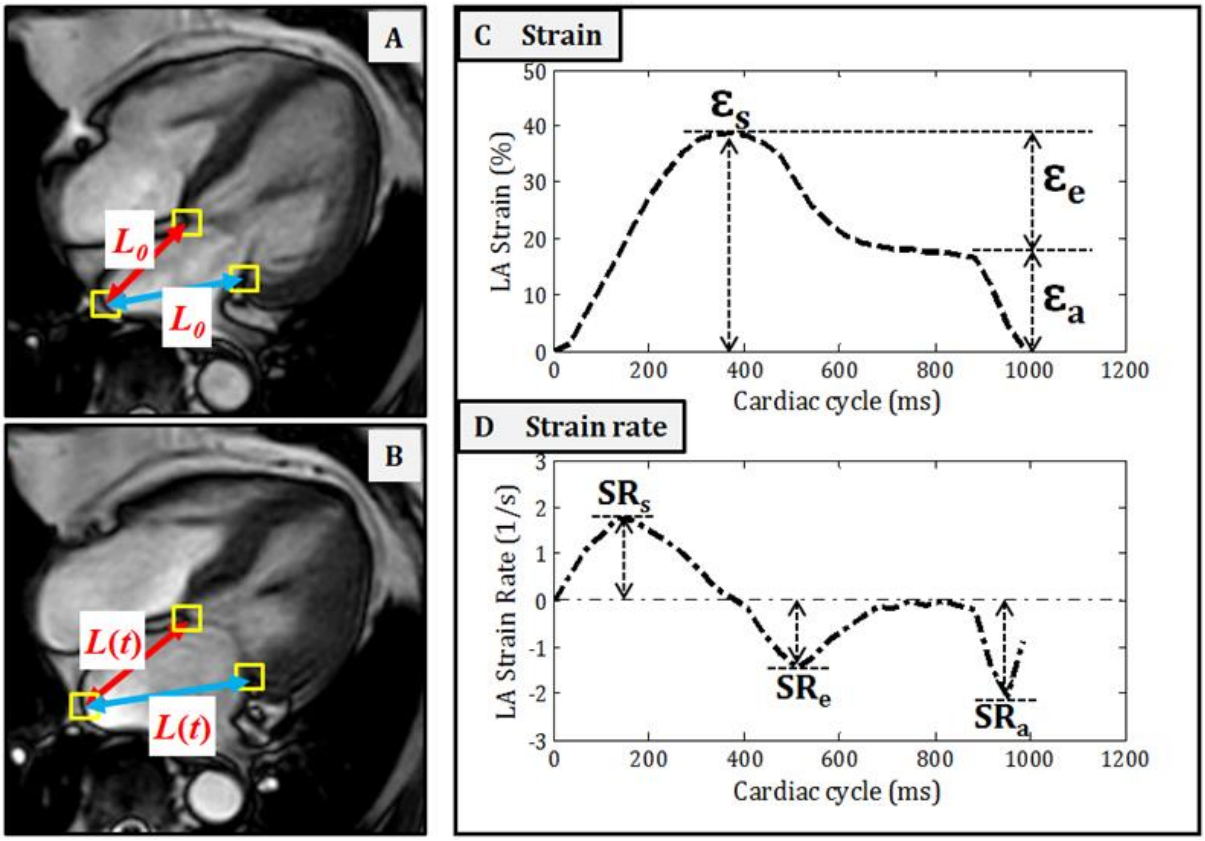


***Figure 1: Derivation of left atrial strain and strain rate parameters:*** *A-B) semi-automatic algorithm to track the distance (L) between the left atrioventricular junction and a user-defined point at the mid posterior left atrial (LA) wall on standard CMR 2- and 4-chamber views. (C-D) Longitudinal strain (ε) at any time point (t) in the cardiac cycle from end-diastole (time 0) was calculated as:* $\varepsilon(t) = (L(t) - L_0)/L_0$. *LA reservoir strain (*$\varepsilon_s$*), conduit strain (*$\varepsilon_e$*) and booster strain (*$\varepsilon_a$*) were calculated at t equals left ventricular end-systole, diastasis and pre-LA systole, respectively, and their corresponding peak strain rates (SR) derived.*

The left atrium is a highly dynamic chamber whose function is conventionally understood in three phases: reservoir, conduit, and booster. In the reservoir phase, the left atrium expands during left ventricular contraction and isovolumetric relaxation to receive venous return from the pulmonary circulation; in the conduit phase, the left atrium drains blood passively into the left ventricle; in the booster phase, the left atrium contracts upon stimulation by a sinus node depolarization, which contribute to 15-30% of left ventricular stroke volume[37].

In our study of community adults who were free of symptomatic cardiovascular disease, left atrial reservoir, conduit, and booster strain and strain rates were differentially associated with age and clinical profiles. We investigated the association between clinical risk factors left atrial function in two steps.

First, simple linear regression with left atrial function as dependent variable was performed for each of the left atrial function phases: reservoir, conduit, and booster function. Univariate analysis was performed and identified clinical risk factors that showed an association with $p < 0.05$. We observed univariate associations between clinical variables and left atrial function. Age and central pulse pressure were significant for reservoir strain; age, ever smoking, hypertension, diabetes, dyslipidaemia, and central systolic blood pressure were significant for conduit strain; heart rate was significant for booster strain. Age, body mass index, heart rate, central diastolic blood pressure, and central pulse pressure were significant for reservoir strain rate. Age, ever smoking, hypertension, diabetes, dyslipidaemia, and central systolic blood pressure were significant for conduit strain rate. Female gender, body mass index, heart rate and central pulse pressure were significant for booster strain rate. Age, ever smoked, diabetes mellitus, dyslipidaemia and central systolic blood pressure were significant for the ratio of conduit strain to booster strain rate.

Table 2 showed multivariate linear regression analysis between significant clinical variables and left atrial function. In multivariate analysis, age was significantly associated with reservoir strain ($\beta = -0.30$, $p < 0.0001$), conduit strain ($\beta = -0.3$, $p < 0.0001$), reservoir strain rate ($\beta = -0.02$, $p < 0.0001$), conduit strain rate ($\beta = 0.04$, $p < 0.0001$) and ratio of conduit strain to booster strain rate ($\beta = -0.01$, $p = 0.012$).

| **Clinical covariates** | **LV function** | **Coef. (95% CI)** | ***P*-value** |
|---|---|---|---|
| **Age (years)** | Ɛs | -0.3 (-0.4, -0.2) | **<0.0001** |
| | Ɛe | -0.3 (-0.4, -0.3) | **<0.0001** |
| | SRs | -0.02 (-0.03, -0.01) | **<0.0001** |
| | SRe | 0.04 (0.04, 0.1) | **<0.0001** |
| | SRe/SRa | -0.01 (-0.02, -0.003) | **0.011** |
| **Female** | SRa | 0.2 (-0.04, 0.5) | 0.098 |
| **Ever smoked** | SRe/SRa | -0.2 (-0.4, 0.1) | 0.14 |

| | | | |
|---|---|---|---|
| **Body mass index ((kg/m$^2$)** | SRs | -0.02 (-0.05, 0.001) | 0.063 |
| | SRa | 0.02 (-0.02, 0.1) | 0.32 |
| **Hypertension** | Ɛe | -0.6 (-2.0, 0.9) | 0.45 |
| | SRs | -0.01 (-0.2, 0.2) | 0.89 |
| | SRe | 0.08 (-0.1, 0.2) | 0.32 |
| **Diabetes mellitus** | SRe/SRa | -0.1 (-0.4, 0.1) | 0.19 |
| **Dyslipidaemia** | SRe/SRa | -0.1 (-0.3, 0.1) | 0.31 |
| **Heart rate (beats per minute)** | Ɛs | 0.1 (0.02, 0.2) | **0.024** |
| | Ɛa | 0.1 (0.1, 0.2) | **0.001** |
| | SRs | 0.01 (-0.00003, 0.01) | 0.051 |
| | SRa | -0.02 (-0.03, -0.01) | **<0.0001** |
| **Central systolic blood pressure (mmHg)** | Ɛe | -0.003 (-0.1, 0.04) | 0.89 |
| | SRe | 0.001 (-0.004, 0.01) | 0.67 |
| | SRe/SRa | -0.004 (-0.01, 0.001) | 0.12 |
| **Central diastolic blood pressure (mmHg)** | SRs | 0.01 (-0.001, 0.01) | 0.099 |
| **Central pulse pressure (mmHg)** | Ɛs | 0.02 (-0.1, 0.1) | 0.61 |
| | SRs | 0.002 (-0.004, 0.01) | 0.59 |
| | SRa | 0.001 (-0.01, 0.01) | 0.85 |

***Table 2: Multivariate analysis of clinical covariates associated with left atrial function*** *.Variables were selected based on simple linear regression with P<0.05 at univariate analysis. Univariable analysis results are described in the text.*

Overall, we found that age was independently associated with reservoir and conduit strain and strain rate, while hypertension, body mass index, dyslipidaemia and gender were not associated with left atrial function. Our real-world data obtained from a community cohort of aged adults, suggest that left atrial function linearly decreases with increasing age. Our observations strengthen other data from another study which similarly found decreases in left atrial reservoir and conduit phases with age[38], extending data of participants from older age groups. Our work implies that future translational studies could use reservoir and conduit strain and strain rates as surrogate targets of the ageing heart.

## b) Overview of omics and metabolomics

Omics refers to a comprehensive analysis of biological molecules which includes genomics, transcriptomics, proteomics, metabolomics, and microbiome.

There are two main analytical approaches used in metabolomics research: targeted and untargeted. Untargeted approaches involve comprehensive analysis of all the measurable analytes in a sample. The advantage to untargeted approaches is broad coverage of potentially important analytes and/or unbiased detection of biomarkers. The disadvantage to untargeted approaches includes a workflow which makes analysing large sample sets difficult, relative quantitation of compounds, a bias towards identifying compounds with high abundance and frequent inability to identify peaks of interest. Targeted approaches involve measuring pre-defined metabolites. Advantages to targeted approaches include use of internal standards which allows identification and absolute quantitation of analytes, including low abundance compounds as well as relatively fast workflow.

Both targeted and untargeted techniques are used to identify these biological molecules. Targeted techniques detect pre-specified known molecules. The disadvantage of targeted metabolomics is that clinically important analytes can be overlooked. In contrast, untargeted techniques discover as many molecules as possible. Untargeted analyses are generally used for discovery of hypothesis-generating data[39]. Typically, targeted techniques are conducted to test specific hypotheses and causal pathways[40].

As a systems biology tool, metabolomics measures large and diverse types of metabolites of different chemical properties. In contrast to genes and proteins that form the genome and proteome, metabolomics represents the metabolome which congregates net gene and protein expression into measurable metabolites in the blood stream. Metabolomics profiles are also influenced by external environments[41-43]. Therefore, metabolomics provides an integrated profile of an individual's biological status including effects from

external environments, which explains the saying: "the genome defines what may happen, the metabolome defines what has happened"[44]. Metabolomics quantifies small molecules that include fatty acids, amino acids, carbohydrates, and other molecules that comprise end products of biochemical cascades linking the genome, transcriptome and proteome to the phenotype. Methods that study the metabolome include gas chromatography coupled to mass spectrometry (GC-MS), liquid chromatography with single-stage mass spectrometry (LC-MS) or nuclear magnetic resonance (NMR) techniques.

### c) Metabolomics implications of left atrial ageing in older adults

While there may be many biomarkers for detecting risk of cardiovascular disease, there are relatively fewer investigations into biomarkers for detecting risk of cardiac ageing. As ageing is a complex physiological and pathological process, the integrated depiction of an individual's biological status that metabolomics can provide is a useful method for understanding mechanisms of ageing.

We used targeted metabolomics profiling for this work. Current evidence points to the importance of fuel metabolism and mitochondrial oxidation pathways for cardiovascular disease, hence we studied panels of acylcarnitine and putative amino acids involved in these pathways.

Antecubital venous blood samples (20–30 ml) were taken from consenting participants in the morning. After collection, the blood samples were immediately placed on ice for transportation and were processed within 6 h to obtain serum samples, which were subsequently stored at −80 °C. Serum metabolomic profiling analysis was performed in the Duke-NUS Metabolomics Facility. Thawed serum samples (100 µl) were spiked with 20 µl deuterium-labelled amino acid/acyl-carnitine mixture and diluted with 800 µl methanol. After centrifugation of the mixture at 17,000 g for 5 mins at 20 °C, the supernatant fraction was collected and divided into two parts: one (100 µl) for acylcarnitine analysis and one (10 µl) of each extracted serum sample. Amino acids were separated using a C8 column (Rapid Resolution HT, 4.5 × 50 mm, 1.8

μm, Zorbax SB-C8) on an Agilent 1290 Infinity LC system (Agilent Technologies, CA, USA) coupled with quadrupole-ion trap mass spectrometer (QTRAP 5500, AB Sciex, DC, USA). Mobile phase A (10/90 Water/Acetonitrile) and Mobile phase B (90/10 Water/ Acetonitrile), both containing 10 mM of Ammonium formate, were used for chromatography separation. Acylcarnitine measurements were made using flow injection tandem mass spectrometry on the Agilent 6430 Triple Quadrupole LC/MS system (Agilent Technologies, CA, USA). The sample analysis was carried out at 0.4 ml/min of 80/20 Methanol/water as mobile phase, and injection of 4 μL of sample. Data acquisition and analysis were performed on Agilent Mass Hunter Workstation B.06.00 Software.

We analysed 83 metabolites comprising 65 acyl-carnitine metabolites, 16 amino acid metabolites and 2 carnitine metabolites. Metabolites with >25% of values below the lower limit of quantification were excluded from analysis (only C10:2 was excluded, hence a total of 83 metabolites were analysed in the final sample). We normalised the distributions of all metabolites by a logarithmic transformation. We identified amino acids associated with LA function, respectively, in 3 ways. Firstly, simple linear regression with LA function as a dependent variable was used respectively to determine the significance of the individual amino acids. Secondly, multivariate linear regression was conducted for each amino acids with $p < 0.05$ in univariate analysis adjusting for significant clinical risk factors identified. Thirdly, multivariate linear regression was conducted including all amino acids that show an association with $p < 0.05$ with LA function in the multivariate analysis adjusting for clinical confounders. To identify metabolites correlations (65 acyl-carnitine metabolites and 2 carnitine metabolites) and reduce the dimensionality of correlated metabolites, we performed sparse principal component analysis (SPCA), which used a penalised matrix decomposition[45]. Compared to the regular principal component analysis that suffers from the fact of a dense loading matrix from all variables, SPCA is capable of producing sparse loadings which makes it more biologically interpretable. Specifically, we set the orthogonality constraint on each component and the number of components to be 10. Description of each component and the proportion of variance-accounted is shown in Table 4.

| Factors | Description | Components | Proportion of variance accounted |
|---|---|---|---|
| **1** | Medium and long-chain carnitines | C8, C8-DC, C12:1, C12, C12-OH/C10-DC, C14:2, C14:1, C14, C16:3, C16:2, C16:1, C18:1 | 11 |
| **2** | Short chain dicarboxyl/hydroxyl carnitines | C3, C4, C5:1, C5, C4-OH, C6, C5OHC3DC, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C103, C81DC, C8-DC | 6.3 |
| **3** | Medium and long chain dicarboxyl/hydroxyl carnitines | C81OHC61DC, C122OHC102DC, C121OH, C142OH, C141OH, C163OHC143DC, C162OH C183OHC163DC, C182OHC162DC, C201, C20, C202OHC182DC, C201OHC181DC, C20OHC18DC, C221 | 7.2 |
| **4** | Long chain carnitines | C16, C183, C182, C181, C18, C204, C203, C202, C201, C202OHC182DC, C225, C224 C223 | 6.0 |
| **5** | Medium and long chain dicarboxyl/hydroxyl carnitines | C4OH, C8OHC6DC, C8DC, C12OHC10DC, C141OH, C14OHC12DC, C162OH, C161OHC141DC, C16OH, C181OHC161DC, C18OHC16DC, C20, C201OHC181DC, C20OHC18DC | 7.4 |
| **6** | Wide spectrum carnitines including odd short chain carnitines | C2, C3, C51, C5, C5OHC3DC, C101, C7DC, C121, C12, C14, C142OH, C163, C162OH, C16OH, C183, C182, C18, C183OHC163DC, C182OHC162DC, C204, C203, C202, C201, C203OHC183DC, C225, C223, C222, C22, Free Carnitine, Total Carnitine | 3.8 |
| **7** | Wide spectrum carnitines including ketone-derived carnitine | C2, C4OH, C6, C81, C5DC, C81OHC61DC, C103, C101, C10, C81DC, C122, C143, C142, C14, C142OH, C14OHC12DC, C162, C161, C16, C162OH, C161OHC141DC, C183, C182, C183OHC163DC, C18OHC16DC, C204, C202, C201OHC181DC, C224, C222, C22 | 4.5 |
| **8** | Wide spectrum carnitines including odd short chain carnitines | C3, C51, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C7DC, C81DC, C8DC, C122, C121, C12OHC10DC, C14OHC12DC, C16, C16OH, C183OHC163DC, C18OHC16DC, C204, C201, C20OHC18DC, C224, C223, C222, C221, Free Carnitine, Total Carnitine | 2.2 |
| **9** | Wide spectrum carnitines including ketone-derived carnitine | C2, C51, C4OH, C6, C5OHC3DC, C81OHC61DC, C101, C81DC, C12, C122OHC102DC, C121OH, C14, C142OH, C14OHC12DC, C162, C183, C182, C181, C183OHC163DC, C182OHC162DC, C181OHC161DC, C18OHC16DC, C204, C203, C201OHC181DC, C20OHC18DC, C225, Free Carnitine, Total Carnitine | 2.3 |
| **10** | Medium and long chain carnitines | C10, C143, C142, C14, C143OHC123DC, C142OH, C163, C16, C181, C18, C182OHC162DC, C204, C203, C201, C20, C221, C22 | 2.3 |

***Table 4. Factors identified by sparse principal component analysis and the associated individual components, description and variance.***

To assess the association between the 10 SPCA factors and LA function, we first performed simple linear regression with LA function as dependent variable, respectively. Further, for each SPCA factor, we performed multivariable linear regression adjusting for significant clinical confounders identified. All statistical analyses were performed using STATA 13 (College Station, Texas, USA), while the SPCA and correlation matrix heatmap showing pairwise Pearson correlations (r) between amino acids and LA function were performed by R. For all analysis, a two-tailed *P* value of <0.05 was considered significant.

Correlations for the 16 amino acids were assessed using the Pearson correlation analysis (Figure 2). We observed that serine was significantly correlated with all LA function except the ratio SRe/SRa (r ranges from −0.36 to 0.32; all $p < 0.05$) whilst arginine, histidine, ornithine, tryptophan and tyrosine were not correlated with any LA function.

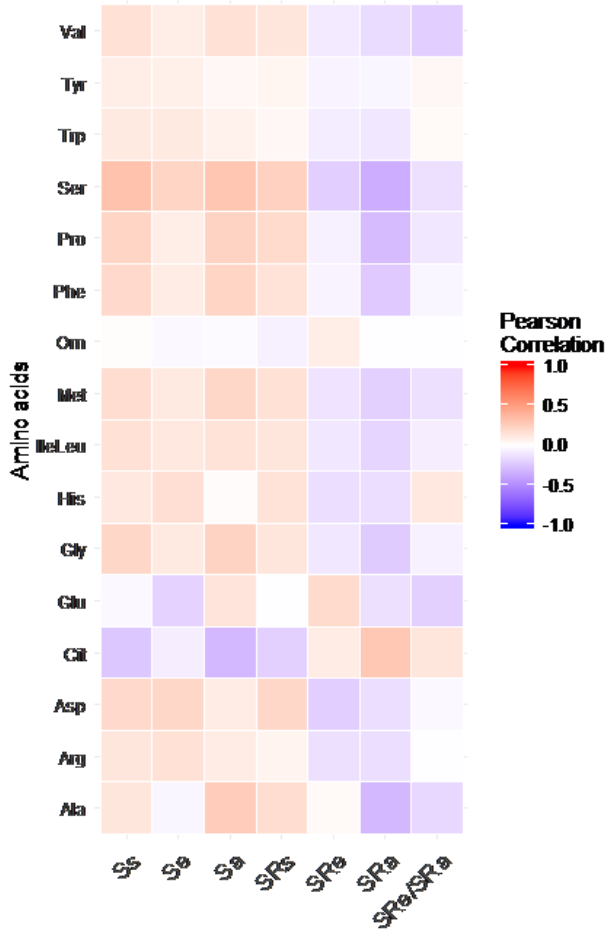


***Figure 2. Correlation matrix heatmap.*** *Heat map of correlations between amino acids and outcomes individually. The correlations increased from purple to red. Significant correlations are coloured while non-significant correlations are colourless.*

Table 3 shows multivariate analysis between individual amino acids and corresponding left atrial functions, adjusting for prior clinical covariates. Higher serine was significantly associated with higher reservoir strain ($\beta$ = 10.1; 95% CI 2.0, 18.2; $p$ = 0.015), reservoir strain rate ($\beta$ = 0.5; 95% CI 0.04, 1.0; $p$ = 0.033) and booster strain rate ($\beta$ = −0.9; 95% CI −1.7, −0.2; $p$ = 0.016). Higher citrulline was associated with lower reservoir strain ($\beta$ = −4.0; 95% CI −7.2, −0.7; p = 0.016), booster strain ($\beta$ = −3.4; 95% CI −5.5, −1.2; $p$ =

0.002) and booster strain rate ($\beta$ = 0.4; 95% CI 0.1, 0.7; $p$ = 0.019). Higher valine was associated with lower ratio of conduit strain rate to booster strain rate ($\beta$ = −0.4; 95% CI −0.7, −0.02; $p$ = 0.039).

| Amino acids | LV function | Coeff (95% CI) | *P*-value |
|---|---|---|---|
| **Ala** | Ɛa | 0.2 (-4.3, 4.7) | 0.93 |
| | SRs | 0.1 (-0.3, 0.5) | 0.57 |
| | SRe/SRa | -0.3 (-0.7, 0.04) | 0.081 |
| **Arg** | - | - | - |
| **Asp** | - | - | - |
| **Cit** | Ɛs | -4.0 (-7.2, -0.7) | **0.016** |
| | Ɛa | -3.4 (-5.5, -1.2) | **0.002** |
| | SRa | 0.4 (0.1, 0.7) | **0.019** |
| **Glu** | - | - | - |
| **Gly** | Ɛs | 1.5 (-5.5, 8.6) | 0.67 |
| | Ɛa | 2.1 (-2.3, 6.6) | 0.35 |
| | SRa | -0.1 (-0.8, 0.6) | 0.76 |
| **His** | - | - | - |
| **IleLeu** | SRa | 0.1 (-0.5, 0.8) | -0.68 |
| | | | |
| **Met** | Ɛa | 0.7 (-2.1, 3.5) | 0.63 |
| | SRa | -0.1 (-0.5, 0.4) | 0.78 |
| **Orn** | - | - | - |
| **Phe** | Ɛs | 5.1 (-1.8, 12.0) | 0.14 |
| | Ɛa | 3.8 (-0.9, 8.6) | 0.11 |
| | SRa | -0.6 (-1.3, 0.2) | 0.13 |
| **Pro** | Ɛs | 0.2 (-5.7, 6.1) | 0.95 |
| | Ɛa | 0.1 (-3.9, 4.2) | 0.95 |
| | SRs | 0.2 (-0.1, 0.6) | 0.20 |
| | SRa | -0.2 (-0.8, 0.4) | 0.56 |
| **Ser** | Ɛs | 10.1 (2.0, 18.2) | **0.015** |
| | Ɛa | 4.5 (-0.8, 9.8) | 0.098 |
| | SRs | 0.5 (0.04, 1.0) | **0.033** |
| | SRa | -0.9 (-1.7, -0.2) | **0.016** |
| **Trp** | - | - | - |
| **Tyr** | - | - | - |
| **Val** | SRe/SRa | -0.4 (-0.7, -0.02) | **0.039** |

***Table 3. Multivariable model for association between individual amino acids and left atrial function.*** *(1) The association of amino acids with LA function was first assessed using simple linear regression, individually. (2) For each amino acids with p<0.05 in univariate analysis, multivariate linear regression was further conducted adjusting for clinical confounders. (3) Finally multivariate linear regression was conducted including all amino acids that show an association with p<0.05 in the multivariable analysis (2).*

After adjustments for significant clinical covariates Factor 3 (medium and long-chain dicarboxyl/hydroxyl acyl-carnitines) was only associated with reservoir strain (εs) (β = −0.6, $p$ = 0.038) (Supplementary Table S5).

| | **Ɛs** | **Ɛe** | **Ɛa** | **SRs** | **SRe** | **SRa** | **SRe/SRa** |
|---|---|---|---|---|---|---|---|
| **Factor 1** | 0.3 (-0.2, 0.8) | 0.3 (-0.1, 0.6) | 0.1 (-0.3, 0.4) | 0.02 (-0.01, 0.05) | -0.03 (-0.1, 0.02) | -0.01 (-0.1, 0.04) | 0.01 (-0.03, 4) |
| **Factor 2** | -0.1 (-0.8, 0.6) | -0.2 (-0.6, 0.3) | -0.03 (-0.5, 0.4) | 0.003 (-0.04, 0.05) | 0.03 (-0.03, 0.1) | 0.02 (-0.05, 0.1) | 0.01 (-0.04, 0.1) |
| **Factor 3** | **-0.8 (-1.4, -0.2)** | **-0.5 (-0.9, -0.1)** | -0.3 (-0.7, 0.04) | **-0.04 (-0.1, -0.01)** | **0.1 (0.01, 0.1)** | **0.1 (0.002, 0.1)** | 0.01 (-0.03, 0.1) |
| **Factor 4** | -0.1 (-0.7, 0.6) | -0.1 (-0.5, 0.4) | -0.1 (-0.5, 0.3) | 0.004 (-0.04, 0.04) | 0.01 (-0.05, 0.1) | 0.01 (-0.05, 0.1) | 0.002 (-0.04, 0.05) |
| **Factor 5** | -0.2 (-0.8, 0.4) | -0.3 (-0.7, 0.1) | 0.1 (-0.2, 0.5) | 0.003 (-0.03, 0.04) | 0.04 (-0.01, 0.1) | -0.02 (-0.1, 0.03) | -0.03 (-0.1, 0.01) |
| **Factor 6** | -0.3 (-1.1, 0.6) | -0.2 (-0.8, 0.4) | -0.02 (-0.6, 0.5) | -0.03 (-0.08, 0.02) | 0.03 (-0.05, 0.1) | -0.002 (-0.1, 0.1) | -0.002 (-0.1, 0.1) |
| **Factor 7** | 0.3 (-0.5, 1.0) | -0.1 (-0.6, 0.4) | 0.3 (-0.2, 0.8) | 0.02 (-0.03, 0.07) | 0.02 (-0.05, 0.1) | -0.05 (-0.1, 0.02) | -0.02 (-0.1, 0.03) |
| **Factor 8** | 0.5 (-0.7, 1.7) | 0.1 (-0.7, 0.9) | 0.2 (-0.6, 0.9) | 0.02 (-0.05, 0.1) | -0.03 (-0.1, 0.1) | -0.01 (-0.1, 0.1) | 0.002 (-0.1, 0.1) |
| **Factor 9** | -0.03 (-1.1, 1.2) | 0.7 (-0.1, 1.5) | -0.5 (-1.3, 0.2) | 0.02 (-0.1, 0.1) | -0.1 (-0.2, 0.03) | 0.1 (-0.03, 0.2) | 0.06 (-0.02, 0.1) |
| **Factor 10** | 0.8 (-0.2, 1.8) | 0.5 (-0.2, 1.2) | 0.4 (-0.2, 1.0) | 0.02 (-0.05, 0.1) | -0.1 (-0.1, 0.02) | -0.1 (-0.1, 0.04) | 0.04 (-0.03, 0.1) |

***Supplementary table S5: Coefficient and 95% confidence generated using linear regression on 10 PCA formed using acylcarnitines with left atrial function .*** *Bold indicates significance at the 5% level.*

Among the specific phases of left atrial function, serine was associated with reservoir function and booster strain rate, citrulline was associated with reservoir, booster and booster strain rate and valine was associated with ratio of conduit strain rate to booster strain rate. A combination of medium to long chain dicarboxyl acylcarnitines were associated with reservoir strain. Our findings are further strengthened by adjustments for clinical variables, known to influence these phases of left atrial function.

Acyl-carnitines reflect upon mitochondrial fuel metabolism and changes in the pattern of individual acyl-carnitine species may reflect both global alterations in mitochondrial function as well as specific changes in patterns of fuel use. Alteration in long-chain acyl carnitines have previously been detected in

symptomatic stroke[46]. The findings underscore important associations between mitochondrial pathways and cardiovascular disease.

On the other hand, the dicarboxyl and hydroxyl acyl-carnitines are a specific class of acyl-carnitines generated via omega and alpha-oxidation. Changes in the dicarboxyl- and hydroxyl-carnitines thus may reflect alterations in pathways spanning the endoplasmic reticulum (ER)[47], the peroxisome and mitochondria[48, 49]. Our study identifies a unique association between a combination of medium and long chain dicarboxyl carnitines and LA reservoir function. This finding highlights potential links between ER, peroxisomal and mitochondrial function and left atrial reservoir function.

The patterns between left atrial function and circulating amino acids observed in our study are novel. Serine is a glucogenic amino acid which can also contribute to the biosynthesis of nucleotides as well as the ceramides, important signalling intermediates which have been linked to the development of cardiovascular disease[50]. Our novel data demonstrates an association between circulating levels of serine with left atrial function preceding clinically manifest atrial disease. This observation is in line with the emerging recognition of serine-related molecules in atrial-related function[51-54]. Our quantitative metabolomics approach suggests that circulating levels of serine are significantly associated with larger magnitude (i.e., beneficial) of left atrial reservoir strain and strain rate. Future studies may use circulating profiles of serine to further investigate associations from a phase of pre-disease, atrial remodelling to clinical atrial dysfunction and disease.

Citrulline was associated with reservoir strain and booster strain rate. Citrulline contributes to the urea cycle, a mitochondrial-based pathway which has been reported to be involved in CVD[55]. Citrulline is a major component of the nitric oxide pathway, which may be important in atrial dysfunction[56]. We also found that valine was associated with ratio of conduit strain rate to booster strain rate. Valine is a branched-

chain amino acid and changes in the branched-chain amino acids have been linked to cardiovascular disease[57] and altered mitochondrial function[58].

Overall, our work highlights the potential importance of mitochondrial fuel metabolism changes in the pathogenesis of altered atrial function among older adults.

I am the principal investigator of the study. My contribution to this work includes obtaining grant funding for this work, setting up the study protocol, recruitment of research participants, obtaining ethical approval, data analyses, manuscript writing and manuscript review.

**PUBLICATION #2**

**Gao F, Kovalik JP, Zhao X, Chow VJ, Chew H, Teo LL, Tan RS, Leng S, Ewe SH, Tan HC, Tan TY, Lee LS, Ching J, Keng BM, Zhong L, Koh WP and Koh AS.**

**Exacerbation of cardiovascular ageing by diabetes mellitus and its associations with acyl-carnitines.** ***Aging (Albany NY).*** **2021;13:14785-14805[59].**

*"Objective: To demonstrate differences in cardiovascular structure and function between diabetic and non-diabetic older adults. To investigate associations between acyl-carnitines and cardiovascular function as indexed by imaging measurements. Methods: A community-based cohort of older adults without cardiovascular disease underwent current cardiovascular imaging and metabolomics acyl-carnitines profiling based on current and archived sera obtained fifteen years prior to examination. Results: A total of 933 participants (women 56%, n=521) with a mean age 63±13 years were studied. Old diabetics compared to old non-diabetics had lower myocardial relaxation (0.8±0.2 vs 0.9±0.3, p=0.0039); lower left atrial conduit strain (12±4.3 vs 14±4.1, p=0.045), lower left atrial conduit strain rate (-1.2±0.4 vs -1.3±0.5, p=0.042) and lower ratio of left atrial conduit strain to left atrial booster strain (0.5±0.2 vs 0.7±0.3, p=0.0029). Higher levels of archived short chain acyl-carnitine were associated with present-day impairments in myocardial relaxation (C5:1; OR 1.03, p=0.011), worse left atrial conduit strain function (C5:1; OR 1.03, p=0.037). Increases in hydroxylated acylcarnitines were associated with worse left atrial conduit strain [(C4-OH; OR 1.05, p=0.0017), (C16:2-OH; OR 1.18, p=0.037)]. Current, archived and changes in long chain acyl-carnitines were associated with cardiovascular functions [(C16; OR 1.02, p=0.002), (C20:3; OR 1.01, p=0.014), (C14:3; OR 1.12, p=0.033), (C18:1; OR 1.01, p=0.018), (C18:2; OR 1.01, p=0.028), (C20:4; OR 1.10, p=0.038)] (all p<0.05). Conclusion: Older diabetic adults had significant impairments in left ventricular myocardial relaxation and left atrial strain, compared to older*

*non-diabetic adults. Short chain and long chain, di-carboxyl and hydroxylated acyl-carnitines were associated with these cardiovascular functional differences."*

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Research Paper

# Exacerbation of cardiovascular ageing by diabetes mellitus and its associations with acyl-carnitines

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## ABSTRACT

**Objective: To demonstrate differences in cardiovascular structure and function between diabetic and non-diabetic older adults. To investigate associations between acyl-carnitines and cardiovascular function as indexed by imaging measurements.**

**Methods: A community-based cohort of older adults without cardiovascular disease underwent current cardiovascular imaging and metabolomics acyl-carnitines profiling based on current and archived sera obtained fifteen years prior to examination.**

**Results: A total of 933 participants (women 56%, n=521) with a mean age 63±13 years were studied. Old diabetics compared to old non-diabetics had lower myocardial relaxation (0.8±0.2 vs 0.9±0.3, p=0.0039); lower left atrial conduit strain (12±4.3 vs 14±4.1, p=0.045), lower left atrial conduit strain rate (-1.2±0.4 vs -1.3±0.5, p=0.042) and lower ratio of left atrial conduit strain to left atrial booster strain (0.5±0.2 vs 0.7±0.3, p=0.0029). Higher levels of archived short chain acyl-carnitine were associated with present-day impairments in myocardial relaxation (C5:1; OR 1.03, p=0.011), worse left atrial conduit strain function (C5:1; OR 1.03, p=0.037). Increases in hydroxylated acyl-carnitines were associated with worse left atrial conduit strain [(C4-OH; OR 1.05, p=0.0017), (C16:2-OH; OR 1.18, p=0.037)]. Current, archived and changes in long chain acyl-carnitines were associated with cardiovascular functions [(C16; OR 1.02, p=0.002), (C20:3; OR 1.01, p=0.014), (C14:3; OR 1.12, p=0.033), (C18:1; OR 1.01, p=0.018), (C18:2; OR 1.01, p=0.028), (C20:4; OR 1.10, p=0.038)] (all p<0.05).**

**Conclusion: Older diabetic adults had significant impairments in left ventricular myocardial relaxation and left atrial strain, compared to older non-diabetic adults. Short chain and long chain, di-carboxyl and hydroxylated acyl-carnitines were associated with these cardiovascular functional differences.**

## INTRODUCTION

Ageing is a well-known cardiovascular risk factor that heightens risks of cardiovascular disease including myocardial infarction, heart failure, and stroke [1]. However, ageing as a risk factor exists in the same milieu as other risk factors, such as obesity and diabetes mellitus. While the individual effects on the

cardiovascular system by these risk factors are known, the extent, of each risk factor, superimposed onto cardiovascular ageing, is not readily appreciated. The extent to which diabetes mellitus modifies the effect of ageing on the heart, deserves deeper investigation. The clinical implication of ageing with or without diabetes may herald differences in cardiovascular phenotype, outcomes and treatments. For example, age-associated decreases in left ventricular volumes, increases in left ventricular mass index, and deteriorations in diastolic function are commonly observed in heart failure among elderly patients [2]. The fact that heart failure can occur without diabetes (or hypertension) as a main driver, implies an urgent need to differentiate the ageing phenotype, from phenotypes related to traditional risk factors [3].

The growing elderly population worldwide highlights the need for unique strategies to confront unresolved risks of heart failure burdens among the elderly [4]. The metabolome represents net profile of diverse chemicals that is influenced by genomics, transcriptomics and proteomic variability [5]. Metabolomic profiles are also influenced by environmental exposure, diet and lifestyle [6–8]. Since metabolomics provides an integrated profile, it may serve as a conglomerating tool for life course phenomena as heterogeneous as ageing. Emerging data have demonstrated the utility of metabolomics in advancing understanding of cardiovascular ageing [9–11], insulin resistance, and diabetes [12, 13]. Therefore, metabolomics may represent a novel approach that dissects new associations between cardiovascular ageing, diabetes and metabolic pathways.

Among a community cohort of participants with risk factors but free of cardiovascular disease, we illustrate structural and functional changes associated with cardiovascular ageing and qualify the impact of diabetes mellitus on cardiovascular ageing changes. In addition, by utilizing cross-sectional and archived metabolomic profiles across the participants' lifespan, we hypothesize that metabolites may be associated with CV changes that differentiate between older adults with diabetes versus older adults without diabetes.

## MATERIALS AND METHODS

### Study population

The subjects were recruited from the Cardiac Ageing Study (CAS)[9], a prospective study initiated in 2014 that examines characteristics and determinants of cardiovascular function in elderly adults. CAS participants were recruited from the prospective, population-based cohort, the Singapore Chinese Health Study (SCHS) [14] and directly from the local community. The current study sample consisted of men and women who participated in the baseline CAS 2014-2017 examination who had no self-reported history of physician-diagnosed cardiovascular disease (such as coronary heart disease, atrial fibrillation), stroke or cancer. We studied the subjects in three groups, comprising of young adults (age < 65 years old), and old adults (age ≥65 years), the latter old group was further categorized into diabetic and non-diabetic adults.

Written informed consent was obtained from participants upon enrolment. The SingHealth Centralised Institutional Review Board (CIRC/2014/628/C) had approved the study protocol.

### Data acquisition

All participants were examined and interviewed on one study visit by trained study coordinators. Participants completed a standardized questionnaire that included medical history and coronary risk factors. Sinus rhythm status was ascertained by resting electrocardiogram. Clinical data were obtained on the same day as assessment of echocardiography and serum collection.

Echocardiography was performed using ALOKA α10 with a 3.5 MHz probe. In each subject, standard echocardiography, which included 2-D, M-mode, pulse Doppler and tissue Doppler imaging, was performed in the standard parasternal and apical (apical 4-chamber, apical 2-chamber and apical long) views, and three cardiac cycles were recorded. E/A ratio was computed as a ratio of peak velocity flow in early diastole E (m/s) to peak velocity flow in late diastole by atrial contraction A (m/s).

Blood samples were collected on the day of echocardiography acquisition. Plasma levels of Galectin-3 (Gal-3) (ARCHITECT Galectin-3; produced by Fujirebio Diagnostics Inc for Abbott Laboratories) and B-type natriuretic peptide (BNP) (ARCHITECT BNP; produced by Fujirebio Diagnostics Inc for Abbott Laboratories) were measured on the Abbott ARCHITECT i2000SR analyzer.

Cine cardiac magnetic resonance (CMR) scans were performed using balanced fast field echo sequence (BFFE). All subjects were imaged on a 3T magnetic resonance imaging system (Ingenia, Philips Healthcare, The Netherlands) with a dStream Torso coil (maximal number of channels 32). Dedicated Qstrain software (version 2.0, Medis) was used in deriving LV and RV longitudinal strain [15]. We developed an in-house semi-automatic algorithm to track the distance (L)

between the left atrioventricular junction and a user-defined point at the mid posterior LA wall on standard CMR 2- and 4-chamber views [9].

**Metabolomics profiling**

Antecubital venous blood samples (20-30 ml) were taken from consenting participants in the morning; fasting was not required before blood collection. After collection, the blood samples were immediately placed on ice for transportation and were processed within 6 h to obtain serum samples, which were subsequently stored at −80° C. Additionally, archived blood samples obtained approximately 15 years prior to this assessment from subjects who had serum samples collected and stored at the time of enrolment were analyzed.

Serum metabolomic profiling analysis for acyl-carnitines was performed in the Duke-NUS Metabolomics Facility. Thawed serum samples (100 µl) were spiked with 20 µl deuterium-labelled acyl-carnitine mixture and diluted with 800 µl methanol. Extraction and measurement of acyl-carnitine were performed as previously described [16]. Data acquisition and analysis were performed on an Agilent MassHunter Workstation B.06.00 Software.

We studied serum samples obtained from our subjects during the current study period (2014-2017) (which we will refer to as *current samples*) as well as from archived samples (1999-2004) (which we will refer to as *archived samples*) collected from the study subjects at the time of their enrolment into the Singapore Chinese Health Study approximately 15 years ago. At the time of their enrolment 15 years ago, mean age of the cohort was 59±3.8 years, mean body mass index was 23±2.9 kg/metre$^2$ and prevalence of diabetes mellitus, hypertension and smoking status were 6.7%, 27% and 28.3% respectively.

**Statistics**

Clinical characteristics are presented as mean and standard deviation (SD) for continuous data and frequency and percentage for categorical data. We assessed the statistical significance of the differences between young and old participants as well as difference between old diabetic and old non-diabetic in old participants. Student t-test was used for continuous data and Chi-square test was used for categorical data.

We determined acyl-carnitine profiles in serum samples from the old participants, focusing on those who had complete archived and current samples (old non-diabetic: n=154; old diabetic: n=53). The list of measured metabolites is presented in Supplementary Table 1. To identify serum metabolites correlations and reduce the dimensionality of correlated metabolites, we performed sparse principal component analysis (SPCA)[9] using a penalized matrix decomposition on data from current serum samples (Supplementary Table 2). Metabolites with >25% of values below the lower limit of quantification were excluded from analysis (C24, C26 and C28 was excluded, hence a total of 66 metabolites were analyzed in the final sample). Other missing metabolites were input with 0.01. In SPCA, we normalized the distributions of all metabolites by a logarithmic transformation. We assessed the component metabolites within the significant PCA factors, between diabetic and non-diabetic using student t-test. For those that show an association with $p<0.05$, we further performed multivariable linear regression adjusted for clinical covariates; female, BMI and 2 or more risk factors (dyslipidemia, hypertension, smoking).

To determine the association between serum metabolomic acyl-carnitine measures to CV function, univariate Cox regression was performed on *archived* metabolites and univariate logistic regression on the change in metabolite levels between current and archived levels. Further multivariate regression model was performed on metabolites that show an association with $p<0.05$ with cardiovascular (CV) function in univariate analysis adjusted for clinical covariates; female, body mass index (BMI), diabetes mellitus (DM) and 2 or more risk factors (dyslipidemia, hypertension, smoking). Two CV functions were analysed in the comparison between old DM and old non-DM groups, (1) myocardial relaxation defined as E/A<=0.9 (mean E/A 0.9 in the Old) and (2) left atrial conduit strain defined as ee<=13.4 (mean ee 13.4 in the Old).

All statistical analyses were performed using STATA 15 (College Station, Texas, USA), while the SPCA were performed by R. For all analysis, a two-tailed *P* value of <0.05 was considered significant.

## RESULTS

We studied a total of 933 participants (women 56%, n=521) with a mean age 63±13 years. Participants were classified into young (n=418) and old (n=515) groups, based on cut off age of 65 years at the time of recruitment in 2014-2017. In the old group, we further categorized them into non-diabetics (n=399) and diabetic (n=116) subgroups. Baseline clinical characteristics of the three groups is shown in Table 1.

In the old group both non-diabetic and diabetic participants were similar in age (73±4.4 vs 73±4.3

Table 1. Baseline clinical characteristics of the overall cohort.

| | Young (n=418) | Old non-diabetic (n=399) | Old diabetic (n=116) | Total (n=933) | P-value (young vs old) | p-value (old diabetic vs old non-diabetic) |
|---|---|---|---|---|---|---|
| **Clinical covariates** | | | | | | |
| Age (years) | 52 (10.6) | 73 (4.4) | 73 (4.3) | 63 (12.9) | <0.0001 | 0.86 |
| Female gender, n(%) | 266 (63.6%) | 207 (51.9%) | 48 (41.4%) | 521 (55.8%) | <0.0001 | 0.046 |
| Body mass index (BMI) (kg/metre²) | 24 (3.7) | 23 (3.4) | 24 (3.8) | 24 (3.6) | 0.76 | 0.027 |
| Systolic blood pressure (mmHg) | 127 (19.0) | 146 (23.7) | 145 (16.3) | 137 (22.9) | <0.0001 | 0.56 |
| Diastolic blood pressure (mmHg) | 76 (12.6) | 74 (11.1) | 70 (11.0) | 75 (11.9) | 0.0023 | 0.0008 |
| Heart rate (beats per minute) | 71 (11.0) | 72 (12.9) | 75 (12.7) | 72 (12.1) | 0.034 | 0.080 |
| Hypertension, n, (%) | 52 (12.4%) | 188 (47.1%) | 94 (81.0%) | 334 (35.8%) | <0.0001 | <0.0001 |
| Dyslipidemia, n, (%) | 92 (22.0%) | 171 (42.9%) | 92 (79.3%) | 355 (38.1%) | <0.0001 | <0.0001 |
| Ever smoked, n, (%) | 14 (4.2%) | 62 (16.1%) | 35 (32.7%) | 111 (13.5%) | <0.0001 | <0.0001 |
| 'CV risk factor >=2' | 39 (9.3%) | 129 (32.3%) | 89 (76.7%) | 257 (27.6%) | <0.0001 | <0.0001 |
| **Biomarkers** | | | | | | |
| B-type natriuretic peptide (BNP) (pg/ml) | 20 (27.1) | 40 (37.3) | 37 (37.5) | 35 (36.0) | <0.0001 | 0.53 |
| Galectin-3 (ng/ml) | 14.4 (7.1) | 16.3 (4.2) | 18.3 (5.1) | 16.2 (5.3) | <0.0001 | 0.0003 |
| Urinary creatinine (mmol/L) | 6.4 (4.5) | 6.6 (5.4) | 7.5 (5.0) | 6.8 (5.3) | 0.66 | 0.22 |
| Urinary albumin (mg/L) | 9.3 (6.8) | 25.8 (62.6) | 23.2 (30.5) | 24.0 (55.2) | 0.15 | 0.75 |
| Urine albumin to creatinine ratio (mg/mmol) | 1.9 (1.4) | 4.7 (10.1) | 4.0 (6.7) | 4.4 (9.1) | 0.14 | 0.56 |
| Glycated hemoglobin (%) | 5.6 (0.7) | 5.9 (0.6) | 6.8 (1.0) | 6.0 (0.8) | 0.010 | <0.0001 |
| Random glucose (mg/dL) | 114 (33.7) | 116 (37) | 175 (71) | 122 (45) | <0.0001 | <0.0001 |

years, p=0.86). The non-diabetic subgroup had more women (52% vs 41%, p=0.046) and had lower body mass index (23±3.4 vs 24±3.8 kg/m$^2$, p=0.027) compared to the diabetic subgroup. Older diabetic participants had higher prevalence of hypertension (81% vs 47%, p<0.0001), dyslipidemia (79% vs 43%, p<0.0001), and smoking history (33% vs 16%, p<0.0001) compared to older non-diabetic subjects. Older diabetic participants had higher galectin levels (18.3±5.1 vs 16.3±4.2 ng/ml, p=0.0003), plasma glucose levels (175±71 vs 116±37 mg/dl, p<0.0001), plasma glycosylated haemoglobin (6.8±1.0 vs 5.9±0.6, %, p<0.0001) but similar BNP (37±37.5 vs 40±37.3, pg/ml, p=0.53), and urine microalbumin to creatinine ratio (4.0±6.7 vs 4.7±10.1, p=0.56) compared to older non-diabetics (Table 1).

Compared to the young group, participants in the overall old group had larger left ventricular wall thickness, LV mass, left atria size and volume, and lower LV function such as lower ratio of peak velocity flow in early diastole to peak velocity flow in late diastole (Supplementary Table 3).

Left ventricular and left atria sizes and structures were similar in non-diabetic and diabetic subgroups (Table 2). However, diabetic participants had lower E/A ratio (0.8±0.2 vs 0.9±0.3, p=0.0039). Lower left atrial functions were observed among diabetics compared to the non-diabetics. Diabetics had lower left atrial conduit strain (12±4.3% vs 14±4.1%, unadjusted p=0.045), lower LA conduit strain rate (-1.2±0.4 s$^{-1}$ vs -1.3±0.5 s$^{-1}$, unadjusted p=0.042) and lower ratio of LA conduit strain to LA booster strain (0.5±0.2 vs 0.7±0.3, adjusted p=0.0029). Pulmonary artery systolic pressure was higher among older non-diabetics, compared to older diabetics (28±7.0 vs 25±6.9 mmHg, p=0.001) (Table 2).

In the acyl-carnitine data from the current samples adjusted linear regression analyses showed that acylcarnitine Factor 4, Factor 5 and Factor 6 differentiated older participants with diabetes from non-diabetics (Table 3). Factor 4 consists of long-chain acyl-carnitines, which are intermediates of fatty acid oxidation [17]. Specifically, we observed that diabetics had lower C18:2 (58.4 vs 67.4, p=0.020), C20:4 (4.2 vs 4.9, p=0.013), C20:3 (4.3 vs 5.3, p=0.002) and C20:2 (3.9 vs 4.4, p=0.037)], compared to non-diabetics. Factor 5 and Factor 6 consists of short chain acyl-carnitines as well as di-carboxyl and hydroxylated acyl-carnitines, which are generated by alpha and omega oxidation pathways [18, 19]. The diabetics had higher C4-OH (25.1 vs 13.0, p<0.0001), C14-OH/C12-DC

Table 2. Cardiovascular characteristics of old non-diabetic vs old diabetic.

| Echocardiography measurements | Old non-diabetic (n=399) | Diabetic (n=116) | Univariate p-value | ~Adjusted P-value |
|---|---|---|---|---|
| Interventricular septum thickness at end diastole (IVSD) (cm) | 0.80 (0.1) | 0.81 (0.2) | 0.52 | - |
| Interventricular septum thickness at end systole (IVSS) (cm) | 1.3 (0.2) | 1.2 (0.2) | 0.76 | - |
| Left ventricular internal diameter end diastole (LVIDD) (cm) | 4.4 (0.6) | 4.3 (0.6) | 0.12 | - |
| Left ventricular internal diameter end systole (LVIDS) (cm) | 2.5 (0.5) | 2.4 (0.5) | 0.41 | - |
| Left ventricular posterior wall end diastole (LVPWD) (cm) | 0.76 (0.1) | 0.77 (0.1) | 0.16 | - |
| Left ventricular posterior wall end systole (LVPWS) (cm) | 1.4 (0.2) | 1.5 (0.2) | 0.28 | - |
| Left ventricular outflow tract (LVOT) (cm) | 2.1 (0.2) | 2.0 (0.2) | 0.26 | - |
| Aortic diameter (AO) (cm) | 3.0 (0.4) | 3.1 (0.4) | 0.084 | - |
| Left atrium (LA) (cm) | 3.6 (0.6) | 3.7 (0.6) | 0.55 | - |
| Left ventricular ejection fraction (LVEF) (%) | 74 (7.7) | 73 (9.2) | 0.11 | - |
| Left ventricular fractional shortening (LVFS) (%) | 44 (7.4) | 42 (7.8) | 0.12 | - |
| Left ventricular mass (grams) | 120 (49) | 116 (40) | 0.41 | - |
| Left ventricular mass index (grams/$m^2$) | 74 (27) | 70 (22) | 0.14 | - |
| Left atrial volume (ml) | 35 (13) | 36 (14) | 0.45 | - |
| Left atrial volume index (ml/$m^2$) | 21 (7.7) | 22 (8.2) | 0.90 | - |
| Isovolumic relaxation time (IVRT) (ms) | 103 (18) | 103 (20) | 0.98 | - |
| Peak velocity flow in early diastole E (MV E peak) (m/s) | 0.71 (0.2) | 0.70 (0.2) | 0.51 | - |
| Peak velocity flow in late diastole by atrial contraction A (MV A peak) (m/s) | 0.81 (0.2) | 0.87 (0.2) | **0.005** | 0.15 |
| Ratio of MV E peak velocity: MV A peak velocity | 0.91 (0.3) | 0.82 (0.2) | **0.003** | **0.039** |
| Mitral valve flow deceleration time (MV DT) (ms) | 213 (40) | 222 (42) | **0.034** | 0.23 |
| Right atrial pressure (mmHg) | 5.0 (1.3) | 4.7 (1.7) | 0.36 | - |
| Pulmonary artery systolic pressure (PASP) (mmHg) | 28 (7.0) | 25 (6.9) | **0.005** | **0.001** |
| Peak systolic septal mitral annular velocity (Septal S') (m/s) | 0.078 (0.02) | 0.077 (0.01) | 0.38 | - |
| Peak early diastolic septal mitral annular velocity (Septal E') (m/s) | 0.074 (0.02) | 0.067 (0.02) | **0.0003** | **0.021** |
| Septal mitral annular velocity during atrial contraction (Septal A') (m/s) | 0.14 (0.6) | 0.11 (0.02) | 0.60 | - |
| Peak systolic lateral mitral annular velocity (m/s) | 0.10 (0.03) | 0.10 (0.03) | 0.10 | - |
| Peak early diastolic lateral mitral annular velocity (m/s) | 0.094 (0.02) | 0.088 (0.02) | **0.019** | 0.094 |
| Lateral mitral annular velocity during atrial contraction (m/s) | 0.12 (0.03) | 0.13 (0.02) | 0.51 | - |
| Ratio of Peak velocity flow in early diastole E (MV E peak) velocity to Peak early diastolic septal mitral annular velocity (Septal E') | 10 (3.3) | 11 (3.1) | **0.022** | 0.34 |
| **CMR measurements** | **(n=187)** | **(n=51)** | | |
| LV global longitudinal strain (LVGLS) (%) | -21 (2.9) | -21 (2.9) | 0.28 | - |
| LV global circumferential strain (LVGCS) (%) | -22 (3.8) | -23 (3.1) | 0.21 | - |
| LV global radial strain (LVGRS) (%) | 104 (25.1) | 104 (19.5) | 0.98 | - |
| Right ventricular global longitudinal strain (RVGLS) (%) | -31 (5.4) | -31 (5.5) | 0.84 | - |
| LA reservoir strain (εs) (%) | 31 (6.9) | 31 (6.2) | 0.98 | - |
| LA conduit strain (εe) (%) | 14 (4.1) | 12 (4.3) | **0.045** | 0.28 |
| LA booster strain (εa) (%) | 17 (4.7) | 18 (3.9) | 0.065 | - |
| Reservoir strain rate (SRs) (1/s) | 1.5 (0.5) | 1.5 (0.4) | 0.92 | - |
| Conduit strain rate (SRe) (1/s) | -1.3 (0.5) | -1.2 (0.4) | **0.042** | 0.30 |
| Booster strain rate (SRa) (1/s) | -2.2 (0.7) | -2.3 (0.6) | 0.19 | - |
| Ratio of SRe/SRa | 0.66 (0.3) | 0.55 (0.2) | **0.006** | **0.029** |
| $LAvolume_{min}$ (ml) | 31 (12.6) | 27 (10.1) | **0.044** | **0.016** |
| $LAvolume_{max}$ (ml) | 64 (18) | 57 (17) | **0.017** | **0.006** |
| LA ejection fraction (%) | 52 (8.9) | 52 (7.2) | 0.92 | - |

~adjusted for female, BMI, CV rf>2.

Table 3. Acyl-carnitine factors and significant components: comparisons between old non-diabetic vs old diabetic.

| Acyl-carnitines | Non-diabetic (n=154) | Diabetic (n=53) | p-value | Adjusted Coef. (95% CI)* | Adjusted P-value* |
|---|---|---|---|---|---|
| **PCA factors** | | | | | |
| X1 | 0.05 (2.7) | -0.1 (2.7) | 0.68 | - | - |
| X2 | 0.06 (2.0) | -0.2 (2.6) | 0.48 | - | - |
| X3 | -0.04 (2.2) | 0.1 (1.8) | 0.61 | - | - |
| X4 | -0.2 (2.1) | 0.7 (1.9) | **0.0080** | 1.0 (0.3, 1.7) | **0.008** |
| X5 | -0.4 (2.0) | 1.1 (2.6) | **<0.0001** | 1.3 (0.6, 2.0) | **<0.0001** |
| X6 | 0.2 (1.1) | -0.5 (2.2) | **0.004** | -0.6 (-1.1, -0.2) | **0.009** |
| X7 | -0.04 (1.7) | 0.1 (1.9) | 0.60 | - | - |
| X8 | -0.01 (1.2) | 0.04 (1.3) | 0.77 | - | - |
| X9 | 0.08 (1.4) | -0.2 (1.2) | 0.17 | - | - |
| X10 | 0.03 (1.4) | -0.1 (1.4) | 0.58 | - | - |
| **Short chain** | | | | | |
| C3 | 543 (180) | 553 (201) | 0.71 | - | - |
| C4 | 338 (144) | 345 (174) | 0.77 | - | - |
| C4-OH | 13.0 (8.0) | 25.1 (16.8) | **<0.0001** | 11.0 (7.5, 14.4) | **<0.0001** |
| C5 | 95.9 (36.0) | 96.5 (39.8) | 0.91 | - | - |
| C5:1 | 15.8 (5.4) | 16.5 (7.3) | 0.47 | - | - |
| **Medium chain** | | | | | |
| C10:1 | 85.3 (55.8) | 86.0 (79.4) | 0.95 | - | - |
| C10:2 | 13.1 (9.4) | 15.1 (11.1) | 0.24 | - | - |
| C12-OH/C10-DC | 2.1 (1.1) | 2.5 (1.2) | **0.012** | 0.3 (-0.06, 0.7) | 0.10 |
| C8:1-OH/C6:1-DC | 27.8 (14.1) | 28.4 (16.2) | 0.81 | - | - |
| C8-DC | 23.3 (13.4) | 26.6 (13.2) | 0.12 | - | - |
| | | | | - | - |
| **Long chain** | | | | | |
| C14:1-OH | 10.6 (6.0) | 11.7 (4.6) | 0.22 | - | - |
| C14:3 | 4.4 (2.6) | 4.2 (2.5) | 0.61 | - | - |
| C14-OH/C12-DC | 5.9 (3.0) | 8.5 (4.6) | **<0.0001** | 2.1 (1.0, 3.2) | **<0.0001** |
| C16 | 106 (26.0) | 102 (29.3) | 0.31 | - | - |
| C16:1-OH/C14:1-DC | 4.8 (1.9) | 5.4 (2.4) | 0.055 | - | - |
| C16:2-OH | 4.3 (1.8) | 4.9 (2.0) | **0.040** | 0.4 (-0.2, 1.0) | 0.17 |
| C16:3-OH/C14:3-DC | 1.3 (0.9) | 1.4 (0.9) | 0.36 | - | - |
| C16-OH | 5.5 (2.7) | 7.3 (3.4) | **0.0001** | 1.6 (0.6, 2.5) | **0.001** |
| C18 | 38.7 (9.8) | 37.3 (8.5) | 0.37 | - | - |
| C18:1 | 116 (33.6) | 109 (28.0) | 0.15 | - | - |
| C18:1-OH/C16:1-DC | 3.8 (1.9) | 4.9 (2.9) | **0.0007** | 0.9 (0.1, 1.6) | **0.019** |
| C18:2 | 67.4 (20.3) | 58.4 (16.2) | **0.004** | -7.7 (-14.2, -1.2) | **0.020** |
| C18:3 | 5.2 (2.1) | 4.6 (2.4) | 0.13 | - | - |
| C18-OH/C16-DC | 4.5 (3.2) | 6.2 (3.1) | **0.012** | 1.3 (0.2, 2.3) | **0.020** |
| C20 | 5.3 (1.8) | 5.0 (1.3) | 0.36 | - | - |
| C20:1 | 6.9 (2.5) | 6.7 (2.2) | 0.49 | - | - |
| C20:1-OH/C18:1-DC | 7.2 (4.1) | 8.0 (3.5) | 0.23 | - | - |
| C20:2 | 4.4 (1.5) | 3.9 (1.2) | **0.033** | -0.5 (-1.0, -0.03) | **0.037** |
| C20:2-OH/C18:2-DC | 2.2 (1.3) | 2.0 (1.0) | 0.37 | - | - |
| C20:3 | 5.3 (2.4) | 4.3 (1.7) | **0.005** | -1.2 (-1.9, -0.5) | **0.002** |
| C20:3-OH/C18:3-DC | 1.2 (0.7) | 1.3 (0.8) | 0.20 | - | - |
| C20:4 | 4.9 (2.0) | 4.2 (1.7) | **0.035** | -0.8 (-1.5, -0.2) | **0.013** |
| C22:1 | 3.1 (2.0) | 2.8 (1.6) | 0.54 | - | - |
| C22:2 | 0.8 (1.2) | 0.7 (0.4) | 0.45 | - | - |
| C22:4 | 1.1 (0.7) | 1.1 (0.6) | 0.80 | - | - |
| C22:5 | 1.8 (0.8) | 1.6 (0.9) | 0.16 | - | - |

*adjusted for female, BMI, CV rf>2.

(8.5 vs 5.9, p<0.0001), C16-OH (7.3 vs 5.5, p=0.001) and C18-OHC/16-DC (6.2 vs 4.5, p=0.020)] compared to non-diabetics. (Table 3).

We next examined the relationship between current serum acyl-carnitine profiles and CV structure and function in older study subjects. The di-carboxyl acyl-carnitines were associated with higher risks of impairments in E/A ratio (C12-OH/C10-DC, p=0.018); C18-OH/C16-DC, p=0.038). Similarly, the di-carboxyl acyl-carnitines were also associated with worse LA conduit strain function (C12-OH/C10-DC, p=0.008); C14-OH/C12-DC, p=0.025); C16:3-OH/C14:3-DC, p=0.018). The short-chain acyl-carnitines and hydroxylated acyl-carnitines were associated with worse LA conduit strain function (C4-OH, p=0.0024); C5, p=0.024). The long chain acyl-carnitines were associated with higher risks of impairments in E/A ratio (C16, p=0.002); C18:1, p=0.046). (Table 4).

To explore associations between archived metabolites and CV function, we analyzed archived serum samples (Supplementary Table 4), as well as computed the metabolites' change over 15-year period and assessed their hazards of association with CV function 15-years later (Supplementary Table 5). We found that higher levels of archived serum long chain acylcarnitine were associated with impairments in E/A ratio (C20:3, p=0.014) (Figure 1A). Longitudinal increases in long chain acylcarnitine over time were also associated with worse LA conduit strain function (C14:3, p=0.033); C18:1, p=0.018); C18:2, p=0.028); C18:3, p=0.019); C20:4, p=0.038); C22:5, p=0.043) (Figure 1D). Higher levels of archived short chain acylcarnitine were associated with larger hazards impairments in E/A ratio (C5:1, p=0.011) as well as with worse LA conduit strain function (C5:1, p=0.037) (Figure 1A). Higher levels of di-carboxylated acyl-carnitines were associated with worse LA conduit strain function (C16:3-OH/C14:3-DC, p=0.019) (Figure 1C). Increases in hydroxylated acyl-carnitines were also associated with worse LA conduit strain function (C4-OH, p=0.017); C16:2-OH, p=0.037) (Figure 1D).

## DISCUSSION

This was a community cohort of older adults without known prevalent cardiovascular disease. Low BNP levels in the cohort provided additional evidence that prevalent undiagnosed heart failure is probably negligible in this cohort. In determining the impact of ageing on cardiovascular structure and function in elderly adults, we recognize the difficulties of studying ageing changes in the absence of comorbidities. We used contemporary and novel imaging markers to define how the aged heart may look like in a general population setting where comorbidities invariably co-exist with human ageing. Our large sample size adjusted for these commonly occurring, age-related comorbidities.

After adjusting for comorbidity burdens, we observed definite increases in left ventricular mass index, left atrial size and left atrial volume index with ageing, along with changes in other cardiovascular *structural* markers such as left ventricular septal thickness, left ventricular internal diameter and aortic diameter. These changes have been previously reported and are thought to be a consequence of ageing [20]. In terms of function, the older adults had significant reductions in left ventricular diastolic function as noted by changes in myocardial relaxation. Observed differences in LV filling pressure did not reach clinically important thresholds, confirming that impairments in myocardial relaxation, instead of gross disturbances in diastolic function commonly observed in elderly patients with heart failure [21], typify cardiovascular ageing in this cohort.

Older adults with diabetes mellitus had similar LV *structure* to their counterparts without diabetes mellitus. In terms of cardiac function, LV and LA functional parameters revealed differences between ageing and diabetes. Older diabetic adults had significant impairments in LV myocardial relaxation, compared to older non-diabetic adults. Using advanced CMR techniques, these functional differences extended upstream to the left atrium. We observed clear differences in left atrial strain as measured by cardiac MRI. This is a novel observation which extends beyond prior reports of metabolic disturbances in left atrial strain [9], Importantly, left atrial conduit strain was the specific disturbance that differentiated diabetics and from non-diabetics, expanding on existing reports that have also observed differences in left atrial atrial strain among diabetics [22], whilst also lending support to prognostic data that has linked left atrial conduit strain to poor prognosis in cardiovascular disease cohorts [23]. This effect of diabetes on left atrial strain, exacerbated over and above effects attributable to solely ageing, should prompt greater efforts that address risks of atrial myopathy in older adults with diabetes, such as incorporating measurement of left atrial strain into clinical protocols [24] or tackling risks of left atrial myopathy in heart failure [25].

Among this cohort of older adults, the profile of acyl-carnitine metabolites differed between diabetics and non-diabetics. Diabetics had lower levels of long-chain acyl-carnitines as compared to non-diabetics. This class of metabolites is generated by mitochondrial oxidation of long-chain fatty acids. Changes in the levels of these

Table 4. Association between current metabolites and cardiovascular function.
i) Outcome: E/A<=0.9.

| Current metabolites | Events/total | OR (95% CI) | p-value | Adjusted OR (95%)* | Adjusted p-value* |
|---|---|---|---|---|---|
| **Short chain** | | | | | |
| C3 | 142/207 | 1.0 (1.0, 1.002) | 0.62 | - | - |
| C4 | 142/207 | 1.0 (1.0, 1.001) | 0.26 | - | - |
| C4-OH | 142/207 | 1.03 (1.001, 1.06) | **0.045** | 1.02 (0.99, 1.05) | 0.25 |
| C5 | 142/207 | 1.001 (0.99, 1.009) | 0.89 | - | - |
| C5:1 | 139/202 | 0.97 (0.93, 1.02) | 0.29 | - | - |
| **Medium chain** | | | | | |
| C10:1 | 137/195 | 1.002 (1.0, 1.008) | 0.42 | - | - |
| C10:2 | 112/168 | 1.03 (0.99, 1.07) | 0.15 | - | - |
| C12-OH/C10-DC | 142/207 | 1.53 (1.11, 2.10) | **0.009** | 1.50 (1.07, 2.09) | **0.018** |
| C8:1-OH/C6:1-DC | 142/207 | 0.99 (0.97, 1.01) | 0.45 | - | - |
| C8-DC | 142/207 | 1.01 (0.99, 1.04) | 0.29 | - | - |
| **Long chain** | | | | | |
| C14:1-OH | 142/207 | 1.03 (0.97, 1.09) | 0.38 | - | - |
| C14:3 | 142/207 | 1.06 (0.94, 1.20) | 0.35 | - | - |
| C14-OH/C12-DC | 142/207 | 1.13 (1.02, 1.25) | **0.016** | 1.11 (1.0, 1.24) | 0.052 |
| C16 | 142/207 | 1.02 (1.007, 1.03) | **0.002** | 1.02 (1.008, 1.03) | **0.002** |
| C16:1-OH/C14:1-DC | 142/207 | 1.16 (0.99, 1.36) | 0.067 | - | - |
| C16:2-OH | 142/207 | 1.06 (0.90-1.25) | 0.48 | - | - |
| C16:3-OH/C14:3-DC | 136/199 | 1.12 (0.79-1.60) | 0.52 | - | - |
| C16-OH | 142/207 | 1.07 (0.97, 1.19) | 0.19 | - | - |
| C18 | 142/207 | 1.03 (0.99, 1.06) | 0.12 | - | - |
| C18:1 | 142/207 | 1.01 (1.0, 1.02) | **0.043** | 1.01 (1.0, 1.02) | **0.046** |
| C18:1-OH/C16:1-DC | 142/207 | 1.23 (1.03, 1.46) | **0.022** | 1.20 (1.0, 1.43) | 0.054 |
| C18:2 | 142/207 | 1.008 (0.99, 1.02) | 0.31 | - | - |
| C18:3 | 139/203 | 1.05 (0.92, 1.21) | 0.46 | - | - |
| C18-OH/C16-DC | 142/207 | 1.22 (1.04, 1.42) | **0.014** | 1.19 (1.01, 1.41) | **0.038** |
| C20 | 142/207 | 1.05 (0.88, 1.25) | 0.60 | - | - |
| C20:1 | 142/207 | 0.97 (0.87, 1.10) | 0.68 | - | - |
| C20:1-OH/C18:1-DC | 142/207 | 1.06 (0.97, 1.15) | 0.19 | - | - |
| C20:2 | 142/207 | 0.97 (0.80, 1.19) | 0.80 | - | - |
| C20:2-OH/C18:2-DC | 141/206 | 1.04 (0.81, 1.33) | 0.76 | - | - |
| C20:3 | 142/207 | 1.09 (0.95, 1.26) | 0.22 | - | - |
| C20:3-OH/C18:3-DC | 132/194 | 0.86 (0.58, 1.29) | 0.47 | - | - |
| C20:4 | 142/207 | 1.03 (0.89, 1.20) | 0.68 | - | - |
| C22:1 | 142/207 | 0.98 (0.84, 1.14) | 0.80 | - | - |
| C22:2 | 137/197 | 1.36 (0.72, 2.56) | 0.35 | - | - |
| C22:4 | 140/203 | 1.11 (0.69, 1.77) | 0.67 | - | - |
| C22:5 | 141/205 | 1.06 (0.74, 1.50) | 0.76 | - | - |

*adjusted for diabetes mellitus, female, BMI, CV rf>2.

ii) Outcome: εe<=13.4 %.

| Current metabolites | Events/total | OR (95% CI) | p-value | Adjusted OR (95% CI) * | Adjusted p-value * |
|---|---|---|---|---|---|
| **Short chain** | | | | | |
| C3 | 87/169 | 1.001 (1.0, 1.002) | 0.52 | - | - |
| C4 | 87/169 | 1.001 (1.0, 1.003) | 0.66 | - | - |
| C4-OH | 87/169 | 1.05 (1.02, 1.09) | **0.002** | 1.04 (1.006, 1.08) | **0.0024** |
| C5 | 87/169 | 1.01 (1.002, 1.02) | **0.022** | 1.01 (1.001, 1.02) | **0.024** |
| C5:1 | 85/166 | 1.02 (0.97, 1.08) | 0.39 | - | - |
| **Medium chain** | | | | | |
| C10:1 | 83/157 | 1.002 (1.0, 1.007) | 0.39 | - | - |
| C10:2 | 69/134 | 1.02 (0.98, 1.06) | 0.32 | - | - |
| C12-OH/C10-DC | 87/169 | 1.70 (1.23, 2.33) | **0.001** | 1.58 (1.13, 2.21) | **0.008** |
| C8:1-OH/C6:1-DC | 87/169 | 0.99 (0.97, 1.02) | 0.54 | - | - |
| C8-DC | 87/169 | 1.02 (1.0, 1.05) | 0.062 | - | - |
| **Long chain** | | | | | |
| C14:1-OH | 87/169 | 1.09 (1.01, 1.17) | **0.019** | 1.07 (1.0, 1.16) | 0.051 |
| C14:3 | 87/169 | 1.05 (0.94, 1.18) | 0.41 | - | - |
| C14-OH/C12-DC | 87/169 | 1.17 (1.06, 1.29) | **0.002** | 1.13 (1.02, 1.25) | **0.025** |
| C16 | 87/169 | 1.004 (0.99, 1.02) | 0.42 | - | - |
| C16:1-OH/C14:1-DC | 87/169 | 1.09 (0.94, 1.26) | 0.28 | - | - |
| C16:2-OH | 87/169 | 1.19 (1.005, 1.41) | **0.043** | 1.15 (0.96, 1.37) | 0.13 |
| C16:3-OH/C14:3-DC | 84/161 | 1.78 (1.16, 2.71) | **0.008** | 1.70 (1.10, 2.64) | **0.018** |
| C16-OH | 87/169 | 1.07 (0.96, 1.18) | 0.21 | - | - |
| C18 | 87/169 | 0.99 (0.96, 1.02) | 0.60 | - | - |
| C18:1 | 87/169 | 1.003 (0.99, 1.01) | 0.49 | - | - |
| C18:1-OH/C16:1-DC | 87/169 | 1.13 (0.98, 1.31) | 0.10 | - | - |
| C18:2 | 87/169 | 0.99 (0.98, 1.008) | 0.33 | - | - |
| C18:3 | 85/167 | 1.05 (0.91, 1.20) | 0.52 | - | - |
| C18-OH/C16-DC | 87/169 | 1.15 (1.008, 1.32) | **0.038** | 1.08 (0.95, 1.23) | 0.25 |
| C20 | 87/169 | 1.08 (0.90, 1.28) | 0.42 | - | - |
| C20:1 | 87/169 | 1.03 (0.92, 1.17) | 0.59 | - | - |
| C20:1-OH/C18:1-DC | 87/169 | 1.05 (0.98, 1.14) | 0.19 | - | - |
| C20:2 | 87/169 | 1.01 (0.82, 1.24) | 0.91 | - | - |
| C20:2-OH/C18:2-DC | 87/168 | 0.95 (0.76, 1.20) | 0.67 | - | - |
| C20:3 | 87/169 | 1.02 (0.90, 1.16) | 0.75 | - | - |
| C20:3-OH/C18:3-DC | 81/159 | 1.13 (0.75, 1.72) | 0.55 | - | - |
| C20:4 | 87/169 | 1.008 (0.87, 1.17) | 0.92 | - | - |
| C22:1 | 87/169 | 1.08 (0.92, 1.26) | 0.35 | - | - |
| C22:2 | 81/160 | 1.29 (0.76, 2.18) | 0.34 | - | - |
| C22:4 | 85/166 | 0.83 (0.52, 1.32) | 0.42 | - | - |
| C22:5 | 85/167 | 1.10 (0.77, 1.56) | 0.61 | - | - |

*adjusted for diabetes mellitus, female, BMI, CV rf>2.

metabolites thus reflects alterations in fatty acid oxidation, which is known to be associated with diabetes and cardiovascular disorders [26]. Older subjects with diabetes also had higher levels of dicarboxyl- and hydroxyl-carnitine metabolites. These metabolites are generally thought to be generated by alpha- and omega- fatty acid oxidation pathways [27]. Notably, these metabolites have previously been shown to be associated with increased risk of recurrent cardiovascular events as well as ischemic stroke [28]. Since patients with diabetes are known to be at higher CV risk it is perhaps understandable that older subjects

with diabetes would have increased levels of these metabolites.

Interestingly, some of these acyl-carnitine metabolites were associated with cardiovascular function. Higher levels of long chain acyl-carnitines were associated with impairments in myocardial relaxation as well as with worse left atrial function. As earlier mentioned, long-chain acyl-carnitines are linked to mitochondrial fatty acid oxidation pathways. This is one of the first reports that links fuel oxidation pathways to changes in directly measured cardiac function, early disturbances in diastolic function. Previous reports have noted links between long chain acyl-carnitines and heart failure [27]. Across the clinical spectrum from heart failure with reduced ejection fraction (HFrEF), to heart failure with preserved ejection fraction (HFpEF), to non-heart failure (HF) controls, long chain acyl-carnitine levels were greater in HFrEF than HFpEF, both of which were greater than non-HF controls [29]. Our observations now directly link long chain acyl-carnitines to imaging markers of diastolic function, a pathophysiological disturbance that predominates across the clinical heart failure spectrum. In addition, levels of long chain acyl-carnitines obtained 15 years ago were associated with present-day abnormalities in these cardiovascular functions. In tandem with baseline levels of long chain acyl-carnitines from 15 years ago, interval increase in long chain acyl-carnitines predicted abnormalities in myocardial relaxation and left atrial conduit strain.

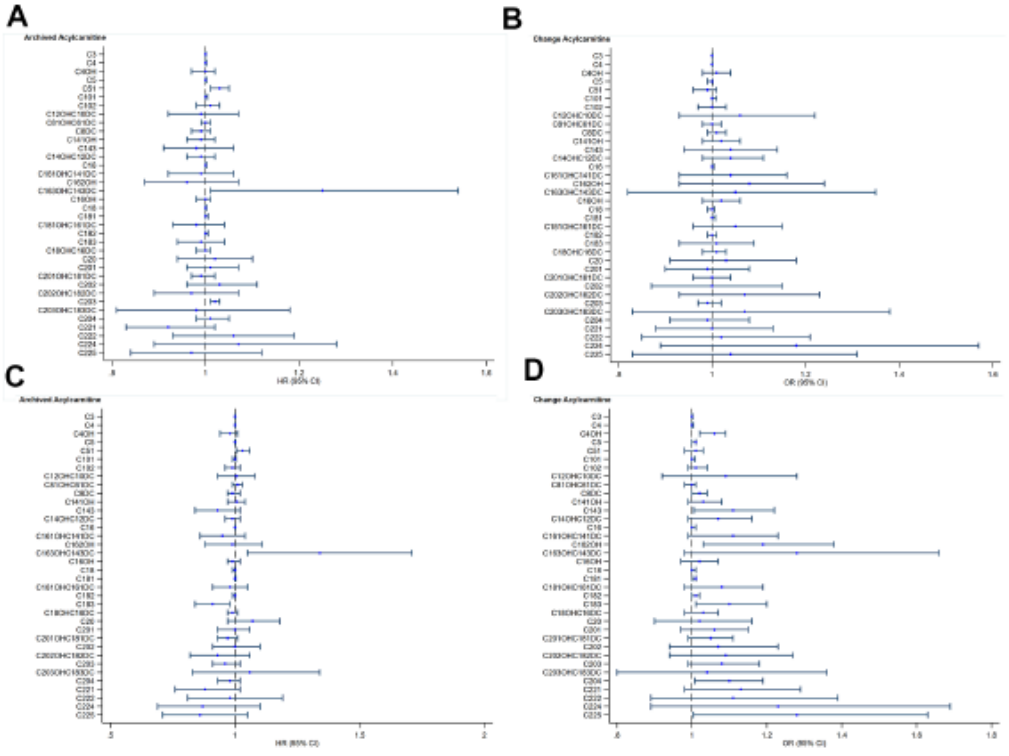


**Figure 1. Acyl-carnitines and cardiovascular function.** (A) Archived Acyl-carnitine and impaired myocardial relaxation. Blue circles and lines represent unadjusted hazard ratios (HR) for one-unit increase in archived acyl-carnitine and its 95% confidence interval (95%CI) on impaired myocardial relaxation. (B) Change in Acyl-carnitine and impaired myocardial relaxation. Blue circles and lines represent unadjusted odds ratios (OR) for one-unit increase in archived acyl-carnitine and its 95% confidence interval (95%CI) on impaired myocardial relaxation. (C) Archived Acyl-carnitine and impaired left atrial conduit strain. Blue circles and lines represent unadjusted hazard ratios (HR) for one-unit increase in archived acylcarnitine and its 95% confidence interval (95%CI) on impaired myocardial relaxation. (D) Change in Acyl-carnitine and impaired left atrial conduit strain. Blue circles and lines represent unadjusted odds ratios (OR) for one-unit increase in archived acyl-carnitine and its 95% confidence interval (95%CI) on impaired myocardial relaxation.

We also noted an association between increased levels of short chain, hydroxylated- and dicarboxyl- acyl-carnitines and impaired LV and atrial function, confirming these observations in baseline levels obtained 15 years ago and interval increases in these metabolites over time. These classes of fuel intermediates are likely generated by the process of alpha- and omega oxidation [18, 19]. Short chain, hydroxylated- and dicarboxyl- acyl-carnitines were specifically higher among older adults with diabetes, highlighting the importance of fuel oxidation pathways in the pathogenesis of diabetes, a connection which has been well described [26]. These pathways may also represent important treatment targets to ameliorate impact of diabetes on cardiovascular outcomes in older adults. Previous reports have noted associations between the presence of atrial fibrillation and generalized changes in metabolic pathways [30]. This is the first study to highlight alpha and omega oxidation, in association with altered left atrial function.

We acknowledge study limitations. Our observational study design does not imply causality between the metabolites and their present-day cardiovascular function. The metabolic perturbations among diabetic samples are complex, and while we could identify key pathways involved from their sera, our observational study design is unable to differentiate between adaptive versus pathogenic responses. The lower levels of certain long chain acyl-carnitines in the diabetic samples, might hypothetically represent either an adaptive response or treatment effect. In addition, metabolomics responses may differ between study groups as a consequence of dietary or lifestyle factors, which we did not account for in this analysis. A prospective longitudinal clinical trial might clarify this, alongside adjustments for medication data and duration of co-morbidities, which we did not correct for as well. However, the generally low levels of glycated haemoglobin among the groups suggest a lower risk cohort. As a community-based study focused on studying asymptomatic individuals prior to disease manifestation, our results reflect asymptomatic or preclinical phase of disease, designed to look for upstream differences that are likely subtle. Even so, true relationships between the groups, and their associations with metabolomics, may only be underestimated, and unlikely overestimated. While there may be analytic differences between non-fasting serum samples (which we used in our study) and fasting serum samples, large cohort studies face challenges in getting community elderly participants to fast for prolonged periods of time. Based on the Health Professionals Follow-up Study and Nurses' Health Study, fasting, season of blood collection, and time of day of blood collection were not important sources of variability in measurements of most metabolites [31]. Finally, we are unable to account for residual confounding factors that may be present in such a study design.

Despite these limitations, our observations lend biological basis to previous reports that have linked fuel oxidation pathways to cardiovascular outcomes [13]. Our results provide basis for future work that explores the role of metabolite analysis in early detection, as a possible preventative strategy upstream in ageing.

## CONCLUSIONS

Distinct alterations in fuel oxidation pathways in short chain and long chain acyl-carnitines, di-carboxyl and hydroxylated acyl-carnitines, were associated with present-day changes in cardiovascular function. These alterations in cardiovascular function distinguished diabetic versus non-diabetic older adults. Targeting distinct fuel oxidation pathways in older adults depending on diabetes status may provide greater precision on therapeutic strategies. Investigations into acyl-carnitines early in the ageing trajectory may represent a window of opportunity to apply preventative and/or screening methods against deteriorations in cardiovascular health with ageing.

## AUTHOR CONTRIBUTIONS

FG, JPK, RST, WPK, ASK contributed to the conception and design of the study, advised on all statistical aspects, and interpreted the data. JPK, XDZ, FG, SL, VC, HC, LYT, SHE, HCT, TYT, LSL, JC, BMK, LZ performed data analyses. All authors critically reviewed the manuscript. All authors approved the final draft for submission.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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## SUPPLEMENTARY MATERIALS

### Supplementary Tables

**Supplementary Table 1. List of measured metabolites.**

| Short name | Name |
|---|---|
| C2 | Acetyl carnitine |
| C3 | Propionyl carnitine |
| C4 | Butyryl carnitine or isobutryl carnitine |
| C5:1 | Tiglyl carnitine or 3-methyl crotonyl carnitine |
| C5 | Isovaleryl, 3-methylbutyryl carnitine , 2-Methylbutyryl, valeryl or pivaloyl carnitine |
| C4-OH | D-3-Hydroxy-butyryl carnitine, L-3-hydroxybutyryl carnitine |
| C6 | Hexanoyl carnitine |
| C5-OH/C3-DC | 3-Hydroxy-isovaleryl carnitine or malonyl carnitine |
| C4-DC/C6-OH | Methylmalonyl carnitine or succinyl carnitine |
| C8:1 | Octenoyl carnitine |
| C8 | Octanoyl carnitine |
| C5-DC | Glutaryl carnitine, ethylmalonyl carnitine |
| C8:1-OH/C6:1-DC | 3-Hydroxy- octenoyl carnitine or hexenedioyl carnitine |
| C8-OH/C6-DC | 3-hydroxy octanoyl carnitine or adipoyl carnitine, 3-methylglutaryl carnitine |
| C10:3 | Decatrienoyl carnitine |
| C10:1 | Decenoyl carnitine |
| C10 | Decanoyl carnitine |
| C7-DC | Pimeloyl carnitine, heptanedioyl carnitine |
| C8:1-DC | Octadecenedioyl carnitine |
| C8-DC | Suberoyl carnitine |
| C12:2 | - |
| C12:1 | Dodecenoyl carnitine |
| C12 | Lauroyl carnitine |
| C12:2-OH/C10:2-DC | - |
| C12:1-OH | Hydroxydodecenoyl carnitine |
| C12-OH/C10-DC | 3-Hydroxy-dodecanoyl carnitine or sebacoyl carnitine |
| C14:3 | - |
| C14:2 | Tetradecadienoyl carnitine |
| C14:1 | Tetradecenoyl carnitine |
| C14 | Myristoyl carnitine |
| C14:3-OH/C12:3-DC | - |
| C14:2-OH | 3-Hydroxytetradecenoylcarnitine |
| C14:1-OH | 3-Hydroxy-tetradecenoyl carnitine |
| C14-OH/C12-DC | 3-Hydroxy-tetradecanoyl carnitine or dodecanedioyl carnitine |
| C16:3 | - |
| C16:2 | Hexadecadienoyl carnitine |
| C16:1 | Palmitoleoyl carnitine |
| C16 | Palmitoyl carnitine |
| C16:3-OH/C14:3-DC | - |

| | |
|---|---|
| C16:2-OH | 3-Hydroxyhexadecadienoyl carnitine |
| C16:1-OH/C14:1-DC | 3-Hydroxy-palmitoleoyl carnitine or cis-5-tetradecenedioyl carnitine |
| C16-OH | 3-Hydroxy-hexadecanoyl carnitine |
| C18:3 | Linolenyl carnitine |
| C18:2 | Linoleyl carnitine |
| C18:1 | Oleyl carnitine |
| C18 | Stearoyl carnitine |
| C18:3-OH/C16:3-DC | 3-Hydroxyl-linolenyl carnitine or |
| C18:2-OH/C16:2-DC | 3-Hydroxy-linoleyl carnitine or hexadecadienedioyl carnitine |
| C18:1-OH/C16:1-DC | 3-Hydroxy-octadecenoyl carnitine or hexadecanedioyl carnitine |
| C18-OH/C16-DC | 3-Hydroxy-octadecanoyl carnitine or hexadecanedioyl carnitine, thapsoyl carnitine |
| C20:4 | Arachidonoyl carnitine |
| C20:3 | Dihomogammalinolenyl carnitine |
| C20:2 | - |
| C20:1 | - |
| C20 | Arachidoyl carnitine, eicosanoyl carnitine |
| C20:3-OH/C18:3-DC | - |
| C20:2-OH/C18:2-DC | - |
| C20:1-OH/C18:1-DC | Octadecenedioyl carnitine |
| C20-OH/C18-DC | 3-Hydroxy-eicosanoyl carnitine or octadecanedioyl carnitine |
| C22:5 | - |
| C22:4 | - |
| C22:3 | - |
| C22:2 | - |
| C22:1 | - |
| C22 | Docosanoyl carnitine, Behenoyl carnitine |

**Supplementary Table 2. Factors identified by sparse principal component analysis and the associated individual components, description and variance.**

| Factors | Description | Components | Percentage of variance accounted |
|---|---|---|---|
| 1 | Medium and long-chain carnitines | C8, C121, C12, C12OHC10DC, C142, C141, C14, C163, C162, C161, C181 | 11 |
| 2 | Short- and medium- chain dicarboxyl/ hydroxyl carnitines | C3, C4, C5, C4OH, C5OHC3DC, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C102, C81DC, C8DC | 6.9 |
| 3 | long chain dicarboxyl/hydroxyl carnitines | C122OHC102DC, C121OH, C142OH, C141OH, C183OHC163DC, C182OHC162DC, C201, C20, C202OHC182DC, C201OHC181DC, C20OHC18DC, C221 | 6.8 |
| 4 | Long chain carnitines | C16, C183, C182, C181, C18, C204, C203, C202, C201, C20, C202OHC182DC, C225, C224 | 6.4 |
| 5 | Medium and long chain dicarboxyl/hydroxyl carnitines | C4, C4OH, C8DC, C12OHC10DC, C141OH, C14OHC12DC, C163OHC143DC, C162OH, C161OHC141DC, C16OH, C181OHC161DC, C18OHC16DC, C203OHC183DC, C201OHC181DC | 7.7 |
| 6 | Wide spectrum carnitines including odd short chain carnitines | C3, C4, C51, C5, C81OHC61DC, C102, C101, C12OHC10DC, C143, C14OHC12DC, C163OHC143DC, C161OHC141DC, C16OH, C183, C18, C181OHC161DC, C18OHC16DC, C204, C203OHC183DC, C201OHC181DC, C225, C222, C221 | 3.1 |
| 7 | Wide spectrum carnitines including odd short chain carnitines | C2, C4OH, C6, C81, C103, C102, C101, C10, C122, C122OHC102DC, C121OH, C143, C142, C14, C141OH, C162, C161, C16, C162OH, C182, C182OHC162DC, C18OHC16DC, C202, C20, C203OHC183DC, C225, C224, C222, C22 | 4.3 |
| 8 | Wide spectrum carnitines including odd short chain carnitines | C3, C4, C5, C4OH, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C7DC, C8DC, C122, C122OHC102DC, C16OH, C183, C203OHC183DC, C202OHC182DC | 2.3 |
| 9 | Wide spectrum carnitines | C101, C81DC, C12OHC10DC, C141OH, C162OH, C16OH, C183, C183OHC163DC, C182OHC162DC, C18OHC16DC, C20, C203OHC183DC, C201OHC181DC, C20OHC18DC, C223, C221 | 2.5 |
| 10 | Medium and long chain carnitines | C102, C10, C12, C121OH, C143, C14, C143OHC123DC, C163, C16, C182, C18, C204, C203, C201, C20, C221, C22 | 2.3 |

Supplementary Table 3. Cardiovascular characteristics of young vs old non-diabetic.

| Echocardiography measurements | Young (n=418) | Old (n=515) | Univariate P-value | ~Adjusted P-value |
|---|---|---|---|---|
| Interventricular septum thickness at end diastole (IVSD) (cm) | 0.77 (0.1) | 0.80 (0.2) | **0.001** | **0.042** |
| Interventricular septum thickness at end systole (IVSS) (cm) | 1.2 (0.2) | 1.3 (0.2) | **0.003** | **0.027** |
| Left ventricular internal diameter end diastole (LVIDD) (cm) | 4.4 (0.4) | 4.4 (0.6) | **0.019** | **0.044** |
| Left ventricular internal diameter end systole (LVIDS) (cm) | 2.7 (0.4) | 2.5 (0.5) | **<0.0001** | **<0.0001** |
| Left ventricular posterior wall end diastole (LVPWD) (cm) | 0.7 (0.1) | 0.8 (0.1) | **0.001** | **0.008** |
| Left ventricular posterior wall end systole (LVPWS) (cm) | 1.4 (0.3) | 1.4 (0.2) | 0.11 | - |
| Left ventricular outflow tract (LVOT) (cm) | 2.1 (1.5) | 2.1 (0.2) | 0.24 | - |
| Aortic diameter (AO) (cm) | 2.8 (0.5) | 3.0 (0.5) | **<0.0001** | **<0.0001** |
| Left atrium (LA) (cm) | 3.4 (0.5) | 3.7 (0.6) | **<0.0001** | **<0.0001** |
| Left ventricular ejection fraction (LVEF) (%) | 71 (8.1) | 74 (8.1) | **<0.0001** | **<0.0001** |
| Left ventricular fractional shortening (LVFS) (%) | 40 (6.9) | 43 (7.5) | **<0.0001** | **<0.0001** |
| Left ventricular mass (grams) | 112 (32) | 120 (47) | **0.004** | **0.047** |
| Left ventricular mass index (grams/m$^2$) | 66 (16) | 73 (26) | **<0.0001** | **<0.0001** |
| Left atrial volume (ml) | 33 (11) | 35 (13) | **0.011** | 0.17 |
| Left atrial volume index (ml/m$^2$) | 20 (6.1) | 21 (7.8) | **<0.0001** | **0.002** |
| Isovolumic relaxation time (IVRT) (ms) | 94 (15) | 103 (18) | **<0.0001** | **<0.0001** |
| Peak velocity flow in early diastole E (MV E peak) (m/s) | 0.8 (0.2) | 0.7 (0.2) | **<0.0001** | **<0.0001** |
| Peak velocity flow in late diastole by atrial contraction A (MV A peak) (m/s) | 0.6 (0.2) | 0.8 (0.2) | **<0.0001** | **<0.0001** |
| Ratio of MV E peak velocity: MV A peak velocity | 1.4 (0.5) | 0.9 (0.3) | **<0.0001** | **<0.0001** |
| Mitral valve flow deceleration time (MV DT) (ms) | 198 (28) | 215 (41) | **<0.0001** | **<0.0001** |
| Right atrial pressure (mmHg) | 4.3 (1.3) | 4.9 (1.4) | **<0.0001** | **<0.0001** |
| Pulmonary artery systolic pressure (PASP) (mmHg) | 22 (5.4) | 27 (7.0) | **<0.0001** | **<0.0001** |
| Peak systolic septal mitral annular velocity (Septal S') (m/s) | 0.09 (0.04) | 0.08 (0.02) | **<0.0001** | **<0.0001** |
| Peak early diastolic septal mitral annular velocity (Septal E') (m/s) | 0.10 (0.03) | 0.07 (0.02) | **<0.0001** | **<0.0001** |
| Septal mitral annular velocity during atrial contraction (Septal A') (m/s) | 0.1 (0.02) | 0.1 (0.6) | 0.50 | - |
| Peak systolic lateral mitral annular velocity (m/s) | 0.1 (0.03) | 0.10 (0.03) | **<0.0001** | **<0.0001** |
| Peak early diastolic lateral mitral annular velocity (m/s) | 0.1 (0.03) | 0.09 (0.02) | **<0.0001** | **<0.0001** |
| Lateral mitral annular velocity during atrial contraction (m/s) | 0.1 (0.03) | 0.1 (0.03) | 0.53 | - |
| Ratio of Peak velocity flow in early diastole E (MV E peak) velocity to Peak early diastolic septal mitral annular velocity (Septal E') | 8.2 (2.5) | 10 (3.3) | **<0.0001** | **<0.0001** |
| **CMR measurements** | **(n=15)** | **(n=224)** | | |
| LV global longitudinal strain (LVGLS) (%) | -21 (3.0) | -21 (2.9) | 1.00 | - |
| LV global circumferential strain (LVGCS) (%) | -21 (4.6) | -22 (3.7) | 0.36 | - |
| LV global radial strain (LVGRS) (%) | 92 (53) | 104 (24) | 0.10 | - |
| Right ventricular global longitudinal strain (RVGLS) (%) | -34 (5.1) | -31 (5.4) | 0.11 | - |
| | **(n=23)** | **(n=217)** | | |
| LA reservoir strain (εs) (%) | 39 (6.2) | 31 (6.8) | **<0.0001** | **<0.0001** |
| LA conduit strain (εe) (%) | 21 (4.8) | 13 (4.2) | **<0.0001** | **<0.0001** |
| LA booster strain (εa) (%) | 17 (4.0) | 17 (4.6) | 0.97 | - |
| Reservoir strain rate (SRs) (1/s) | 2.0 (0.5) | 1.5 (0.5) | **<0.0001** | **<0.0001** |
| Conduit strain rate (SRe) (1/s) | -2.5 (0.7) | -1.3 (0.5) | **<0.0001** | **<0.0001** |
| Booster strain rate (SRa) (1/s) | -2.4 (0.7) | -2.2 (0.7) | 0.26 | - |
| Ratio of SRe/SRa | 1.1 (0.4) | 0.6 (0.3) | **<0.0001** | **<0.0001** |
| $LAvolume_{min}$ (ml) | 28 (8.8) | 31 (12) | 0.30 | - |
| $LAvolume_{max}$ (ml) | 65 (14) | 62 (18) | 0.47 | - |
| LA ejection fraction (%) | 58 (8.1) | 52 (8.6) | **0.002** | **0.004** |

~adjusted for female, BMI, CV rf>2.

Supplementary Table 4. Association between archived metabolites and cardiovascular function.
i) Outcome: E/A<=0.9.

| Archived metabolites | Events/total | HR (95% CI) | p-value | Adjusted HR (95%)* | Adjusted p-value* |
|---|---|---|---|---|---|
| **Short chain** | | | | | |
| C3 | 124/180 | 1.0 (1.0, 1.001) | 0.45 | - | - |
| C4 | 124/180 | 1.0 (1.0, 1.002) | 0.62 | - | - |
| C4-OH | 124/180 | 1.0 (0.97, 1.02) | 0.59 | - | - |
| C5 | 124/180 | 1.0 (1.0, 1.002) | 0.50 | - | - |
| C5:1 | 119/173 | 1.03 (1.01, 1.05) | **0.003** | 1.03 (1.01, 1.05) | **0.011** |
| **Medium chain** | | | | | |
| C10:1 | 119/168 | 1.0 (1.0, 1.004) | 0.97 | - | - |
| C10:2 | 96/143 | 1.01 (0.98, 1.03) | 0.53 | - | - |
| C12-OH/C10-DC | 124/180 | 0.99 (0.92, 1.07) | 0.88 | - | - |
| C8:1-OH/C6:1-DC | 124/180 | 1.0 (0.99, 1.01) | 0.81 | - | - |
| C8-DC | 124/180 | 0.99 (0.97, 1.01) | 0.50 | - | - |
| **Long chain** | | | | | |
| C14:1-OH | 124/180 | 0.99 (0.96, 1.02) | 0.47 | - | - |
| C14:3 | 124/180 | 0.98 (0.91, 1.06) | 0.69 | - | - |
| C14-OH/C12-DC | 124/180 | 0.99 (0.96, 1.02) | 0.57 | - | - |
| C16 | 124/180 | 1.0 (1.0, 1.002) | 0.60 | - | - |
| C16:1-OH/C14:1-DC | 124/180 | 0.99 (0.92, 1.06) | 0.74 | - | - |
| C16:2-OH | 124/180 | 0.96 (0.87, 1.07) | 0.48 | - | - |
| C16:3-OH/C14:3-DC | 118/172 | 1.25 (1.01, 1.54) | **0.036** | 1.19 (0.97, 1.46) | 0.10 |
| C16-OH | 124/180 | 1.0 (0.98, 1.01) | 0.61 | - | - |
| C18 | 124/180 | 1.0 (1.0, 1.002) | 0.95 | - | - |
| C18:1 | 124/180 | 1.0 (1.0, 1.005) | 0.17 | - | - |
| C18:1-OH/C16:1-DC | 124/180 | 0.98 (0.93, 1.04) | 0.55 | - | - |
| C18:2 | 124/180 | 1.0 (1.0, 1.005) | 0.87 | - | - |
| C18:3 | 121/176 | 0.99 (0.94, 1.04) | 0.71 | - | - |
| C18-OH/C16-DC | 124/180 | 1.0 (0.98, 1.01) | 0.88 | - | - |
| C20 | 124/180 | 1.02 (0.94, 1.10) | 0.62 | - | - |
| C20:1 | 124/180 | 1.01 (0.96, 1.07) | 0.62 | - | - |
| C20:1-OH/C18:1-DC | 124/180 | 0.99 (0.97, 1.02) | 0.53 | - | - |
| C20:2 | 124/180 | 1.03 (0.96, 1.11) | 0.37 | - | - |
| C20:2-OH/C18:2-DC | 123/179 | 0.97 (0.89, 1.07) | 0.55 | - | - |
| C20:3 | 124/180 | 1.02 (1.01, 1.03) | **0.003** | 1.01 (1.003, 1.03) | **0.014** |
| C20:3-OH/C18:3-DC | 115/168 | 0.98 (0.81, 1.18) | 0.83 | - | - |
| C20:4 | 124/180 | 1.01 (0.98, 1.05) | 0.46 | - | - |
| C22:1 | 124/180 | 0.92 (0.83, 1.02) | 0.10 | - | - |
| C22:2 | 119/170 | 1.06 (0.93, 1.19) | 0.44 | - | - |
| C22:4 | 121/176 | 1.07 (0.89, 1.28) | 0.47 | - | - |
| C22:5 | 123/178 | 0.97 (0.84, 1.12) | 0.64 | - | - |

*Correct for diabetes mellitus, female, BMI, CV rf>2.

ii) Outcome: εe<=13.4 %.

| Archived metabolites | Events/total | HR (95% CI) | p-value | Adjusted HR (95% CI) * | Adjusted p-value * |
|---|---|---|---|---|---|
| **Short chain** | | | | | |
| C3 | 85/163 | 1.0 (1.0, 1.001) | 0.98 | - | - |
| C4 | 85/163 | 1.0 (1.0, 1.001) | 0.42 | - | - |
| C4-OH | 85/163 | 0.98 (0.94, 1.01) | 0.22 | - | - |
| C5 | 85/163 | 1.0 (1.0, 1.002) | 0.56 | - | - |
| C5:1 | 83/159 | 1.03 (1.005, 1.06) | **0.018** | 1.03 (1.002, 1.06) | **0.037** |
| **Medium chain** | | | | | |
| C10:1 | 81/151 | 1.0 (0.99, 1.004) | 0.68 | - | - |
| C10:2 | 68/130 | 0.99 (0.96, 1.02) | 0.44 | - | - |
| C12-OH/C10-DC | 85/163 | 1.005 (0.93, 1.08) | 0.90 | - | - |
| C8:1-OH/C6:1-DC | 85/163 | 1.01 (0.99, 1.03) | 0.20 | - | - |
| C8-DC | 85/163 | 0.99 (0.97, 1.02) | 0.66 | - | - |
| **Long chain** | | | | | |
| C14:1-OH | 85/163 | 1.006 (0.97, 1.04) | 0.73 | - | - |
| C14:3 | 85/163 | 0.93 (0.84, 1.02) | 0.12 | - | - |
| C14-OH/C12-DC | 85/163 | 0.99 (0.96, 1.02) | 0.66 | - | - |
| C16 | 85/163 | 1.0 (1.0, 1.001) | 0.27 | - | - |
| C16:1-OH/C14:1-DC | 85/163 | 0.95 (0.86, 1.04) | 0.26 | - | - |
| C16:2-OH | 85/163 | 0.99 (0.88, 1.11) | 0.83 | - | - |
| C16:3-OH/C14:3-DC | 82/155 | 1.34 (1.05, 1.71) | **0.017** | 1.32 (1.05, 1.67) | **0.019** |
| C16-OH | 85/163 | 0.99 (0.97, 1.02) | 0.57 | - | - |
| C18 | 85/163 | 1.0 (0.99, 1.002) | 0.32 | - | - |
| C18:1 | 85/163 | 1.0 (1.0, 1.003) | 0.64 | - | - |
| C18:1-OH/C16:1-DC | 85/163 | 0.98 (0.91, 1.05) | 0.51 | - | - |
| C18:2 | 85/163 | 1.0 (0.99, 1.001) | 0.12 | - | - |
| C18:3 | 83/161 | 0.91 (0.84, 0.98) | **0.019** | 0.89 (0.82, 0.96) | **0.005** |
| C18-OH/C16-DC | 85/163 | 0.99 (0.97, 1.01) | 0.50 | - | - |
| C20 | 85/163 | 1.07 (0.97, 1.18) | 0.16 | - | - |
| C20:1 | 85/163 | 1.0 (0.93, 1.06) | 0.89 | - | - |
| C20:1-OH/C18:1-DC | 85/163 | 0.97 (0.93, 1.01) | 0.19 | - | - |
| C20:2 | 85/163 | 1.0 (0.91, 1.10) | 0.99 | - | - |
| C20:2-OH/C18:2-DC | 85/162 | 0.93 (0.82, 1.06) | 0.29 | - | - |
| C20:3 | 85/163 | 0.96 (0.91, 1.02) | 0.20 | - | - |
| C20:3-OH/C18:3-DC | 79/153 | 1.06 (0.83, 1.34) | 0.66 | - | - |
| C20:4 | 85/163 | 0.98 (0.93, 1.02) | 0.33 | - | - |
| C22:1 | 85/163 | 0.88 (0.76, 1.02) | 0.096 | - | - |
| C22:2 | 79/154 | 0.98 (0.81, 1.19) | 0.85 | - | - |
| C22:4 | 83/160 | 0.87 (0.69, 1.10) | 0.24 | - | - |
| C22:5 | 83/161 | 0.86 (0.71, 1.05) | 0.14 | - | - |

*adjusted for diabetes mellitus, female, BMI, CV rf>2.

Supplementary Table 5. Association between change in metabolites and cardiovascular function.
i) Outcome: εe<=13.4 %.

| Changes from archived to current | Events/total | OR (95% CI) | p-value | Adjusted OR (95% CI) * | Adjusted p-value * |
|---|---|---|---|---|---|
| **Short chain** | | | | | |
| C3 | 85/163 | 1.0 (1.0, 1.002) | 0.37 | - | - |
| C4 | 85/163 | 1.0 (1.0, 1.004) | 0.14 | - | - |
| C4-OH | 85/163 | 1.06 (1.02, 1.09) | **0.001** | 1.05 (1.01, 1.08) | **0.017** |
| C5 | 85/163 | 1.01 (1.0, 1.01) | 0.077 | - | - |
| C5:1 | 83/159 | 1.01 (0.98, 1.03) | 0.66 | - | - |
| **Medium chain** | | | | | |
| C10:1 | 81/151 | 1.0 (1.0, 1.008) | 0.25 | - | - |
| C10:2 | 68/130 | 1.01 (0.99, 1.04) | 0.32 | - | - |
| C12-OH/C10-DC | 85/163 | 1.09 (0.92, 1.28) | 0.32 | - | - |
| C8:1-OH/C6:1-DC | 85/163 | 1.0 (0.98, 1.01) | 0.55 | - | - |
| C8-DC | 85/163 | 1.02 (1.0, 1.04) | 0.092 | - | - |
| **Long chain** | | | | | |
| C14:1-OH | 85/163 | 1.03 (0.99, 1.08) | 0.13 | - | - |
| C14:3 | 85/163 | 1.11 (1.005, 1.22) | **0.040** | 1.12 (1.009, 1.25) | **0.033** |
| C14-OH/C12-DC | 85/163 | 1.07 (0.99, 1.16) | 0.076 | - | - |
| C16 | 85/163 | 1.0 (1.0, 1.01) | 0.11 | - | - |
| C16:1-OH/C14:1-DC | 85/163 | 1.11 (0.99, 1.23) | 0.068 | - | - |
| C16:2-OH | 85/163 | 1.19 (1.03, 1.38) | **0.017** | 1.18 (1.01, 1.37) | **0.037** |
| C16:3-OH/C14:3-DC | 82/155 | 1.28 (0.98, 1.66) | 0.067 | - | - |
| C16-OH | 85/163 | 1.02 (0.97, 1.07) | 0.40 | - | - |
| C18 | 85/163 | 1.0 (1.0, 1.01) | 0.36 | - | - |
| C18:1 | 85/163 | 1.01 (1.001, 1.01) | **0.027** | 1.01 (1.001, 1.01) | **0.018** |
| C18:1-OH/C16:1-DC | 85/163 | 1.08 (0.98, 1.19) | 0.12 | - | - |
| C18:2 | 85/163 | 1.01 (1.0, 1.02) | **0.047** | 1.01 (1.001, 1.02) | **0.028** |
| C18:3 | 83/161 | 1.10 (1.01, 1.20) | **0.036** | 1.12 (1.02, 1.23) | **0.019** |
| C18-OH/C16-DC | 85/163 | 1.03 (0.98, 1.07) | 0.28 | - | - |
| C20 | 85/163 | 1.02 (0.90, 1.16) | 0.76 | - | - |
| C20:1 | 85/163 | 1.06 (0.97, 1.15) | 0.23 | - | - |
| C20:1-OH/C18:1-DC | 85/163 | 1.05 (0.99, 1.11) | 0.10 | - | - |
| C20:2 | 85/163 | 1.07 (0.94, 1.23) | 0.31 | - | - |
| C20:2-OH/C18:2-DC | 85/162 | 1.09 (0.94, 1.27) | 0.26 | - | - |
| C20:3 | 85/163 | 1.08 (0.99, 1.18) | 0.070 | - | - |
| C20:3-OH/C18:3-DC | 79/153 | 1.04 (0.80, 1.36) | 0.77 | - | - |
| C20:4 | 85/163 | 1.10 (1.007, 1.19) | **0.033** | 1.10 (1.01, 1.20) | **0.038** |
| C22:1 | 85/163 | 1.13 (0.98, 1.29) | 0.092 | - | - |
| C22:2 | 79/154 | 1.11 (0.89, 1.39) | 0.35 | - | - |
| C22:4 | 83/160 | 1.23 (0.89, 1.69) | 0.21 | - | - |
| C22:5 | 83/161 | 1.28 (1.004, 1.63) | **0.046** | 1.31 (1.01, 1.71) | **0.043** |

*adjusted for diabetes mellitus, female, BMI, CV rf>2.

**PUBLICATION #3**

**Kovalik JP, Zhao X, Gao F, Leng S, Chow V, Chew H, Teo LLY, Tan RS, Ewe SH, Tan HC, Wee HN, Lee LS, Ching J, Keng BMH, Koh WP, Zhong L and Koh AS.**

**Amino acid differences between diabetic older adults and non-diabetic older adults and their associations with cardiovascular function.**

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*"Background: Ageing and insulin resistant states such as diabetes mellitus frequently coexist and increase the risk of cardiovascular disease development among older adults. Here we investigate metabolic differences in amino acid profiles between ageing and diabetes mellitus, and their associations with cardiovascular function. Methods: In a group of community older adults we performed echocardiography, cardiac magnetic resonance imaging as well as cross sectional and longitudinal metabolomics profiling based on current and archived sera obtained fifteen years prior to examination. Results: We studied a total of 515 participants (women 50%, n = 255) with a mean age 73 (SD = 4.3) years. Diabetics had higher alanine (562 vs 448, p < 0.0001), higher glutamate (107 vs 95, p = 0.016), higher proline (264 vs 231, p = 0.008) and lower arginine (107 vs 117, p = 0.043), lower citrulline (30 vs 38, p = 0.006) levels (μM) compared to non-diabetics. Over time, changes in amino acid profiles differentiated diabetic older adults from non-diabetic older adults, with greater accumulation of alanine (p = 0.002), proline (p = 0.008) and (non-significant) trend towards greater accumulation of glycine (p = 0.057) among the older diabetics compared to the older non-diabetics. However, independent of diabetes status, amino acids were associated with cardiovascular functions in ageing, [archived valine (p = 0.011), leucine (p = 0.011), archived isoleucine (p = 0.0006), archived serine (p = 0.008), archived glycine (p = 0.006) methionine (p = 0.003)] which were associated with impairments in E/A ratio. Conclusion: Markers of branched chain amino acids and one -carbon metabolism pathways were associated with changes in cardiovascular function in older adults regardless of diabetes status. However, nitrogen handling pathways were specifically altered among*

*older adults with diabetes. These findings broaden our understanding into specific amino acid pathways that may be altered between diabetic and non-diabetic older adults, and their relevance to cardiovascular function in ageing."*

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# Amino acid differences between diabetic older adults and non-diabetic older adults and their associations with cardiovascular function

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ABSTRACT

*Background:* Ageing and insulin resistant states such as diabetes mellitus frequently coexist and increase the risk of cardiovascular disease development among older adults. Here we investigate metabolic differences in amino acid profiles between ageing and diabetes mellitus, and their associations with cardiovascular function.

*Methods:* In a group of community older adults we performed echocardiography, cardiac magnetic resonance imaging as well as cross sectional and longitudinal metabolomics profiling based on current and archived sera obtained fifteen years prior to examination.

*Results:* We studied a total of 515 participants (women 50%, $n = 255$) with a mean age 73 (SD = 4.3) years. Diabetics had higher alanine (562 vs 448, $p < 0.0001$), higher glutamate (107 vs 95, $p = 0.016$), higher proline (264 vs 231, $p = 0.008$) and lower arginine (107 vs 117, $p = 0.043$), lower citrulline (30 vs 38, $p = 0.006$) levels (μM) compared to non-diabetics. Over time, changes in amino acid profiles differentiated diabetic older adults from non-diabetic older adults, with greater accumulation of alanine ($p = 0.002$), proline ($p = 0.008$) and (non-significant) trend towards greater accumulation of glycine ($p = 0.057$) among the older diabetics compared to the older non-diabetics. However, independent of diabetes status, amino acids were associated with cardiovascular functions in ageing, [archived valine ($p = 0.011$), leucine ($p = 0.011$), archived isoleucine ($p = 0.0006$), archived serine ($p = 0.008$), archived glycine ($p = 0.006$) methionine ($p = 0.003$)] which were associated with impairments in E/A ratio.

*Conclusion:* Markers of branched chain amino acids and one -carbon metabolism pathways were associated with changes in cardiovascular function in older adults regardless of diabetes status. However, nitrogen handling pathways were specifically altered among older adults with diabetes. These findings broaden our understanding into specific amino acid pathways that may be altered between diabetic and non-diabetic older adults, and their relevance to cardiovascular function in ageing.

Trial registration: ClinicalTrials.gov Identifier: NCT02791139

## 1. Introduction

The rapidly ageing population worldwide emphasizes the need for precise strategies to tackle burdens of heart disease among older adults [1]. While there are community-based studies that have looked at the association between metabolomics signatures, cardiovascular disease (CVD) and cardiovascular function [2,3], few have studied older adults. In addition, ageing processes complicate the heart failure phenotype that accompanies concomitant risk factors such as diabetes mellitus. As both ageing and diabetes are risk factors that contribute to CVD

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development, it is plausible that comparing the sera of both entities will provide clarity on biological mechanisms that underlie disease development in older adults who may also have concomitant diabetes. This is supported by recent studies that have used metabolomics to advance understanding of cardiovascular ageing [4–6], insulin resistance, and diabetes [7–12]. Investigations into joint or disparate metabolic pathways that are involved in ageing and diabetes would provide basis for future precision medicine that is targeted towards distinct mechanisms [13–15]. Biologically, the heart is a muscular organ with unique amino acid requirements such that interactions between pathways such as branched chain amino acids, one-carbon and nitrogen disposal pathways may be relevant to both ageing- and diabetes mellitus- related cardiovascular dysfunction.

Prior studies that include older adults are either cross-sectional or not pre-specified to study CVD prior to disease onset [16–18]. To overcome limitations in causality between biochemical pathways and cardiovascular disease development, pre-specified community cohorts that include samples obtained before disease development may strengthen discovery of pathways associated with ageing-related CVD.

Among a community cohort of participants with risk factors but free of cardiovascular disease, we investigate metabolic differences between ageing and diabetes mellitus, hypothesizing that amino acid profiles would unravel metabolic interactions between these entities.

## 2. Methods

### 2.1. Study population

The subjects were recruited from the Cardiac Ageing Study (CAS) (4), a prospective study initiated in 2014 that examines characteristics and determinants of cardiovascular function in older adults. CAS participants were recruited from the prospective, population-based cohort, the Singapore Chinese Health Study (SCHS) [19] and directly from the local community. The current study sample consisted of men and women who participated in the baseline CAS 2014–2017 examination who had no self-reported history of physician-diagnosed cardiovascular disease (such as coronary heart disease, atrial fibrillation), stroke or cancer (Supplementary Fig. A flow chart). Written informed consent was obtained from participants upon enrolment. The SingHealth Centralised Institutional Review Board (CIRC/2014/628/C) had approved the study protocol.

### 2.2. Data acquisition

All participants were examined and interviewed on one study visit by trained study coordinators. Participants completed a standardized questionnaire that included medical history and coronary risk factors. Hypertension was defined by current use of antihypertensive drugs or physician-diagnosed hypertension. Diabetes mellitus was defined by current use of anti-diabetic agents or physician-diagnosed diabetes mellitus. Dyslipidemia was defined by current use of lipid-lowering agents or physician-diagnosed dyslipidemia. Smoking history was defined as ever smokers (former or current smoking) or never smokers. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Sinus rhythm status was ascertained by resting electrocardiogram. Clinical data were obtained on the same day as assessment of echocardiography and serum collection.

Echocardiography was performed using ALOKA α10 with a 3.5 MHz probe. In each subject, standard echocardiography, which included 2-D, M-mode, pulse Doppler and tissue Doppler imaging, was performed in the standard parasternal and apical (apical 4-chamber, apical 2-chamber and apical long) views, and three cardiac cycles were recorded. Left ventricular ejection fraction (LVEF), left atrial (LA) volume and LA volume index were measured. The trans-mitral flow E and A wave with the sample volume position at the tip of the mitral valve leaflets from the apical 4-chamber view were recorded by Doppler echocardiography. E/A ratio was computed as a ratio of peak velocity flow in early diastole E (m/s) to peak velocity flow in late diastole by atrial contraction A (m/s). Pulsed wave tissue Doppler imaging was performed with the sample volume at the septal and lateral annulus from the apical 4-chamber view. The frame rate was between 80 and 100 frames per second. The tissue velocity patterns were recorded and expressed as E', and A'. All measurements were measured by the same operator and the measurements were averaged over three cardiac cycles and adjusted by the RR interval.

Blood samples were collected on the day of echocardiography acquisition. Plasma levels of Galectin-3 (Gal-3) (ARCHITECT Galectin-3; produced by Fujirebio Diagnostics Inc. for Abbott Laboratories) and B-type natriuretic peptide (BNP) (ARCHITECT BNP; produced by Fujirebio Diagnostics Inc. for Abbott Laboratories) were measured on the Abbott ARCHITECT i2000SR analyser. Glucose, glycated haemoglobin, urinary microalbumin were measured using standard assays.

Cine cardiac magnetic resonance (CMR) scans were performed using balanced fast field echo sequence (BFFE). All subjects were imaged on a 3 T magnetic resonance imaging system (Ingenia, Philips Healthcare, The Netherlands) with a dStream Torso coil (maximal number of channels 32). BFFE end-expiratory breath hold cine images were acquired in multi-planar long-axis views (2-, 3-, and 4-chamber views). Typical parameters were as follows: TR/TE 3/1 ms; flip angle, 45°; in-plane spatial resolution, 1.0 mm × 1.0 mm to 1.5 mm × 1.5 mm; slice thickness, 8 mm; pixel bandwidth, 1797 Hz; field of view, 300 mm; frame rate, 30 or 40 per cardiac cycle. Dedicated Qstrain software (version 2.0, Medis) was used in deriving LV longitudinal strain including LV global longitudinal strain (LVGLS), circumferential strain (LVGCS), radial strain (LVGRS) and right ventricular global longitudinal strain (RVGLS) [20]. We developed an in-house semi-automatic algorithm to track the distance (L) between the left atrioventricular junction and a user-defined point at the mid posterior LA wall on standard CMR 2- and 4-chamber views (4); [21,22]. Both 2- and 4-chamber views were used to generate the average strain and strain rate results. Longitudinal strain ($\varepsilon$) at any time point ($t$) in the cardiac cycle from end-diastole (time 0) was calculated as: $\varepsilon(t) = (L(t) - L_0)/L_0$. LA reservoir strain ($\varepsilon_s$), conduit strain ($\varepsilon_e$) and booster strain ($\varepsilon_a$) were calculated at $t$ equals left ventricular end-systole, diastasis and pre-LA systole, respectively. Peak values of the first time derivative of the strain-time curve at systole, diastasis and LA contraction corresponded to the respective peak strain rates (SR). Strain and strain rate parameters from both 2- and 4-chamber views were averaged to obtain mean results for analysis.

### 2.3. Metabolomics profiling

Antecubital venous blood samples (20–30 ml) were taken from consenting participants in the morning; fasting was not required before blood collection. After collection, the blood samples were immediately placed on ice for transportation and were processed within 6 h to obtain serum samples, which were subsequently stored at −80 °C. Additionally, archived blood samples obtained approximately 15 years prior to this assessment from subjects who had serum samples collected and stored at the time of enrolment were analyzed.

Serum metabolomic profiling analysis was performed in the Duke-NUS Metabolomics Facility. Thawed serum samples (50 μl) were spiked with 10 μl deuterium-labelled amino acid mixture and diluted with 400 μl methanol. After centrifugation of the mixture at 17,000*g* for 5 mins at 4 °C, the supernatant fraction was collected (10 μl) for amino acid analysis. A pooled quality control (QC) sample was prepared by mixing equal amounts (10 μl) of each extracted serum sample. Extraction and measurement of amino acid panels (quantified in units of μM) were performed as previously described [23]. The methanol extracts were derivatised with 3 M Hydrochloric acid in butanol (Sigma Aldrich, USA) for amino acid analysis and diluted in water for analysis in LC-MS. For amino acid analysis, a C18 column (Phenomenex, 100 × 2.1 mm, 1.6 μm, Luna® Omega) on a Agilent 1290 Infinity LC system (Agilent

**Table 1**
Baseline clinical characteristics of the overall cohort.

| | Old Non-DM (n = 399) | Old DM (n = 116) | p-value (Old DM vs Old non-DM) |
|---|---|---|---|
| Clinical covariates | | | |
| Age (years) | 73 (4.4) | 73 (4.3) | 0.86 |
| Female gender, n(%) | 207 (51.9%) | 48 (41.4%) | 0.046 |
| Body mass index (kg/m$^2$) | 23 (3.4) | 24 (3.8) | 0.027 |
| Systolic blood pressure (mmHg) | 146 (23.7) | 145 (16.3) | 0.56 |
| Diastolic blood pressure (mmHg) | 74 (11.1) | 70 (11.0) | 0.0008 |
| Heart rate (beats per minute) | 72 (12.9) | 75 (12.7) | 0.080 |
| Hypertension, n, (%) | 188 (47.1%) | 94 (81.0%) | <0.0001 |
| Dyslipidemia, n, (%) | 171 (42.9%) | 92 (79.3%) | <0.0001 |
| Ever smoked, n, (%) | 62 (16.1%) | 35 (32.7%) | <0.0001 |
| 'CV risk factor ≥2' | 129 (32.3%) | 89 (76.7%) | <0.0001 |
| Biomarkers | | | |
| BNP (pg/ml) | 40 (37.3) | 37 (37.5) | 0.53 |
| Galectin-3 (ng/ml) | 16.3 (4.2) | 18.3 (5.1) | 0.0003 |
| Urinary creatinine (mmol/L) | 6.6 (5.4) | 7.5 (5.0) | 0.22 |
| Urinary albumin (mg/L) | 25.8 (62.6) | 23.2 (30.5) | 0.75 |
| Urine albumin to creatinine ratio (mg/mmol) | 4.7 (10.1) | 4.0 (6.7) | 0.56 |
| Glycated haemoglobin (%) | 5.9 (0.6) | 6.8 (1.0) | <0.0001 |
| Random glucose (mg/dL) | 116 (37) | 175 (71) | <0.0001 |

Technologies, CA, USA) coupled with quadrupole-ion trap mass spectrometer (QTRAP 5500, AB Sciex, DC, USA) were used. Mobile phase A (Water) and Mobile phase B (Acetonitrile) both containing 0.1% Formic acid were used for chromatography separation. The LC run was performed at a flow rate of 0.4 mL min$^{-1}$ with initial gradient of 2% B for 0.8 min, then increased to 15% B in 0.1 min, 20% B in 5.7 min, 50% B in 0.5 min, 70% B in 0.5 min, followed by re-equilibration of the column to the initial run condition (2% B) for 0.9 min. All compounds were ionized in positive mode using electrospray ionization. The chromatograms were integrated using MultiQuant™ 3.0.3 software (AB Sciex, DC, USA).

We studied serum samples obtained from our subjects during the current study period (2014–2017) (which we will refer to as *current samples*) as well as from archived samples (1999–2004) (which we will refer to as *archived samples*) collected from the study subjects at the time of their enrolment approximately 15 years ago. These archived samples refer to samples from participants who were recruited from the Singapore Chinese Health Study, where they had provided blood samples in the period 1999–2004 (Supplementary Fig. A).

### 2.4. Statistics

Clinical characteristics are presented as mean and standard deviation (SD) for continuous data and frequency and percentage for categorical data. We assessed the statistical significance of the differences between old participants (aged>65 years old at the time of recruitment in 2014–2017) with diabetes versus old participants without diabetes. Student *t*-test was used for continuous data and Chi-square test was used for categorical data.

We determined amino acid profiles in serum samples from the old participants, focusing on those who had complete archived and current samples (old non-DM: $n = 154$; old DM: $n = 53$). We assessed the component metabolites, between DM and non-DM using student *t*-test. For those that show an association with $p < 0.05$, we further performed multivariable linear regression adjusted for clinical covariates; female, BMI and 2 or more risk factors (dyslipidemia, hypertension, smoking).

The associations between amino acid metabolites and cardiovascular functions were assessed by univariate Cox regression performed on *archived* metabolites and univariate logistic regression on longitudinal change in the metabolites (Supplementary Table B). Further multivariate regression model was performed on metabolites that show an association with $p < 0.05$ with cardiovascular (CV) function in univariate analysis adjusted for clinical covariates; female, body mass index (BMI), diabetes mellitus (DM) and 2 or more risk factors (dyslipidemia, hypertension, smoking). Two CV functions were analyzed in the comparison between old DM and old non-DM groups, (1) myocardial relaxation defined as E/A ≤0.9 (mean E/A 0.9 in the study subjects) and (2) left atrial conduit strain defined as εe ≤13.4 (mean εe 13.4 in the study subjects).

All statistical analyses were performed using STATA 15 (College Station, Texas, USA). For all analysis, a two-tailed *P* value of <0.05 was considered significant.

**Table 2**
Amino acids: Comparisons between Old Non-DM vs Old DM (Current sample).

| Amino acids (μM) | Non-DM (n = 154) | DM (n = 53) | p-value | Adjusted Coef. (95% CI)[a] | Adjusted P-value[a] |
|---|---|---|---|---|---|
| Alanine | 448 (111) | 562 (145) | **<0.0001** | 107 (66.6, 148) | **<0.0001** |
| Arginine | 117 (29.6) | 107 (27.3) | **0.034** | −10.0 (−19.7, −0.3) | **0.043** |
| Aspartate | 21.3 (6.1) | 21.8 (6.3) | 0.57 | – | – |
| Citrulline | 38.2 (12.5) | 30.3 (21.3) | **0.001** | −7.2 (−12.3, −2.0) | **0.006** |
| Glutamate | 95.1 (22.7) | 107 (27.3) | **0.002** | 9.4 (1.8–17.0) | **0.016** |
| Glycine | 227 (55.4) | 235 (49.9) | 0.36 | – | – |
| Histidine | 79.7 (22.1) | 78.8 (15.5) | 0.79 | – | – |
| Leucine | 120 (36.7) | 135 (43.8) | 0.14 | – | – |
| Ileleucine | 121 (55.9) | 120 (49.6) | 0.93 | – | – |
| Methionine | 26.0 (9.2) | 26.0 (10.4) | 0.98 | – | – |
| Ornithine | 93.2 (39.4) | 80.1 (20.8) | **0.022** | −10.2 (−22.2, 1.7) | 0.093 |
| Phenylalanine | 77.9 (17.2) | 74.5 (12.9) | 0.19 | – | – |
| Proline | 231 (56.9) | 264 (97.3) | **0.003** | 31.6 (8.2, 55.0) | **0.008** |
| Serine | 119 (25.3) | 119 (25.5) | 0.93 | – | – |
| Tryptophan | 54.8 (14.2) | 50.2 (14.0) | **0.039** | −4.0 (−8.7, 0.8) | 0.10 |
| Tyrosine | 72.5 (21.2) | 67.4 (20.7) | 0.14 | – | – |
| Valine | 247 (61.8) | 248 (61.2) | 0.97 | – | – |

[a] Adjusted for female, BMI, CV rf > 2.

## 3. Results

We studied a total of 515 participants (women 50%, $n = 255$) with a mean age 73 years (SD = 4.3 years) who entered the Cardiac Ageing Study and had cardiovascular imaging performed. Participants were classified into old group without diabetes ($n = 399$) and old group with diabetes ($n = 116$). Baseline clinical characteristics of the two groups is shown in Table 1. Diabetic participants had lower E/A ratio (0.8 ± 0.2 vs 0.9 ± 0.3, $p = 0.0039$), lower left atrial conduit strain (12 ± 4.3% vs 14 ± 4.1%, unadjusted $p = 0.045$), lower LA conduit strain rate (−1.2 ± 0.4 s$^{-1}$ vs −1.3 ± 0.5 s$^{-1}$, unadjusted $p = 0.042$) and lower ratio of LA conduit strain to LA booster strain (0.5 ± 0.2 vs 0.7 ± 0.3, adjusted $p = 0.0029$). Pulmonary artery systolic pressure was higher among older non-diabetics, compared to older diabetics (28 ± 7.0 vs 25 ± 6.9 mmHg, $p = 0.001$) (Supplementary Table A).

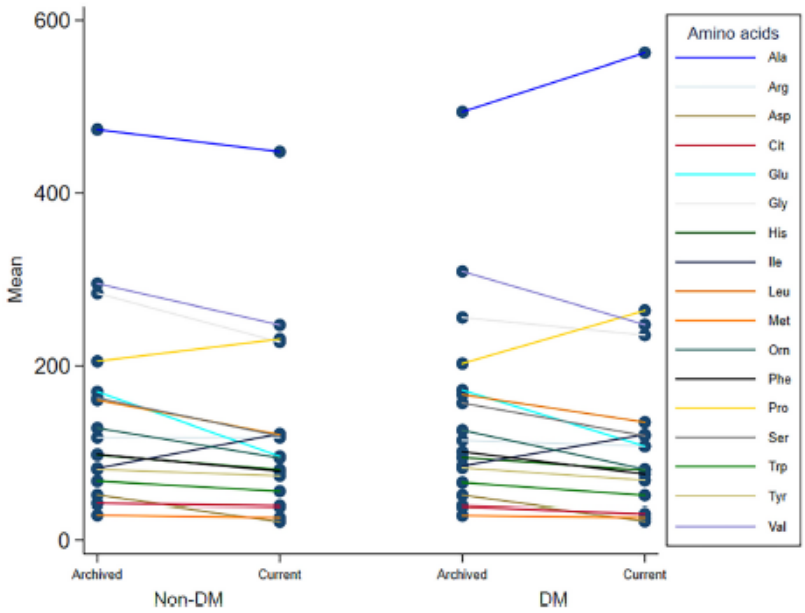


**Fig. 1.** Longitudinal trends in amino acid profiles differentiated diabetic older adults with non-diabetic older adults.

Analyses from current samples based on adjusted linear regression showed that alanine, arginine, citrulline, glutamate and proline differentiated older participants with diabetes from non-diabetics. Specifically, we observed that diabetics had higher alanine (562 vs 448, p < 0.0001), higher glutamate (107 vs 95, $p = 0.016$), higher proline (264 vs 231, $p = 0.008$) and lower arginine (107 vs 117, $p = 0.043$), lower citrulline (30 vs 38, $p = 0.006$) levels compared to non-diabetics (Table 2).

Next, we performed similar profiling on archived serum samples, as well as computed the metabolites' longitudinal change over 15-year period (Fig. 1). Longitudinal trends in amino acid profiles differentiated diabetic older adults with non-diabetic older adults. Over time, there was greater accumulation of alanine (OR 1.004, 95%CI 1.002–1.007, $p = 0.002$), proline (OR 1.01, 95%CI 1.002–1.01, $p = 0.008$) and (non-significant) trend towards greater accumulation of glycine (OR 1.0, 95%CI 0.9998–1.01, $p = 0.057$) among the older diabetics compared to the older non-diabetics. Reductions in citrulline levels were more pronounced among the older diabetics compared to the older non-diabetics (OR 0.96, 95%CI 0.9–0.99, $p = 0.005$).

We next examined if these amino acid differences between older diabetics and older non-diabetics were associated with their CV differences. Lower current levels of arginine were associated with impairments in E/A ratio (HR 0.98, 95%CI 0.97–0.99, $p = 0.002$) (Table 3). We observed that higher levels of archived alanine (HR 1.003, 95%CI 1.001–1.004, $p = 0.001$) and higher levels of archived glycine (HR 1.004, 95%CI 1.001–1.006, $p = 0.006$) were associated with higher risks of impairments in E/A ratio (Table 4). In addition, there was an (non-significant) trend towards higher risks of impairments in E/A ratio with longitudinal increase in proline (HR 1.004, 95%CI 1.0–1.01, $p = 0.073$) over time (Supplementary Table B).

However, we found a range of amino acids that were found to be associated with CV functions present in older adults, independent of diabetes status. Longitudinal increases in valine (OR 1.0, 95%CI 0.99–1.0, $p = 0.021$) (Supplementary Table B) over time, as well as in higher current levels of valine (OR 1.0, 95%CI 0.99–1.0, $p = 0.032$) (Table 3) and higher levels of archived valine (HR 1.004, 95%CI 1.001–1.007, $p = 0.011$) (Table 4) were associated with impairments in E/A ratio. We observed similar associations in other members of the branched chain amino acids, such as leucine and isoleucine. Higher levels of archived leucine (HR 1.01, 95%CI 1.002–1.02, p = 0.011) and archived isoleucine (HR 1.01, 95%CI 1.003–1.02, $p = 0.0006$) were associated with impairments in E/A ratio (Table 4). Higher levels of archived serine (HR 1.008, 95%CI 1.002–1.01, $p = 0.008$) and archived glycine (HR 1.004, 95%CI 1.001–1.006, $p = 0.006$) were associated with impairments in E/A ratio (Table 4). Lower current levels of histidine (OR 0.97, 95%CI 0.95–0.99, $p = 0.009$) were associated with worse LA conduit strain (Table 3). For methionine, lower current levels of methionine (OR 0.97, 95%CI 0.94–1.0, $p = 0.039$) (Table 3) were associated with impairments with E/A ratio, although higher archived levels of methionine (HR 1.04, 95%CI 1.01–1.07, $p = 0.003$) (Table 4) seem to be associated with impairments in E/A ratio as well.

Higher archived levels of phenylalanine (HR 1.01, 95%CI 1.002–1.02, $p = 0.015$), archived tryptophan (HR 1.02, 95%CI 1.003–1.03, $p = 0.016$), and archived tyrosine (HR 1.02, 95%CI 1.01–1.03, $p < 0.0001$) were associated with higher risks of impairments in E/A ratio in old age (Table 4). The association between aspartate and CV function was mixed. While higher levels of archived aspartate were associated with higher risks of impairments in E/A (HR 1.04, 95%CI 1.02–1.05, $p < 0.0001$) and worse LA conduit strain (HR 1.02, 95%CI 1.002–1.04, $p = 0.033$) (Table 4), lower current levels of aspartate (OR 0.94, 95%CI 0.90–0.99, $p = 0.02$) were associated with higher risks of E/A in old age (Table 3).

## 4. Discussion

In a large study of community-dwelling older adults without cardiovascular disease, we integrated phenotypic observations with cross-sectional and longitudinal metabolomic profiling to identify distinguishing metabolic pathways altered in older adults with diabetes.

Our study showed that in older subjects, higher alanine, glutamate

**Table 3**
Association between current metabolites and CV function.

i) Outcome: E/A ≤0.9

| Current metabolites | Events/ total | OR (95% CI) | p-value | Adjusted OR (95%)* | Adjusted p-value* |
|---|---|---|---|---|---|
| Alanine | 142/ 207 | 1.002 (0.999, 1.004) | 0.17 | – | – |
| Arginine | 142/ 207 | 0.98 (0.97, 0.99) | **0.001** | 0.98 (0.97, 0.99) | **0.002** |
| Aspartate | 142/ 207 | 0.95 (0.90, 0.99) | **0.024** | 0.94 (0.90, 0.99) | **0.020** |
| Citrulline | 142/ 207 | 0.98 (0.97, 1.003) | 0.10 | – | – |
| Glutamate | 142/ 207 | 1.005 (0.99, 1.02) | 0.39 | – | – |
| Glycine | 142/ 207 | 0.995 (0.99, 1.00003) | 0.051 | – | – |
| Histidine | 142/ 207 | 0.996 (0.98, 1.01) | 0.56 | – | – |
| Leucine | 53/82 | 0.99 (0.98, 1.002) | 0.11 | – | – |
| Ileleucine | 142/ 207 | 0.998 (0.99, 1.004) | 0.55 | – | – |
| Methionine | 142/ 207 | 0.97 (0.94, 0.997) | **0.034** | 0.97 (0.94, 0.998) | **0.039** |
| Ornithine | 142/ 207 | 0.999 (0.99, 1.01) | 0.90 | – | – |
| Phenylalanine | 142/ 207 | 0.99 (0.97, 1.01) | 0.37 | – | – |
| Proline | 142/ 207 | 1.001 (0.997, 1.01) | 0.50 | – | – |
| Serine | 142/ 207 | 0.998 (0.99, 1.01) | 0.80 | – | – |
| Tryptophan | 142/ 207 | 0.98 (0.96, 1.001) | 0.059 | – | – |
| Tyrosine | 142/ 207 | 0.99 (0.98, 1.01) | 0.46 | – | – |
| Valine | 142/ 207 | 0.99 (0.99, 0.9998) | **0.043** | 0.99 (0.99, 0.9995) | **0.032** |

ii) Outcome: LA conduit strain, ee ≤13.4%

| Current metabolites | Events/ total | OR (95% CI) | p-value | Adjusted OR (95% CI)[a] | Adjusted p-value[a] |
|---|---|---|---|---|---|
| Alanine | 87/169 | 1.001 (1.0, 1.003) | 0.30 | – | – |
| Arginine | 87/169 | 0.99 (0.98, 1.01) | 0.30 | – | – |
| Aspartate | 87/169 | 1.02 (0.97, 1.07) | 0.46 | – | – |
| Citrulline | 87/169 | 0.98 (0.96, 1.002) | 0.068 | – | – |
| Glutamate | 87/169 | 1.01 (0.998, 1.02) | 0.11 | – | – |
| Glycine | 87/169 | 1.002 (0.996, 1.01) | 0.55 | – | – |
| Histidine | 87/169 | 0.97 (0.96, 0.99) | **0.009** | 0.97 (0.95, 0.99) | **0.009** |
| Leucine | 21/54 | 1.001 (0.99, 1.01) | 0.85 | – | – |
| Ileleucine | 87/169 | 1.004 (0.999, 1.01) | 0.12 | – | – |
| Methionine | 87/169 | 1.005 (0.97, 1.04) | 0.76 | – | – |
| Ornithine | 87/169 | 0.99 (0.98, 1.002) | 0.13 | – | – |

**Table 3** (*continued*)

ii) Outcome: LA conduit strain, ee ≤13.4%

| Current metabolites | Events/ total | OR (95% CI) | p-value | Adjusted OR (95% CI)[a] | Adjusted p-value[a] |
|---|---|---|---|---|---|
| Phenylalanine | 87/169 | 1.001 (0.98, 1.02) | 0.95 | – | – |
| Proline | 87/169 | 0.9997 (0.996, 1.004) | 0.90 | – | – |
| Serine | 87/169 | 0.998 (0.99, 1.01) | 0.69 | – | – |
| Tryptophan | 87/169 | 0.995 (0.98, 1.02) | 0.66 | – | – |
| Tyrosine | 87/169 | 0.997 (0.98, 1.01) | 0.62 | – | – |
| Valine | 87/169 | 1.003 (0.998, 1.01) | 0.28 | – | – |

[a] Adjusted for diabetes mellitus, female, BMI, CV rf > 2.

and proline as well as lower arginine and citrulline distinguished diabetics from non-diabetics. Additionally, increasing alanine and proline and decreasing citrulline between archived and current samples also distinguished diabetics from non-diabetics. We did not find any significant associations between the BCAA and large neutral amino acids and diabetes. This is different from other studies which show a clear association between higher serum leucine, isoleucine, phenylalanine, tyrosine and tryptophan and insulin resistance (9;24). Our cohort is comprised primarily of older adults which is in contrast to most other studies which have examined serum BCAA and insulin resistance in younger subjects (age 46–51) [24], (age 50–51) (9). BCAA changes with ageing are less well characterized [25] but studies have associated older age with declining serum BCAA [26–28]. Some studies associate decreasing serum BCAA with ageing-related frailty and increased risk of death while other studies show decreased serum BCAA in healthy aged subjects. The average age of our study participants was 73 years so the failure to detect increased BCAA with insulin resistance may be related to ageing processes in our subjects.

Higher serum glutamate(9); [29], alanine (9); [30] and proline (9) have been associated with insulin resistance and risk of type two diabetes mellitus. Decreased citrulline (9) and changes in various components of nitrogen handling [30] have also been associated with diabetes risk. Changes in alanine and glutamate have also been linked to increased BCAA levels (9) and this has been explained to be as a result of interactions between all of these metabolites in central carbon pathways. In our study this association does not hold, perhaps due to ageing-related effects on BCAA. It may also mean that BCAA, alanine and glutamate are not as intimately associated as previously assumed. The relationship between nitrogen handling and insulin resistance and diabetes is not well established. Some studies have made a link between gut microbiome-derived nitrogen metabolites and diabetes risk [31] which raises a possible connection between our findings of altered nitrogen handling, diabetes risk and gut microbiome composition and activity [32].

In the entire cohort several amino-acid related metabolite patterns emerged in association with worsening myocardial function. Current and/or archived valine, leucine and isoleucine was directly associated with changes in E/A, reflecting alterations in myocardial relaxation. Additionally, archived tryptophan and tyrosine showed the same association. These 'large neutral amino acids' including the branched-chain amino acids (BCAA), tryptophan and tyrosine have been consistently shown to be elevated in insulin resistant states (9). Since ageing is considered a risk factor for diabetes and insulin resistance [33] it is

**Table 4**
Association between archived metabolites and CV function.

| i) Outcome: E/A ≤0.9 | | | | | |
|---|---|---|---|---|---|
| Archived metabolites | Events/ total | HR (95% CI) | p-value | Adjusted HR (95%)* | Adjusted *p*-value* |
| Alanine | 124/ 180 | 1.002 (1.001, 1.003) | **0.003** | 1.003 (1.001, 1.004) | **0.001** |
| Arginine | 94/140 | 1.0001 (0.99, 1.01) | 0.98 | – | – |
| Aspartate | 124/ 180 | 1.03 (1.02, 1.05) | **<0.0001** | 1.04 (1.02, 1.05) | **<0.0001** |
| Citrulline | 124/ 180 | 0.998 (0.98, 1.01) | 0.77 | – | – |
| Glutamate | 124/ 180 | 1.004 (0.9995, 1.01) | 0.078 | – | – |
| Glycine | 124/ 180 | 1.003 (1.0001, 1.01) | **0.041** | 1.004 (1.001, 1.01) | **0.006** |
| Histidine | 124/ 180 | 1.002 (0.99, 1.01) | 0.79 | – | – |
| Leucine | 40/61 | 1.01 (1.002, 1.02) | **0.010** | 1.01 (1.002, 1.02) | **0.011** |
| Ileleucine | 124/ 180 | 1.01 (1.004, 1.02) | **0.003** | 1.01 (1.003, 1.02) | **0.006** |
| Methionine | 124/ 180 | 1.04 (1.01, 1.06) | **0.004** | 1.04 (1.01, 1.07) | **0.003** |
| Ornithine | 124/ 180 | 1.001 (0.997, 1.01) | 0.58 | – | – |
| Phenylalanine | 124/ 180 | 1.01 (1.002, 1.02) | **0.013** | 1.01 (1.002, 1.02) | **0.015** |
| Proline | 124/ 180 | 1.002 (0.999, 1.01) | 0.15 | – | – |
| Serine | 124/ 180 | 1.006 (1.001, 1.01) | **0.024** | 1.008 (1.002, 1.01) | **0.008** |
| Tryptophan | 124/ 180 | 1.01 (1.001, 1.03) | **0.028** | 1.02 (1.003, 1.03) | **0.016** |
| Tyrosine | 124/ 180 | 1.01 (1.005, 1.02) | **0.002** | 1.02 (1.01, 1.03) | **<0.0001** |
| Valine | 124/ 180 | 1.004 (1.001, 1.01) | **0.005** | 1.004 (1.001, 1.01) | **0.011** |

| Outcome: LA conduit strain, ee ≤13.4% | | | | | |
|---|---|---|---|---|---|
| Archived metabolites | Events/ total | HR (95% CI) | *p*- value | Adjusted HR (95% CI)* | Adjusted *p*-value* |
| Alanine | 85/163 | 1.002 (0.99997, 1.003) | 0.054 | – | – |
| Arginine | 66/125 | 1.006 (0.995, 1.02) | 0.28 | – | – |
| Aspartate | 85/163 | 1.02 (1.0001, 1.04) | **0.048** | 1.02 (1.002, 1.04) | **0.033** |
| Citrulline | 85/163 | 0.99 (0.97, 1.01) | 0.32 | – | – |
| Glutamate | 85/163 | 1.0003 (0.99, 1.01) | 0.90 | – | – |
| Glycine | 85/163 | 1.001 (0.998, 1.004) | 0.43 | – | – |
| Histidine | 85/163 | 0.99 (0.98, 1.01) | 0.18 | – | – |
| Leucine | 21/53 | 1.004 (0.99, 1.01) | 0.45 | – | – |
| Ileleucine | 85/163 | 1.01 (1.002, 1.02) | **0.020** | 1.01 (0.999, 1.02) | 0.063 |
| Methionine | 85/163 | 1.02 (0.99, 1.05) | 0.22 | – | – |
| Ornithine | 85/163 | 0.996 (0.99, 1.002) | 0.19 | – | – |
| Phenylalanine | 85/163 | 1.01 (0.998, 1.02) | 0.13 | – | – |
| Proline | 85/163 | 1.003 (0.999, 1.01) | 0.12 | – | – |
| Serine | 85/163 | 1.002 (0.996, 1.01) | 0.49 | – | – |
| Tryptophan | 85/163 | 1.01 (0.99, 1.02) | 0.29 | – | – |
| Tyrosine | 85/163 | 1.01 (0.997, 1.02) | 0.15 | – | – |
| Valine | 85/163 | 1.003 (0.999, 1.01) | 0.095 | – | – |

*Correct for diabetes mellitus, female, BMI, CV rf > 2.
*Adjusted for diabetes mellitus, female, BMI, CV rf > 2.

possible that ageing-associated insulin resistance, even in those not diagnosed with DM, is contributing to the relaxation abnormalities noted in this cohort [34]. Changes in myocardial BCAA metabolism have also been directly implicated in heart failure both in human subjects [35] as well as in mouse models [36].

In the entire cohort archived or current serine, glycine and methionine were also associated with E/A. These amino acids contribute towards many distinct pathways but as a whole can be mapped back to one-carbon metabolism (Fig. 2). Elements of the one carbon metabolism pathway have been implicated in human LV dysfunction [37], [38], and a mouse model of heart failure [39]. Changes in homocysteine and the choline-related methylamine metabolites are linked to increased risk of atherosclerosis [11,40]. Our observations among a sample of community-based participants expand upon these prior findings and suggest that these pathways may be important to look at in preventative studies. Our results lend support to another similar cohort of aged subjects, which has also reported associations between serine, arginine and tyrosine with ageing [41].

The ageing DM group showed increased levels of alanine, glutamine, proline with decreased arginine and citrulline. These amino acids all play a role in nitrogen transfer and excretion. The increased alanine and decreased arginine noted in the ageing DM group was also found to be associated with E/A function. Both of these amino acids contribute to body nitrogen handling via inter-tissue nitrogen transfer (alanine) or urea cycle (arginine) (Fig. 2) and have been implicated in HFpEF [37,42]. Other studies of heart failure have found associations with nitrogen pathways [38,43]. Finally, a longitudinal study of normal ageing has shown arginine and ornithine declining in subjects as they age [37]. These findings point to a potential role for changes in nitrogen handling in the pathogenesis of diabetes-related heart failure in older subjects.

The strengths of our study include the large sample size and multiple time point sampling of participants. Other strengths include a largely quantitative technique that provides absolute determination of

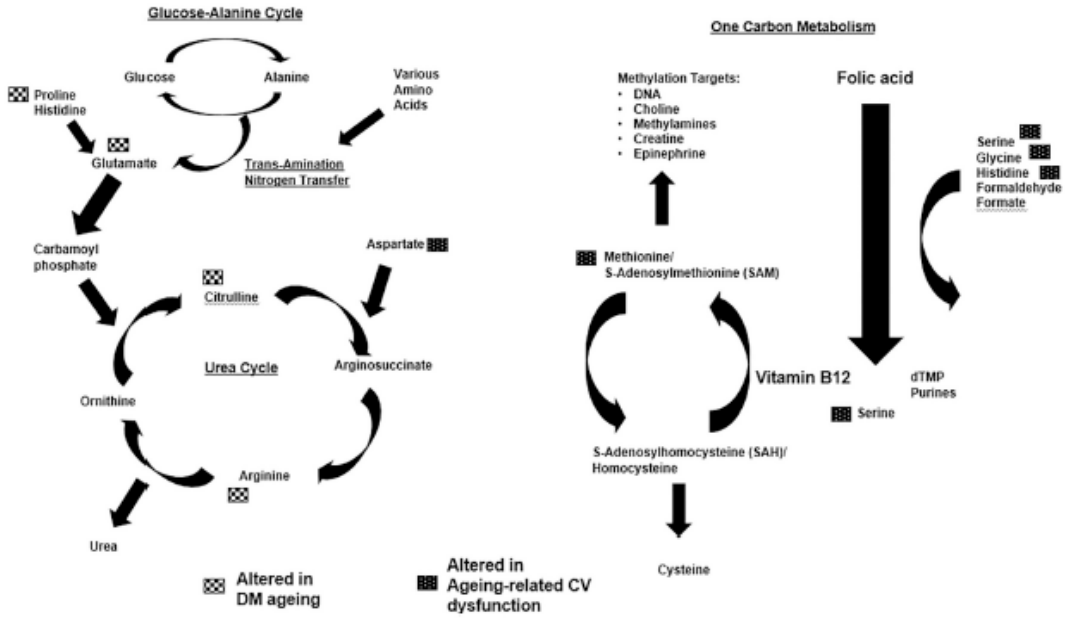


**Fig. 2.** Overview of nitrogen handling and one carbon metabolism pathways. Nitrogen handling: Nitrogen is funnelled into glutamine via transamination reactions involving various amino acids, the glucose-alanine cycle and catabolism of amino acids such as proline and histidine. Glutamine and aspartate contribute nitrogen to the urea cycle. One carbon metabolism: Folic acid accepts one carbon groups from various sources. One carbon groups are transferred from folic acid to purine, serine and vitamin B12. Vitamin B12 transfers methyl groups to homocysteine to 're-charge' methionine. S-adenosylmethionine transfers methyl groups to various targets including DNA, choline the methylamines (ie betaine, sarcosine), creatine and epinephrine.

metabolite which is useful for precise correlations between metabolite levels and cardiovascular outcomes. Despite these strengths our study has limitations. The observational study design does not imply causality between the metabolites and their present-day cardiovascular function. Adaptive versus pathogenic responses cannot be differentiated based on this clinical study design, although it probably reflects generalizable real-world data. Actual mechanistic studies such as animal studies might provide greater insights into adaptive versus pathogenic responses. However, our human clinical data is useful for narrowing down to specific metabolic pathways, relevant to cardiovascular health states. Similar studies from other cohorts would be necessary to confirm our observations, in addition to improving generalizability. We did not correct for details such as medication data so that effects arising from medication treatment are unknown. However, this is a low risk community cohort (based on biomarkers such as glycated haemoglobin), for which the observed relationships are more likely to be underestimated than overestimated. The marginal values in some of the observed risk ratios may also reflect underestimation rather than overestimation of clinical significance.

The fasting status of the subjects in our cohort was neither prescribed nor ascertained so that effects of post-prandial rise in amino acids contributing to the signal cannot be precluded. Prior studies that reported increased BCAA and large neutral amino acids in patients with insulin resistance used fasting samples for the analysis(9;24). In these studies the BCAA leucine/isoleucine and valine as well as the large neutral amino acids phenylalanine, tyrosine and tryptophan were elevated in obesity and insulin resistant states. In our study there were no differences in BCAA while tryptophan was lower in diabetic versus non-diabetics. This difference may have come about due to our use of samples which were not ascertained to be fasting. Another consideration, is the age difference in our cohort (average age 73 years) vs previous reported studies as discussed above. Among previous studies that have examined cardiovascular function and serum metabolites, fasting status was not consistent. One study that used fasting samples found association between BCAA and heart failure subtypes [8]. A second study which did not specify use of fasting samples identified various amino acids including components of nitrogen handling as increased in heart failure with preserved ejection fraction versus control [37]. A separate study which also did not specify use of fasting samples found lower histidine and increased phenylalanine associated with heart failure diagnosis [44]. Our current study demonstrated associations between various amino acid levels and discrete measurements of cardiovascular function, in particular left ventricle relaxation. From an upstream perspective, the left ventricle defects may represent early and perhaps important drivers preceding clinical heart failure in older adults.

Finally, while there may be analytic differences between non-fasting serum samples and fasting serum samples, a study had shown that fasting, season of blood collection, and time of day of blood collection were not important sources of variability in measurements of most metabolites [45].

## 5. Conclusion

Community-dwelling older adults with diabetes but no known cardiovascular disease had alterations in nitrogen handling metabolic pathways that were associated with changes in cardiovascular function. In ageing subjects, markers of BCAA and one -carbon metabolism pathways were associated with changes in cardiovascular function regardless of diabetes status. These findings can help broaden our

understanding of how tissue metabolic pathways contribute to ageing-related changes to cardiovascular function in the presence or absence of diabetes.

## Ethics approval and consent to participate

Written informed consent was obtained from participants upon enrolment. The SingHealth Centralised Institutional Review Board (CIRC/2014/628/C) had approved the study protocol.

## Consent for publication

Not applicable.

## Contributors

JPK, FG, RST, WPK, LZ, ASK contributed to the conception and design of the study, advised on all statistical aspects, and interpreted the data. JPK, XDZ, FG, SL, VC, HC, LYT, SHE, HCT, WHN, LSL, JC, BMK analyses. All authors critically reviewed previous drafts. All authors approved the final draft for submission.

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## Declaration of Competing Interest

None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yjmcc.2021.05.009.

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**COMMENTARY (PUBLICATIONS #2 AND #3)**

**Main findings of both publications #2 and #3:**

**a) Metabolomics differentiated older adults with ageing and older adults with diabetes mellitus**

Both ageing and diabetes mellitus are risk factors that contribute to cardiovascular diseases in older adults. The sera of both ageing and diabetes exist in the same circulatory milieu but individual or superimposed effects of either are poorly characterised. Metabolomics, however, has the potential to map out joint or disparate metabolic pathways. This is supported by emerging studies that have used metabolomics to understand diabetes, insulin resistance and cardiovascular ageing[46, 61-64]. The clinical implication of ageing with or without diabetes is significant, as there may be differences in cardiovascular phenotype, and treatment. In older adults, age-associated decreases in left ventricular volumes, increases in left ventricular mass index and deteriorations in diastolic function frequently accompany heart failure in ageing[65].

Our work in these studies differentiated cardiovascular characteristics of older adults with and without diabetes. All participants were examined and interviewed on one study visit by trained study coordinators. Participants completed a standardised questionnaire that included medical history and coronary risk factors. Sinus rhythm status was ascertained by resting electrocardiogram. Clinical data were obtained on the same day as assessment of echocardiography and serum collection. Echocardiography was performed using ALOKA α10 with a 3.5 MHz probe. In each subject, standard echocardiography, which included 2-D, M-mode, pulse Doppler and tissue Doppler imaging, was performed in the standard parasternal and apical (apical 4-chamber, apical 2-chamber and apical long) views, and three cardiac cycles were recorded. E/A ratio was computed as a ratio of peak velocity flow in early diastole E (m/s) to peak velocity flow in late diastole by atrial contraction A (m/s). Cine cardiac magnetic resonance (CMR) scans were performed using

balanced fast field echo sequence (BFFE). All subjects were imaged on a 3T magnetic resonance imaging system (Ingenia, Philips Healthcare, The Netherlands) with a dStream Torso coil (maximal number of channels 32). Dedicated Qstrain software (version 2.0, Medis) was used in deriving LV and RV longitudinal strain[66]. We developed an in-house semi-automatic algorithm to track the distance (L) between the left atrioventricular junction and a user-defined point at the mid posterior LA wall on standard CMR 2- and 4-chamber views.

There were non-diabetics (n=399) and diabetic (n=116) as shown in Table 2. Compared to the younger group, participants in the overall older group had larger left ventricular wall thickness, left ventricular mass, left atria size and volume, and poorer left diastolic function such as lower ratio of peak velocity flow in early diastole to peak velocity flow in late diastole. Left ventricular and left atria sizes and structures were similar in non-diabetic and diabetic subgroups. However, older adults with diabetes had lower E/A ratio (0.8±0.2 vs 0.9±0.3, p=0.0039). Lower left atrial functions were observed among older adults with diabetes compared to older adults without diabetes . Older adults with diabetes  had lower left atrial conduit strain (12±4.3% vs 14±4.1%, unadjusted p=0.045), lower LA conduit strain rate (-1.2±0.4 s-1 vs -1.3±0.5 s-1, unadjusted p=0.042) and lower ratio of LA conduit strain to LA booster strain (0.5±0.2 vs 0.7±0.3, adjusted p=0.0029). Pulmonary artery systolic pressure was higher among older adults without diabetes , compared to older adults with diabetes (28±7.0 vs 25±6.9 mmHg, p=0.001) (Table 2).

| **Echocardiography measurements** | **Older adults without diabetes (n=399)** | **Older adults with diabetes (n=116)** | **Univariate p-value** | **~Adjusted P-value** |
|---|---|---|---|---|
| Interventricular septum thickness at end diastole (IVSD) (cm) | 0.80 (0.1) | 0.81 (0.2) | 0.52 | - |
| Interventricular septum thickness at end systole (IVSS) (cm) | 1.3 (0.2) | 1.2 (0.2) | 0.76 | - |
| Left ventricular internal diameter end diastole (LVIDD) (cm) | 4.4 (0.6) | 4.3 (0.6) | 0.12 | - |
| Left ventricular internal diameter end systole (LVIDS) (cm) | 2.5 (0.5) | 2.4 (0.5) | 0.41 | - |
| Left ventricular posterior wall end diastole (LVPWD) (cm) | 0.76 (0.1) | 0.77 (0.1) | 0.16 | - |
| Left ventricular posterior wall end systole (LVPWS) (cm) | 1.4 (0.2) | 1.5 (0.2) | 0.28 | - |
| Left ventricular outflow tract (LVOT) (cm) | 2.1 (0.2) | 2.0 (0.2) | 0.26 | - |
| Aortic diameter (AO) (cm) | 3.0 (0.4) | 3.1 (0.4) | 0.084 | - |
| Left atrium (LA) (cm) | 3.6 (0.6) | 3.7 (0.6) | 0.55 | - |
| Left ventricular ejection fraction (LVEF) (%) | 74 (7.7) | 73 (9.2) | 0.11 | - |
| Left ventricular fractional shortening (LVFS) (%) | 44 (7.4) | 42 (7.8) | 0.12 | - |
| Left ventricular mass (grams) | 120 (49) | 116 (40) | 0.41 | - |
| Left ventricular mass index (grams/$m^2$) | 74 (27) | 70 (22) | 0.14 | - |
| Left atrial volume (ml) | 35 (13) | 36 (14) | 0.45 | - |
| Left atrial volume index (ml/$m^2$) | 21 (7.7) | 22 (8.2) | 0.90 | - |
| Isovolumic relaxation time (IVRT) (ms) | 103 (18) | 103 (20) | 0.98 | - |
| Peak velocity flow in early diastole E (MV E peak) (m/s) | 0.71 (0.2) | 0.70 (0.2) | 0.51 | - |
| Peak velocity flow in late diastole by atrial contraction A (MV A peak) (m/s) | 0.81 (0.2) | 0.87 (0.2) | **0.005** | 0.15 |
| Ratio of MV E peak velocity: MV A peak velocity | 0.91 (0.3) | 0.82 (0.2) | **0.003** | **0.039** |
| Mitral valve flow deceleration time (MV DT) (ms) | 213 (40) | 222 (42) | **0.034** | 0.23 |
| Right atrial pressure (mmHg) | 5.0 (1.3) | 4.7 (1.7) | 0.36 | - |
| Pulmonary artery systolic pressure (PASP) (mmHg) | 28 (7.0) | 25 (6.9) | **0.005** | **0.001** |
| Peak systolic septal mitral annular velocity (Septal S′) (m/s) | 0.078 (0.02) | 0.077 (0.01) | 0.38 | - |
| Peak early diastolic septal mitral annular velocity (Septal E') (m/s) | 0.074 (0.02) | 0.067 (0.02) | **0.0003** | **0.021** |
| Septal mitral annular velocity during atrial contraction (Septal A') (m/s) | 0.14 (0.6) | 0.11 (0.02) | 0.60 | - |
| Peak systolic lateral mitral annular velocity (m/s) | 0.10 (0.03) | 0.10 (0.03) | 0.10 | - |
| Peak early diastolic lateral mitral annular velocity (m/s) | 0.094 (0.02) | 0.088 (0.02) | **0.019** | 0.094 |
| Lateral mitral annular velocity during atrial contraction (m/s) | 0.12 (0.03) | 0.13 (0.02) | 0.51 | - |
| Ratio of Peak velocity flow in early diastole E (MV E peak) velocity to Peak early diastolic septal mitral annular velocity (Septal E') | 10 (3.3) | 11 (3.1) | **0.022** | 0.34 |
| | | | | |
| **CMR measurements** | **(n=187)** | **(n=51)** | | |
| LV global longitudinal strain (LVGLS) (%) | -21 (2.9) | -21 (2.9) | 0.28 | - |
| LV global circumferential strain (LVGCS) (%) | -22 (3.8) | -23 (3.1) | 0.21 | - |
| LV global radial strain (LVGRS) (%) | 104 (25.1) | 104 (19.5) | 0.98 | - |
| Right ventricular global longitudinal strain (RVGLS) (%) | -31 (5.4) | -31 (5.5) | 0.84 | - |
| LA reservoir strain (εs) (%) | 31 (6.9) | 31 (6.2) | 0.98 | - |
| LA conduit strain (εe) (%) | 14 (4.1) | 12 (4.3) | **0.045** | 0.28 |
| LA booster strain (εa) (%) | 17 (4.7) | 18 (3.9) | 0.065 | - |
| Reservoir strain rate (SRs) (1/s) | 1.5 (0.5) | 1.5 (0.4) | 0.92 | - |
| Conduit strain rate (SRe) (1/s) | -1.3 (0.5) | -1.2 (0.4) | **0.042** | 0.30 |

| | | | | |
|---|---|---|---|---|
| Booster strain rate (SRa) (1/s) | -2.2 (0.7) | -2.3 (0.6) | 0.19 | - |
| Ratio of SRe/SRa | 0.66 (0.3) | 0.55 (0.2) | **0.006** | **0.029** |
| $LAvolume_{min}$ (ml) | 31 (12.6) | 27 (10.1) | **0.044** | **0.016** |
| $LAvolume_{max}$ (ml) | 64 (18) | 57 (17) | **0.017** | **0.006** |
| LA ejection fraction (%) | 52 (8.9) | 52 (7.2) | 0.92 | - |

***Table 2: Cardiovascular characteristics of older adults without diabetes vs older adults with diabetes.*** ~ adjusted for female, BMI, CV rf>2

Next, we investigated the association between acylcarnitines and cardiac function.

Acylcarnitines are intermediates of fatty acids and branched-chain amino acid metabolism. Acylcarnitines are essential for beta-oxidation and energy metabolism[67]. They act as carriers to transport long chain fatty acids into mitochondria for beta-oxidation to provide energy for cellular metabolism[68]. Reductions in mitochondrial bioenergetics is an important hallmark of ageing[69]. Therefore, abnormal acylcarnitine levels are biomarkers of mitochondrial dysfunction which have been used to study age-related conditions such as frailty[70] and Alzheimer's disease[71], in addition to type 2 diabetes mellitus[72]. Acylcarnitines may be biologically classified based on their subspecies after catabolic metabolism, consisting of very long chain (C≥24), long chain (C12-22), medium chain (C8-10), short chain (C2-6), or short chain di-carboxyl and hydroxylated species (-DC, -OH)[73].

Blood samples were collected simultaneously with cardiovascular imaging acquisition. After collection, the blood samples were immediately placed on ice for transportation and were processed within 6 hours to obtain serum samples. Serum metabolomic profiling analysis for acyl-carnitines was performed in a dedicated metabolomics facility. A pooled quality control (QC) sample was prepared by mixing equal amounts (10µl) of each extracted serum sample. For acyl-carnitines, serum samples (50µl) were spiked with 10µl deuterium-labelled acyl-carnitine mixture and diluted with 400µl methanol. Data acquisition and analysis were performed on an Agilent MassHunter Workstation B.06.00 Software.

To identify acylcarnitines profiles and reduce the dimensionality of correlated metabolites, we performed sparse principal component analysis (SPCA) using a penalised matrix decomposition. In SPCA, we normalised the distributions of all metabolites by a logarithmic transformation. We assessed the component metabolites within the significant PCA factors, between diabetic and non-diabetic using student t-test. For those that show an association with $p<0.05$, we further performed multivariable linear regression adjusted for clinical covariates; female, body mass index and risk factors (dyslipidaemia, hypertension, smoking). To determine the association between serum metabolomic acyl-carnitine measures to cardiac function, univariate Cox regression was performed on baseline and change in metabolite levels. Further multivariate regression model was performed on metabolites that show an association with $p<0.05$ with cardiac function in univariate analysis adjusted for clinical covariates; female, body mass index body mass index, diabetes mellitus and risk factors (dyslipidaemia, hypertension, smoking). All statistical analyses were performed using STATA 15 (College Station, Texas, USA), while the SPCA were performed by R. For all analysis, a two-tailed P value of $<0.05$ was considered significant.

| Factors | Description | Components | Percentage of variance accounted |
|---|---|---|---|
| **1** | Medium and long-chain carnitines | C8, C121, C12, C12OHC10DC, C142, C141, C14, C163, C162, C161, C181 | 11 |
| **2** | Short- and medium- chain dicarboxyl/ hydroxyl carnitines | C3, C4, C5, C4OH, C5OHC3DC, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C102, C81DC, C8DC | 6.9 |
| **3** | long chain dicarboxyl/hydroxyl carnitines | C122OHC102DC, C121OH, C142OH, C141OH, C183OHC163DC, C182OHC162DC, C201, C20, C202OHC182DC, C201OHC181DC, C20OHC18DC, C221 | 6.8 |
| **4** | Long chain carnitines | C16, C183, C182, C181, C18, C204, C203, C202, C201, C20, C202OHC182DC, C225, C224 | 6.4 |
| **5** | Medium and long chain dicarboxyl/hydroxyl carnitines | C4, C4OH, C8DC, C12OHC10DC, C141OH, C14OHC12DC, C163OHC143DC, C162OH, C161OHC141DC, C16OH, C181OHC161DC, C18OHC16DC, C203OHC183DC, C201OHC181DC | 7.7 |
| **6** | Wide spectrum carnitines including odd short chain carnitines | C3, C4, C51, C5, C81OHC61DC, C102, C101, C12OHC10DC, C143, C14OHC12DC, C163OHC143DC, C161OHC141DC, C16OH, C183, C18, C181OHC161DC, C18OHC16DC, C204, C203OHC183DC, C201OHC181DC, C225, C222, C221 | 3.1 |
| **7** | Wide spectrum carnitines including odd short chain carnitines | C2, C4OH, C6, C81, C103, C102, C101, C10, C122, C122OHC102DC, C121OH, C143, C142, C14, C141OH, C162, C161, C16, C162OH, C182, C182OHC162DC, C18OHC16DC, C202, C20, C203OHC183DC, C225, C224, C222, C22 | 4.3 |
| **8** | Wide spectrum carnitines including odd short chain carnitines | C3, C4, C5, C4OH, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C7DC, C8DC, C122, C122OHC102DC, C16OH, C183, C203OHC183DC, C202OHC182DC | 2.3 |
| **9** | Wide spectrum carnitines | C101, C81DC, C12OHC10DC, C141OH, C162OH, C16OH, C183, C183OHC163DC, C182OHC162DC, C18OHC16DC, C20, C203OHC183DC, C201OHC181DC, C20OHC18DC, C223, C221 | 2.5 |
| **10** | Medium and long chain carnitines | C102, C10, C12, C121OH, C143, C14, C143OHC123DC, C163, C16, C182, C18, C204, C203, C201, C20, C221, C22 | 2.3 |

***Supplementary Table 2: Factors identified by sparse principal component analysis and the associated individual components, description and variance.***

Based on adjusted linear regression analyses, long chain acylcarnitines (Factor 4), short chain acyl-carnitines as well as di-carboxyl and hydroxylated acyl-carnitines (Factor 5 and 6) differentiated older adults without diabetes from older adults with diabetes. For long chain acylcarnitine subspecies, participants with diabetes had lower C18:2 (58.4 vs 67.4, p=0.020), C20:4 (4.2 vs 4.9, p=0.013), C20:3 (4.3 vs 5.3, p=0.002) and C20:2 (3.9 vs 4.4, p=0.037)], compared to participants without diabetes. In terms of short chain acyl-carnitines and di-carboxyl and hydroxylated acyl-carnitines, the participants with diabetes had higher C4-OH (25.1 vs 13.0, p<0.0001), C14-OH/C12-DC and C18-OHC/16-DC (6.2 vs 4.5, p=0.020)] compared to participants without diabetes.

| **Acyl-carnitines** | **Non-Diabetic (n=154)** | **Diabetic (n=53)** | **p-value** | **Adjusted Coef. (95% CI)*** | **Adjusted P-value*** |
|---|---|---|---|---|---|
| **PCA factors** | | | | | |
| X1 | 0.05 (2.7) | -0.1 (2.7) | 0.68 | - | - |
| X2 | 0.06 (2.0) | -0.2 (2.6) | 0.48 | - | - |
| X3 | -0.04 (2.2) | 0.1 (1.8) | 0.61 | - | - |
| X4 | -0.2 (2.1) | 0.7 (1.9) | **0.0080** | 1.0 (0.3, 1.7) | **0.008** |
| X5 | -0.4 (2.0) | 1.1 (2.6) | **<0.0001** | 1.3 (0.6, 2.0) | **<0.0001** |
| X6 | 0.2 (1.1) | -0.5 (2.2) | **0.004** | -0.6 (-1.1, -0.2) | **0.009** |
| X7 | -0.04 (1.7) | 0.1 (1.9) | 0.60 | - | - |
| X8 | -0.01 (1.2) | 0.04 (1.3) | 0.77 | - | - |
| X9 | 0.08 (1.4) | -0.2 (1.2) | 0.17 | - | - |
| X10 | 0.03 (1.4) | -0.1 (1.4) | 0.58 | - | - |
| | | | | | |
| **Short chain** | | | | | |
| C3 | 543 (180) | 553 (201) | 0.71 | - | - |
| C4 | 338 (144) | 345 (174) | 0.77 | - | - |
| C4-OH | 13.0 (8.0) | 25.1 (16.8) | **<0.0001** | 11.0 (7.5, 14.4) | **<0.0001** |
| C5 | 95.9 (36.0) | 96.5 (39.8) | 0.91 | - | - |
| C5:1 | 15.8 (5.4) | 16.5 (7.3) | 0.47 | - | - |
| | | | | | |
| **Medium chain** | | | | | |
| C10:1 | 85.3 (55.8) | 86.0 (79.4) | 0.95 | - | - |
| C10:2 | 13.1 (9.4) | 15.1 (11.1) | 0.24 | - | - |
| C12-OH/C10-DC | 2.1 (1.1) | 2.5 (1.2) | **0.012** | 0.3 (-0.06, 0.7) | 0.10 |
| C8:1-OH/C6:1-DC | 27.8 (14.1) | 28.4 (16.2) | 0.81 | - | - |
| C8-DC | 23.3 (13.4) | 26.6 (13.2) | 0.12 | - | - |
| | | | | - | - |
| **Long chain** | | | | | |
| C14:1-OH | 10.6 (6.0) | 11.7 (4.6) | 0.22 | - | - |
| C14:3 | 4.4 (2.6) | 4.2 (2.5) | 0.61 | - | - |
| C14-OH/C12-DC | 5.9 (3.0) | 8.5 (4.6) | **<0.0001** | 2.1 (1.0, 3.2) | **<0.0001** |
| C16 | 106 (26.0) | 102 (29.3) | 0.31 | - | - |

| | | | | | |
|---|---|---|---|---|---|
| C16:1-OH/C14:1-DC | 4.8 (1.9) | 5.4 (2.4) | 0.055 | - | - |
| C16:2-OH | 4.3 (1.8) | 4.9 (2.0) | **0.040** | 0.4 (-0.2, 1.0) | 0.17 |
| C16:3-OH/C14:3-DC | 1.3 (0.9) | 1.4 (0.9) | 0.36 | - | - |
| C16-OH | 5.5 (2.7) | 7.3 (3.4) | **0.0001** | 1.6 (0.6, 2.5) | **0.001** |
| C18 | 38.7 (9.8) | 37.3 (8.5) | 0.37 | - | - |
| C18:1 | 116 (33.6) | 109 (28.0) | 0.15 | - | - |
| C18:1-OH/C16:1-DC | 3.8 (1.9) | 4.9 (2.9) | **0.0007** | 0.9 (0.1, 1.6) | **0.019** |
| C18:2 | 67.4 (20.3) | 58.4 (16.2) | **0.004** | -7.7 (-14.2, -1.2) | **0.020** |
| C18:3 | 5.2 (2.1) | 4.6 (2.4) | 0.13 | - | - |
| C18-OH/C16-DC | 4.5 (3.2) | 6.2 (3.1) | **0.012** | 1.3 (0.2, 2.3) | **0.020** |
| C20 | 5.3 (1.8) | 5.0 (1.3) | 0.36 | - | - |
| C20:1 | 6.9 (2.5) | 6.7 (2.2) | 0.49 | - | - |
| C20:1-OH/C18:1-DC | 7.2 (4.1) | 8.0 (3.5) | 0.23 | - | - |
| C20:2 | 4.4 (1.5) | 3.9 (1.2) | **0.033** | -0.5 (-1.0, -0.03) | **0.037** |
| C20:2-OH/C18:2-DC | 2.2 (1.3) | 2.0 (1.0) | 0.37 | - | - |
| C20:3 | 5.3 (2.4) | 4.3 (1.7) | **0.005** | -1.2 (-1.9, -0.5) | **0.002** |
| C20:3-OH/C18:3-DC | 1.2 (0.7) | 1.3 (0.8) | 0.20 | - | - |
| C20:4 | 4.9 (2.0) | 4.2 (1.7) | **0.035** | -0.8 (-1.5, -0.2) | **0.013** |
| C22:1 | 3.1 (2.0) | 2.8 (1.6) | 0.54 | - | - |
| C22:2 | 0.8 (1.2) | 0.7 (0.4) | 0.45 | - | - |
| C22:4 | 1.1 (0.7) | 1.1 (0.6) | 0.80 | - | - |
| C22:5 | 1.8 (0.8) | 1.6 (0.9) | 0.16 | - | - |

***Table 3: Acyl-carnitine Factors and significant components: Comparisons between older adults without diabetes vs older adults with diabetes.*** *Adjusted for female, BMI, CV rf>2

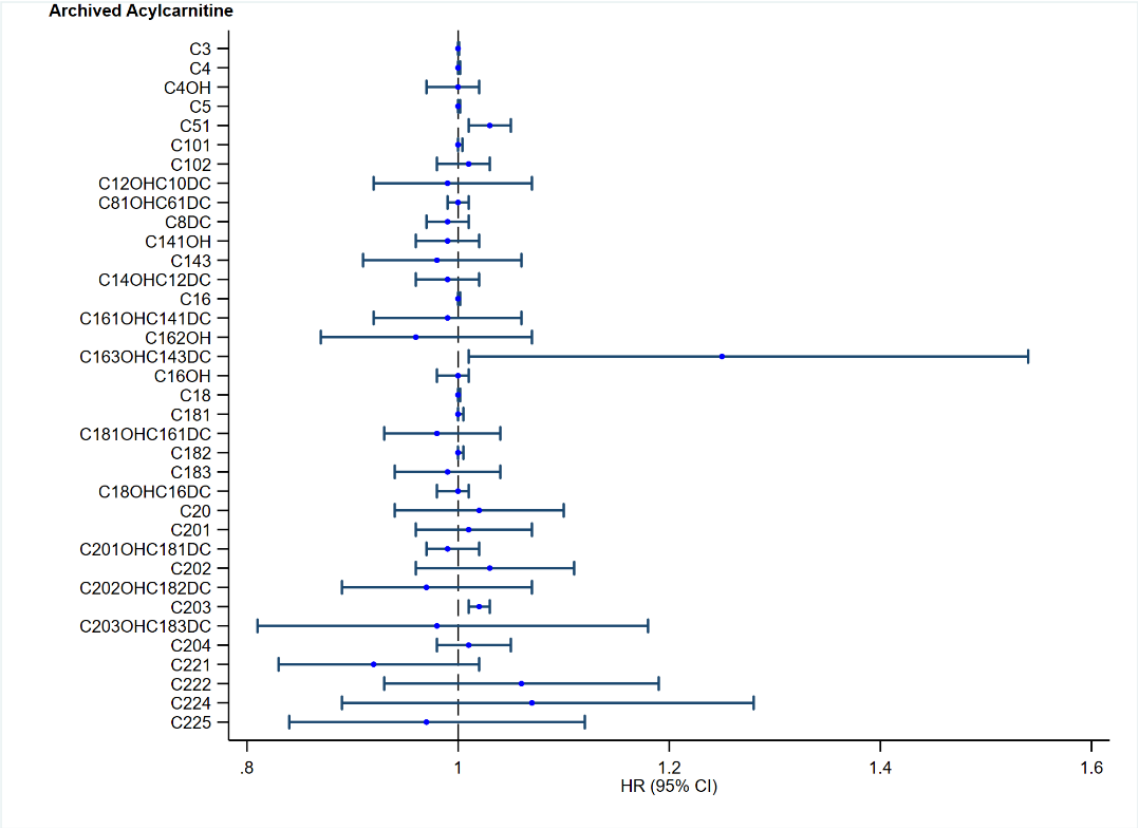
We next examined the relationship between current serum acyl-carnitine profiles and CV structure and function in older study subjects. Higher di-carboxyl acyl-carnitines were associated with higher risks of impairments in E/A ratio (C12-OH/C10-DC, p=0.018); C18-OH/C16-DC, p=0.038). Similarly, higher di-carboxyl acyl-carnitines were also associated with worse LA conduit strain function (C12-OH/C10-DC, p=0.008); C14-OH/C12-DC, p=0.025); C16:3-OH/C14:3-DC, p=0.018). The short-chain acyl-carnitines and hydroxylated acyl-carnitines were associated with worse LA conduit strain function (C4-OH, p=0.0024); C5, p=0.024). Higher long chain acyl-carnitines were associated with higher risks of impairments in E/A ratio (C16, p=0.002); C18:1, p=0.046). (Table 4).

Longitudinal associations between baseline acylcarnitines, delta change in acylcarnitine levels, and cardiac function were further analysed. Higher levels of baseline long chain acylcarnitines were associated with impairments in left ventricular relaxation (C20:3, p=0.014) (Figure 1A). Delta increases in long chain

acylcarnitine were also associated with worse left atrial conduit strain function (C14:3, p=0.033); C18:1, p=0.018); C18:2, p=0.028); C18:3, p=0.019); C20:4, p=0.038); C22:5, p=0.043) (Figure 1D). Higher levels of baseline short chain acylcarnitine were associated with larger hazards impairments in left ventricular relaxation (C5:1, p=0.011) as well as with worse left atrial conduit strain function (C5:1, p=0.037) (Figure 1A). Higher levels of di-carboxylated acyl-carnitines were associated with worse LA conduit strain function (C16:3-OH/C14:3-DC, p=0.019) (Figure 1C). Increases in hydroxylated acyl-carnitines were also associated with worse LA conduit strain function (C4-OH, p=0.017); C16:2-OH, p=0.037) (Figure 1D).

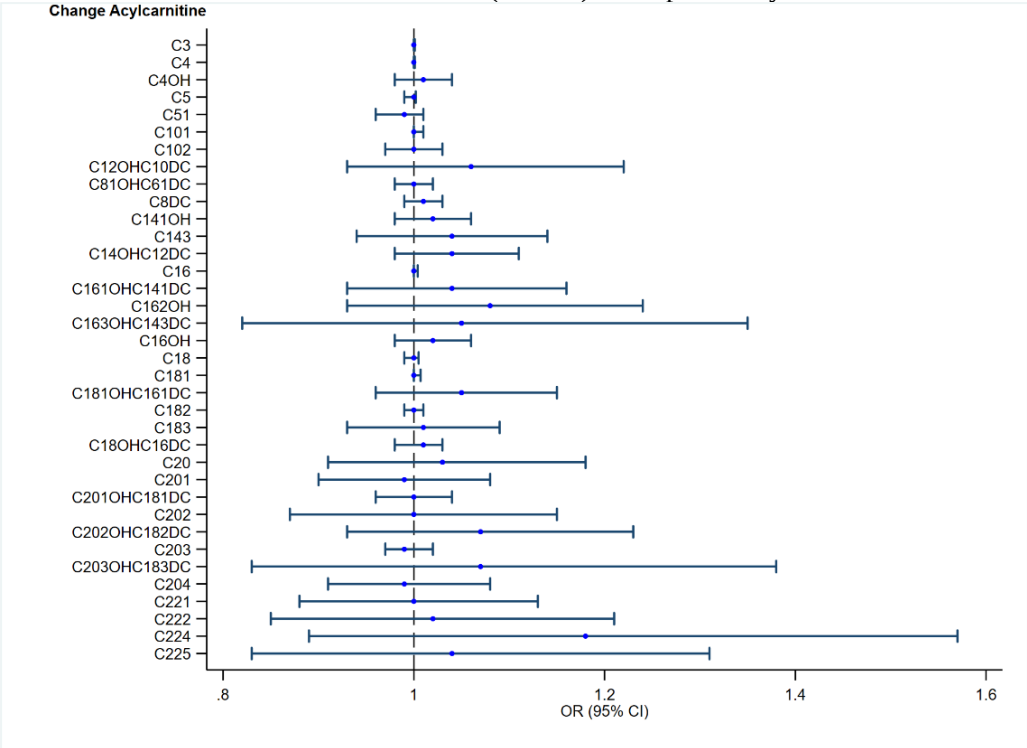
**Figure 1: Acyl-carnitines and cardiovascular function**

**1a) Archived Acyl-carnitine and impaired myocardial relaxation**
Blue circles and lines represent unadjusted hazard ratios (HR) for one-unit increase in archived acyl-carnitine and its 95% confidence interval (95%CI) on impaired myocardial relaxation.



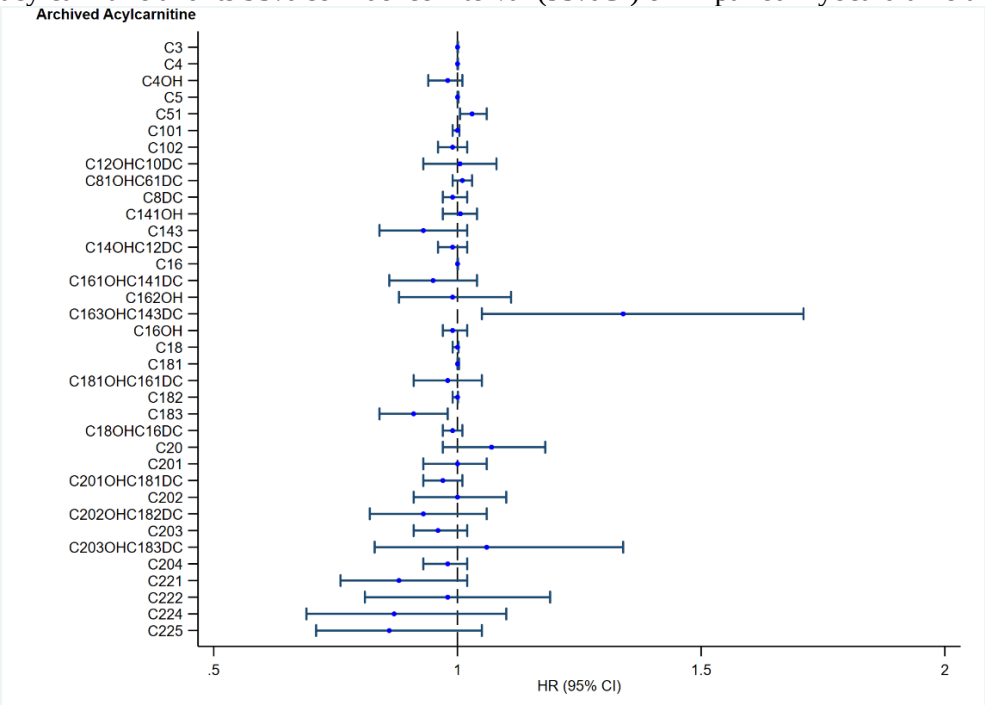
**1b) Change in Acyl-carnitine and impaired myocardial relaxation**

Blue circles and lines represent unadjusted odds ratios (OR) for one-unit increase in archived acyl-carnitine and its 95% confidence interval (95%CI) on impaired myocardial relaxation.



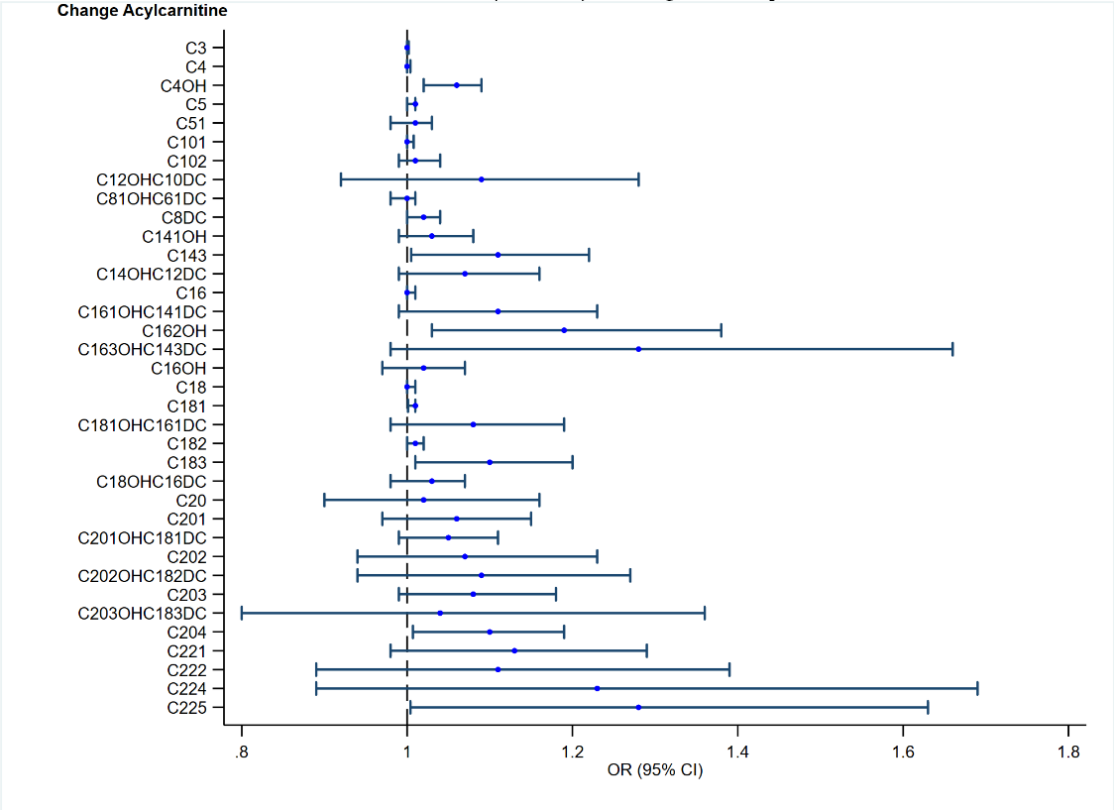

**1c) Archived Acyl-carnitine and impaired left atrial conduit strain**

Blue circles and lines represent unadjusted hazard ratios (HR) for one-unit increase in archived acylcarnitine and its 95% confidence interval (95%CI) on impaired myocardial relaxation.



**1d) Change in Acyl-carnitine and impaired left atrial conduit strain**

Blue circles and lines represent unadjusted odds ratios (OR) for one-unit increase in archived acyl-carnitine and its 95% confidence interval (95%CI) on impaired myocardial relaxation.




***Amino acids***

Biologically, the heart is a muscular organ with unique amino acid requirements such that interactions between pathways such as branched chain amino acids, one-carbon and nitrogen disposal pathways may be relevant to both ageing- and diabetes mellitus- related cardiovascular dysfunction. We hypothesised that amino acid profiles would further unravel metabolic signatures of ageing-related cardiovascular dysfunction.

Thawed serum samples (50 µl) were spiked with 10 µl deuterium-labelled amino acid mixture and diluted with 400 µl methanol. After centrifugation of the mixture at 17,000*g* for 5 mins at 4 ◦C, the supernatant fraction was collected (10 µl) for amino acid analysis. A pooled quality control (QC) sample was prepared by mixing equal amounts (10 µl) of each extracted serum sample. Extraction and measurement of amino acid panels (quantified in units of µM) were performed. The methanol extracts were derivatised with 3 M

Hydrochloric acid in butanol (Sigma Aldrich, USA) for amino acid analysis and diluted in water for analysis in LC-MS. For amino acid analysis, a C18 column (Phenomenex, 100 × 2.1 mm, 1.6 µm, Luna® Omega) on an Agilent 1290 Infinity LC system (AgilentTechnologies, CA, USA) coupled with quadrupole-ion trap mass spectrometer (QTRAP 5500, AB Sciex, DC, USA) were used. Mobile phase A (Water) and Mobile phase B (Acetonitrile) both containing 0.1% Formic acid were used for chromatography separation. The LC run was performed at a flow rate of 0.4 mL/min with initial gradient of 2% B for 0.8 min, then increased to 15% B in 0.1 min, 20% B in 5.7 min, 50% B in 0.5 min, 70% B in 0.5 min, followed by re-equilibration of the column to the initial run condition (2% B) for 0.9 min. All compounds were ionised in positive mode using electrospray ionization. The chromatograms were integrated using MultiQuant™ 3.0.3 software (AB Sciex, DC, USA).

Based on adjusted linear regression, alanine, arginine, citrulline, glutamate and proline differentiated older participants without diabetes. In the presence of ageing, alanine, glutamate, proline were lower while arginine and citrulline were higher, compared to older adults with diabetes (Table 2).

| **Amino acids (µM)** | **Non-DM (n=154)** | **DM (n=53)** | **p-value** | **Adjusted Coef. (95%CI)*** | **Adjusted P value*** |
|---|---|---|---|---|---|
| Alanine | 448 (111) | 562 (145) | **<0.0001** | 107 (66.6, 148) | **<0.0001** |
| Arginine | 117 (29.6) | 107 (27.3) | **0.034** | -10.0 (-19.7, -0.3) | **0.043** |
| Aspartate | 21.3 (6.1) | 21.8 (6.3) | 0.57 | - | - |
| Citrulline | 38.2 (12.5) | 30.3 (21.3) | **0.001** | -7.2 (-12.3, -2.0) | **0.006** |
| Glutamate | 95.1 (22.7) | 107 (27.3) | **0.002** | 9.4 (1.8-17.0) | **0.016** |
| Glycine | 227 (55.4) | 235 (49.9) | 0.36 | - | - |
| Histidine | 79.7 (22.1) | 78.8 (15.5) | 0.79 | - | - |
| Leucine | 120 (36.7) | 135 (43.8) | 0.14 | - | - |
| Ileleucine | 121 (55.9) | 120 (49.6) | 0.93 | - | - |
| Methionine | 26.0 (9.2) | 26.0 (10.4) | 0.98 | - | - |
| Ornithine | 93.2 (39.4) | 80.1 (20.8) | **0.022** | -10.2 (-22.2, 1.7) | 0.093 |
| Phenylalanine | 77.9 (17.2) | 74.5 (12.9) | 0.19 | - | - |
| Proline | 231 (56.9) | 264 (97.3) | **0.003** | 31.6 (8.2, 55.0) | **0.008** |
| Serine | 119 (25.3) | 119 (25.5) | 0.93 | - | - |
| Tryptophan | 54.8 (14.2) | 50.2 (14.0) | **0.039** | -4.0 (-8.7, 0.8) | 0.10 |
| Tyrosine | 72.5 (21.2) | 67.4 (20.7) | 0.14 | - | - |
| Valine | 247 (61.8) | 248 (61.2) | 0.97 | - | - |

***Table 2: Amino acids: Comparisons between Older adults without diabetes vs Older adults with diabetes (Current sample).*** *Adjusted for female, BMI, CV rf>2

Computing the metabolites' longitudinal change over 15-year period (Fig. 1) further differentiated older adults without diabetes with older adults with diabetes. Over time, there was lower accumulation of alanine (OR 1.004, 95%CI 1.002–1.007, $p$ = 0.002), proline (OR 1.01, 95%CI 1.002–1.01, $p$ = 0.008) and (non-significant) trend towards lower accumulation of glycine (OR 1.0, 95%CI 0.9998–1.01, $p$ = 0.057) among the older adults without diabetes compared to the older adults with diabetes. Increases in citrulline levels were more pronounced among the older adults without diabetes compared to the older adults with diabetes (OR 0.96, 95%CI 0.9–0.99, $p$ = 0.005). Future interaction analyses that study changes in metabolites with time between the groups would strengthen these observations.

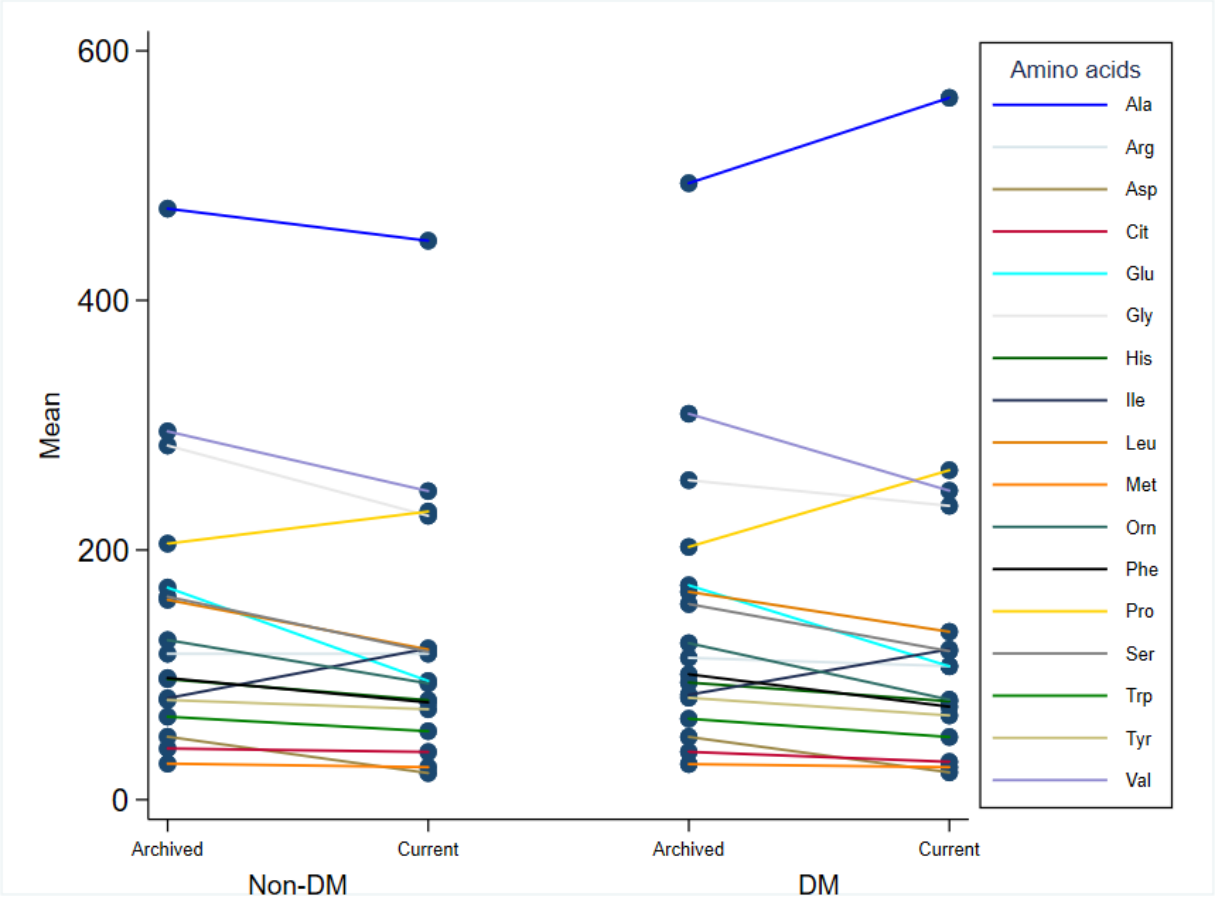



***Figure 1: Longitudinal trends in amino acid profiles differentiated diabetic older adults with or without diabetes.***

We next examined if these amino acid differences between older diabetics and older non-diabetics were associated with their CV differences.

Lower current levels of arginine were associated with impairments in E/A ratio (HR 0.98, 95%CI 0.97–0.99, $p = 0.002$) (Table 3). Higher levels of alanine (HR 1.003, 95%CI 1.001–1.004, $p = 0.001$) and higher levels of glycine (HR 1.004, 95%CI 1.001–1.006, $p = 0.006$) were associated with higher risks of impairments in E/A ratio (Table 4).

Importantly, we found a range of amino acids that were found to be associated with CV functions present in older adults, independent of diabetes status. Longitudinal increases in valine (OR 1.0, 95%CI 0.99–1.0, $p = 0.021$) over time (Table 3) and higher levels of valine (HR 1.004, 95%CI 1.001–1.007, $p = 0.011$) (Table 4) were associated with impairments in E/A ratio. We observed similar associations in other members of the branched chain amino acids, such as leucine and isoleucine. Higher levels of leucine (HR 1.01, 95%CI 1.002–1.02, p = 0.011) and isoleucine (HR 1.01, 95%CI 1.003–1.02, $p = 0.0006$) were associated with impairments in E/A ratio (Table 4). Higher levels of serine (HR 1.008, 95%CI 1.002–1.01, $p = 0.008$) and glycine (HR 1.004, 95%CI 1.001–1.006, $p = 0.006$) were associated with impairments in E/A ratio (Table 4). For methionine, lower levels of methionine (OR 0.97, 95%CI 0.94–1.0, $p = 0.039$) (Table 3) were associated with impairments with E/A ratio. Higher levels of phenylalanine (HR 1.01, 95%CI 1.002–1.02, $p = 0.015$), tryptophan (HR 1.02, 95%CI 1.003–1.03, $p = 0.016$), and tyrosine (HR 1.02, 95%CI 1.01–1.03, $p < 0.0001$) were associated with higher risks of impairments in E/A ratio in old age (Table 4).

**Table 3: Association between current metabolites and CV function**

**i) Outcome: E/A<=0.9**

| Current metabolites | Events/total | OR (95% CI) | p-value | Adjusted OR (95%)* | Adjusted p-value* |
|---|---|---|---|---|---|
| Alanine | 142/207 | 1.002 (0.999, 1.004) | 0.17 | - | - |
| Arginine | 142/207 | 0.98 (0.97, 0.99) | **0.001** | 0.98 (0.97, 0.99) | **0.002** |
| Aspartate | 142/207 | 0.95 (0.90, 0.99) | **0.024** | 0.94 (0.90, 0.99) | **0.020** |
| Citrulline | 142/207 | 0.98 (0.97, 1.003) | 0.10 | - | - |
| Glutamate | 142/207 | 1.005 (0.99, 1.02) | 0.39 | - | - |
| Glycine | 142/207 | 0.995 (0.99, 1.00003) | 0.051 | - | - |
| Histidine | 142/207 | 0.996 (0.98, 1.01) | 0.56 | - | - |
| Leucine | 53/82 | 0.99 (0.98, 1.002) | 0.11 | - | - |
| Ileleucine | 142/207 | 0.998 (0.99, 1.004) | 0.55 | - | - |
| Methionine | 142/207 | 0.97 (0.94, 0.997) | **0.034** | 0.97 (0.94, 0.998) | **0.039** |
| Ornithine | 142/207 | 0.999 (0.99, 1.01) | 0.90 | - | - |
| Phenylalanine | 142/207 | 0.99 (0.97, 1.01) | 0.37 | - | - |
| Proline | 142/207 | 1.001 (0.997, 1.01) | 0.50 | - | - |
| Serine | 142/207 | 0.998 (0.99, 1.01) | 0.80 | - | - |
| Tryptophan | 142/207 | 0.98 (0.96, 1.001) | 0.059 | - | - |
| Tyrosine | 142/207 | 0.99 (0.98, 1.01) | 0.46 | - | - |
| Valine | 142/207 | 0.99 (0.99, 0.9998) | **0.043** | 0.99 (0.99, 0.9995) | **0.032** |

*Adjusted for diabetes mellitus, female, BMI, CV rf>2

**Table 4: Association between archived metabolites and CV function**

**i) Outcome: E/A<=0.9**

| Archived metabolites | Events/total | HR (95% CI) | p-value | Adjusted HR (95%)* | Adjusted p-value* |
|---|---|---|---|---|---|
| Alanine | 124/180 | 1.002 (1.001, 1.003) | **0.003** | 1.003 (1.001, 1.004) | **0.001** |
| Arginine | 94/140 | 1.0001 (0.99, 1.01) | 0.98 | - | - |
| Aspartate | 124/180 | 1.03 (1.02, 1.05) | **<0.0001** | 1.04 (1.02, 1.05) | **<0.0001** |
| Citrulline | 124/180 | 0.998 (0.98, 1.01) | 0.77 | - | - |
| Glutamate | 124/180 | 1.004 (0.9995, 1.01) | 0.078 | - | - |
| Glycine | 124/180 | 1.003 (1.0001, 1.01) | **0.041** | 1.004 (1.001, 1.01) | **0.006** |
| Histidine | 124/180 | 1.002 (0.99, 1.01) | 0.79 | - | - |
| Leucine | 40/61 | 1.01 (1.002, 1.02) | **0.010** | 1.01 (1.002, 1.02) | **0.011** |
| Ileleucine | 124/180 | 1.01 (1.004, 1.02) | **0.003** | 1.01 (1.003, 1.02) | **0.006** |
| Methionine | 124/180 | 1.04 (1.01, 1.06) | **0.004** | 1.04 (1.01, 1.07) | **0.003** |
| Ornithine | 124/180 | 1.001 (0.997, 1.01) | 0.58 | - | - |

| | | | | | |
|---|---|---|---|---|---|
| Phenylalanine | 124/180 | 1.01 (1.002, 1.02) | **0.013** | 1.01 (1.002, 1.02) | **0.015** |
| Proline | 124/180 | 1.002 (0.999, 1.01) | 0.15 | - | **-** |
| Serine | 124/180 | 1.006 (1.001, 1.01) | **0.024** | 1.008 (1.002, 1.01) | **0.008** |
| Tryptophan | 124/180 | 1.01 (1.001, 1.03) | **0.028** | 1.02 (1.003, 1.03) | **0.016** |
| Tyrosine | 124/180 | 1.01 (1.005, 1.02) | **0.002** | 1.02 (1.01, 1.03) | **<0.0001** |
| Valine | 124/180 | 1.004 (1.001, 1.01) | **0.005** | 1.004 (1.001, 1.01) | **0.011** |

*Adjusted for diabetes mellitus, female, BMI, CV rf>2

**b) Specific metabolic pathway of relevance to cardiovascular ageing**

*Distinct alterations in fuel oxidation pathways in short chain and long chain acyl-carnitines, di-carboxyl and hydroxylated acyl-carnitines*

For the first time, we report links between fuel oxidation pathways in older adults to changes in their cardiovascular function with ageing. Higher levels of long chain acylcarnitines were associated with impairments in myocardial relaxation and worse left atrial function, likely reflecting early disturbances in diastolic function.

For years, long chain acylcarnitines have been linked across the clinical spectrum of heart failure, from heart failure with reduced ejection fraction (HFrEF), to heart failure with preserved ejection fraction (HFpEF), to non-heart failure (HF) controls: long chain acyl-carnitine levels have been observed to be greater in HFrEF than HFpEF, both of which were greater than non-HF controls[74].

Our observations now directly link long chain acyl-carnitines to imaging markers of diastolic function, a pathophysiological disturbance that predominates across the clinical heart failure spectrum. In addition, levels of long chain acyl-carnitines obtained at baseline and also longitudinally, were associated with these cardiovascular functions. We further observed that interval increase in long chain acyl-carnitines predicted abnormalities in myocardial relaxation and left atrial conduit strain.

The short chain, hydroxylated- and dicarboxyl- acyl-carnitines are fuel intermediates which are generated by the process of alpha- and omega oxidation[75, 76]. Short chain, hydroxylated- and dicarboxyl- acyl-carnitines were specifically higher among older adults with diabetes, highlighting the importance of fuel oxidation pathways in the pathogenesis of diabetes, a connection which has been well described[77]. These

pathways may also represent important treatment targets to ameliorate impact of diabetes on cardiovascular outcomes in older adults.

*Amino acids correlate to specific metabolic pathways: one-carbon pathways and nitrogen handling pathways*

Serine, glycine and methionine contribute towards many distinct pathways but may be mapped to one-carbon metabolism (Fig. 2). Serine is a non-essential amino acid that is produced by the serine biosynthesis pathway, from a branch of glycolysis that can be converted into glycine, providing carbon units for one-carbon metabolism. Recent observations have linked deficiencies in the one-carbon metabolic pathway to heart failure in both animal models and in human patients[78-80]. Our data reveals a correlation between these metabolites involved in one-carbon pathway and ageing heart functions among a sample of community-based participants. Our work concurs with another cohort of older women and men which had also observed long term changes in serine as an ageing-associated metabolite that is independent from chronological age[81].

In contrast, older adults with diabetes had increased levels of alanine, glutamine, proline with decreased levels of arginine and citrulline. These amino acids are involved in nitrogen handling pathways. These amino acids contribute to body nitrogen handling via inter-tissue nitrogen transfer (alanine) or urea cycle (arginine) (Fig. 2) and have been implicated in HFpEF [74, 82]. Other studies of heart failure have found associations with nitrogen pathways[83, 84]. Finally, a longitudinal study of normal ageing has shown arginine and ornithine declining in subjects as they age[74]. These findings point to a potential role for changes in nitrogen handling in the pathogenesis of heart failure in older subjects, among diabetics.

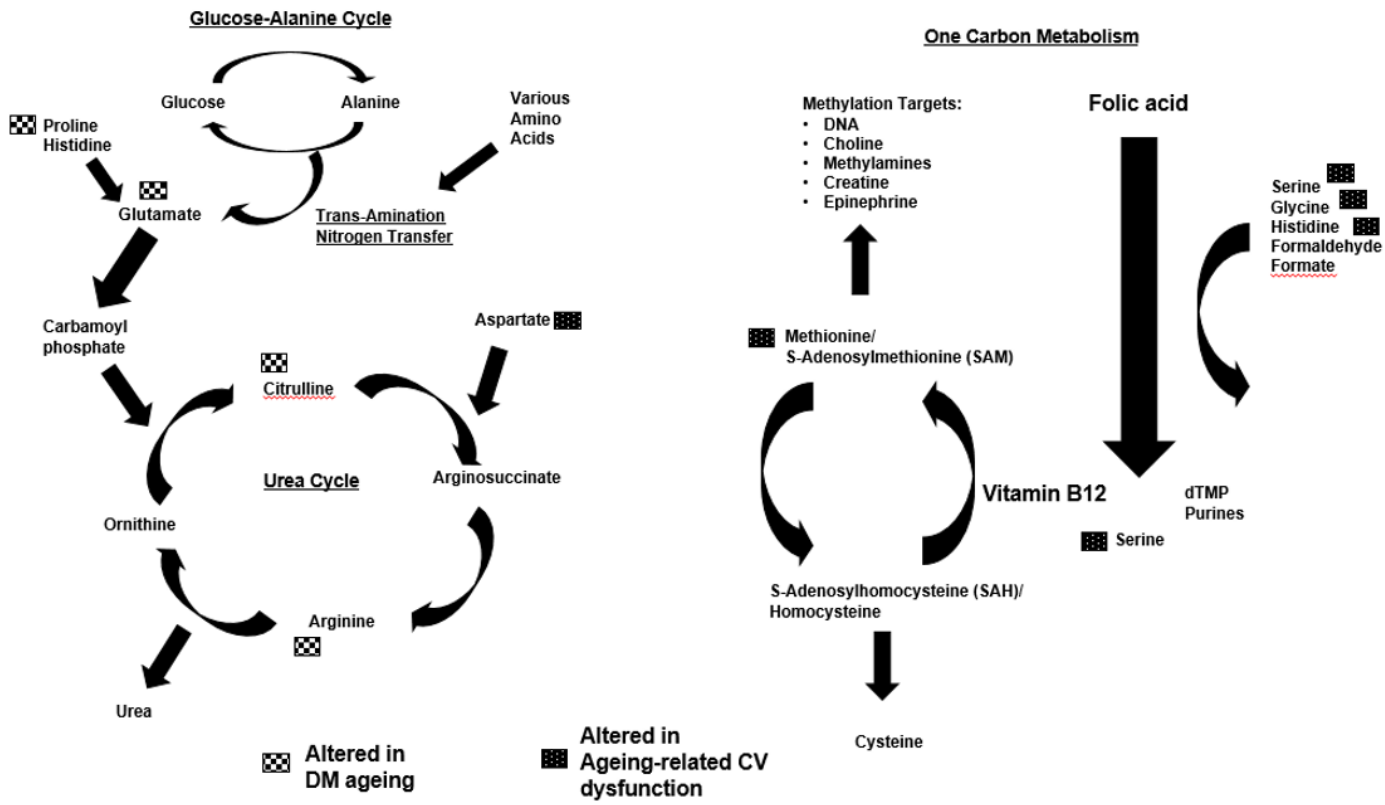



***Figure 2. Overview of nitrogen handling and one carbon metabolism pathways.*** *Nitrogen handling: Nitrogen is funnelled into glutamine via transamination reactions involving various amino acids, the glucose-alanine cycle and catabolism of amino acids such as proline and histidine. Glutamine and aspartate contribute nitrogen to the urea cycle. One carbon metabolism: Folic acid accepts one carbon groups from various sources. One carbon groups are transferred from folic acid to purine, serine and vitamin B12. Vitamin B12 transfers methyl groups to homocysteine to 're-charge' methionine. S-adenosylmethionine transfers methyl groups to various targets including DNA, choline the methylamines (i.e. betaine, sarcosine), creatine and epinephrine.*

Overall, we observe associations between the various amino acids levels with measurements of cardiovascular function associated with left ventricular relaxation. These left ventricle defects in ageing may represent upstream changes that precede clinical heart failure in older adults.

I am the principal investigator of the study. My contribution to publications #2 and #3 includes obtaining grant funding for this work, setting up the study protocol, recruitment of research participants, obtaining ethical approval, data analyses, and manuscript review.

**Research Question #2:**

**Would metabolomics biomarkers identified in (1) differentiate between physical activity levels, i.e., high versus low physical activity practices, among older adults with cardiovascular ageing?**

**PUBLICATION #4**

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**Metabolomic correlates of aerobic capacity among elderly adults. *Clin Cardiol*. 2018;41:1300-1307[85].**

*"Background: Aerobic capacity is a powerful predictor of cardiovascular disease and all-cause mortality, and it declines with advancing age. Hypothesis: Since physical activity alters body metabolism, metabolism markers will likely differ between subjects with high vs low aerobic capacities.*

*Methods: Community-based participants without physician-diagnosed heart disease, stroke or cancer underwent same-day multimodal assessment of cardiovascular function (by echocardiography and magnetic resonance feature tracking of left atrium) and aerobic capacity by peak oxygen uptake (VO2) metrics. Associations between VO2 and cardiovascular and metabolomics profiles were studied in adjusted models including standard covariates. Results: We studied 141 participants, of whom 82 (58.2%) had low VO2, while 59 (41.8%) had high VO2. Compared to participants with high VO2, participants with low VO2 had more adverse cardiovascular parameters, such as lower ratio of peak velocity flow in early diastole to peak velocity flow in late diastole by atrial contraction of >0.8 (76% vs 35%, adjusted odd ratio [OR] = 4.1, 95% confidence interval [CI] [1.7-9.5], P = 0.001) and lower left atrial conduit strain (11.3±4.0 vs 15.6±6.1%, adjusted OR = 1.1, 95% CI [1.002-1.3], P = 0.045). High VO2 was associated with lower accumulation of wide-spectrum acyl-carnitines (OR = 0.6, 95% CI [0.4-0.9], P = 0.013), alanine (OR = 0.1, 95% CI [0.01-0.9], P = 0.044) and glutamine /glutamate (OR = 0.1, 95% CI [0.01-0.5], P = 0.007), compared to low VO2. Conclusion: Elderly adults with low VO2 have adverse cardiovascular and metabolic parameters compared to their counterparts with high VO2. Combined cardiac and metabolomics phenotyping may be a promising tool to provide insights into physiological states, useful for tracking future interventions related to physical activity among community cohorts."*

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WILEY CLINICAL CARDIOLOGY

CLINICAL INVESTIGATIONS

# Metabolomic correlates of aerobic capacity among elderly adults

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**Background:** Aerobic capacity is a powerful predictor of cardiovascular disease and all-cause mortality, and it declines with advancing age.
**Hypothesis:** Since physical activity alters body metabolism, metabolism markers will likely differ between subjects with high vs low aerobic capacities.
**Methods:** Community-based participants without physician-diagnosed heart disease, stroke or cancer underwent same-day multimodal assessment of cardiovascular function (by echocardiography and magnetic resonance feature tracking of left atrium) and aerobic capacity by peak oxygen uptake ($VO_2$) metrics. Associations between $VO_2$ and cardiovascular and metabolomics profiles were studied in adjusted models including standard covariates.
**Results:** We studied 141 participants, of whom 82 (58.2%) had low $VO_2$, while 59 (41.8%) had high $VO_2$. Compared to participants with high $VO_2$, participants with low $VO_2$ had more adverse cardiovascular parameters, such as lower ratio of peak velocity flow in early diastole to peak velocity flow in late diastole by atrial contraction of >0.8 (76% vs 35%, adjusted odd ratio [OR] = 4.1, 95% confidence interval [CI] [1.7-9.5], $P = 0.001$) and lower left atrial conduit strain (11.3 ± 4.0 vs 15.6 ± 6.1%, adjusted OR = 1.1, 95% CI [1.002-1.3], $P = 0.045$). High $VO_2$ was associated with lower accumulation of wide-spectrum acyl-carnitines (OR = 0.6, 95% CI [0.4-0.9], $P = 0.013$), alanine (OR = 0.1, 95% CI [0.01-0.9], $P = 0.044$) and glutamine /glutamate (OR = 0.1, 95% CI [0.01-0.5], $P = 0.007$), compared to low $VO_2$.
**Conclusion:** Elderly adults with low $VO_2$ have adverse cardiovascular and metabolic parameters compared to their counterparts with high $VO_2$. Combined cardiac and metabolomics phenotyping may be a promising tool to provide insights into physiological states, useful for tracking future interventions related to physical activity among community cohorts.

KEYWORDS
aerobic capacity, aging, cardiovascular, elderly, metabolomics

## 1 | INTRODUCTION

Low aerobic capacity is a strong predictor of cardiovascular disease (CVD) and all-cause mortality,[1,2] while increases in aerobic capacity are associated with increased survival.[2] Among aged populations, poor aerobic capacity indicates closer proximity to future declines in cardiovascular and other health indices.[3,4]

Physical activity is associated with many physiologic changes including increased aerobic capacity and alterations in fuel metabolism. Exercise-associated changes in fuel metabolism can be tracked using different methodologies, including metabolomics. Metabolomics has also been used to study biochemical changes that occur in different disease states, particularly insulin resistance, type 2 diabetes and CVD.[5-8] However, there have been fewer studies that examine biochemical changes prior to the development of overt disease.[9] While significant achievements have been made in

metabolomics research, particularly in providing molecular insights into CVD pathogenesis,[8,10,11] there is still little data on metabolomics research into physiological states prior to disease development.

We were interested in understanding the relationship between peak oxygen uptake ($VO_2$) and markers of cardiometabolic disease in otherwise healthy elderly subjects. We surmised that levels of endogenous metabolites as well as measures of cardiovascular function may change according to $VO_2$ in otherwise healthy older adults.

This study was designed to characterize the metabolic profile of community elderly adults with high $VO_2$ in relation to their cardiovascular profiles obtained by detailed cardiovascular assessment. These results may help advance mechanistic understanding of how $VO_2$ drives future CVD and mortality risks, providing translatable knowledge for future therapeutics and/or preventative treatments.

## 2 | METHODS

The subjects were recruited from the cardiac aging study (CAS),[12] a prospective study initiated in 2014 that examines characteristics and determinants of cardiovascular function in elderly adults.

The study sample consisted of men and women who participated in the baseline CAS 2014 examination who had no self-reported history of physician-diagnosed CVD (such as coronary heart disease and stroke) or cancer[13]. Written informed consent was obtained from participants upon enrolment. The SingHealth Centralized Institutional Review Board (2014/628/C) had approved the study protocol. All methods were performed in accordance with the relevant guidelines and regulations.

All participants were examined and interviewed on one study visit by trained study coordinators. Participants completed a standardized questionnaire that included medical history and coronary risk factors. Hypertension was defined by current use of antihypertensive drugs or physician-diagnosed hypertension. Diabetes mellitus was defined by current use of antidiabetic agents or physician-diagnosed diabetes mellitus. Dyslipidaemia was defined by current use of lipid-lowering agents or physician-diagnosed dyslipidaemia. Smoking history was defined as ever smokers (former or current smoking) or never smokers. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Sinus rhythm status was ascertained by resting electrocardiogram. Clinical data were obtained on the same day as assessment of echocardiography, cardiac magnetic resonance (CMR) imaging, and serum collection.

### 2.1 | Assessment of peak oxygen uptake ($VO_2$)

We used a validated nonexercise prediction model comprising physical activity questionnaire to estimate peak oxygen uptake, $VO_2$ milliliter/kg/minute (ml/kg/min).[14,15] This simple physical activity questionnaire consisted of age, gender, height, weight, estimated maximum heart rate, frequency of exercise, length of time for each workout, intensity of each workout, waistline diameter, and resting heart rate. The calculator is available online (https://www.worldfitnesslevel.org).

### 2.2 | Transthoracic echocardiography imaging

Echocardiography was performed using ALOKA α10 (Hitachi Medical, Wallington, CT, USA) with a 3.5-MHz probe. In each subject, standard echocardiography, which included 2-D, M-mode, pulse Doppler, and tissue Doppler imaging, was performed in the standard parasternal and apical (apical 4-chamber, apical 2-chamber, and apical long) views, and three cardiac cycles were recorded. The left ventricular ejection fraction, left atrial (LA) volume, and LA volume index were measured. The trans-mitral flow E and A wave with the sample volume position at the tip of the mitral valve leaflets from the apical 4-chamber view were recorded by Doppler echocardiography. Pulsed wave tissue Doppler imaging was performed with the sample volume at the septal and lateral annulus from the apical 4-chamber view. The frame rate was between 80 and 100 frames per second. The tissue velocity patterns were recorded and expressed as E′ and A′. All measurements were measured by the same operator (ie, J.I.W.), and the measurements were averaged over three cardiac cycles and adjusted by the interbeat interval.

### 2.3 | CMR imaging

Cine CMR scans were performed using balanced fast field echo sequence (BFFE). All subjects were imaged on a 3 T magnetic resonance imaging system (Ingenia, Philips Healthcare, Amsterdam, Netherlands) with a dStream Torso coil (a maximal number of channels 32). BFFE end-expiratory breath hold cine images were acquired in multiplanar long-axis views (2-, 3-, and 4-chamber views). Typical parameters were as follows: Repetition time/ echo time 3/1 ms; flip angle, 45°; in-plane spatial resolution, 1.0 mm × 1.0 mm to 1.5 mm × 1.5 mm; slice thickness, 8 mm; pixel bandwidth, 1797 Hz; field of view, 300 mm; frame rate, 30 or 40 per cardiac cycle. We developed an in-house semiautomatic algorithm to track the distance (L) between the left atrioventricular junction and a user-defined point at the mid posterior LA wall on standard CMR 2- and 4-chamber views.[16,17] Both 2- and 4-chamber views were used to generate the average strain and strain rate results. Longitudinal strain ($\varepsilon$) at any time point ($t$) in the cardiac cycle from end diastole (time 0) was calculated as: $\varepsilon(t) = (L(t) - L_0)/L_0$. LA reservoir strain ($\varepsilon_s$), conduit strain ($\varepsilon_e$), and booster strain ($\varepsilon_a$) were calculated at t equals left ventricular end systole, diastasis, and pre-LA systole, respectively, and their corresponding peak strain rates (SRs) derived (Supporting Information Figure S1). Strain and strain rate (SR) parameters from both 2- and 4-chamber views were averaged to obtain mean results for analysis. Using data from 20 randomly selected subjects, intra- and interobserver comparability was assessed. Two independent observers analyzed all cases in the evaluation of interobserver variability (Shuang Leng and Xiaodan Zhao), while intraobserver variability was assessed from a repeated analysis by the first observer (Shuang Leng) after 7 days (Supporting Information Table S1a). This technique has been validated against volumetric measurements and the strain results obtained from commercial software. Details about the technique can be found in Supporting Information Table S1b and Figure S2.

### 2.4 | Metabolomics profiling

Antecubital venous blood samples (20-30 mL) were taken from consenting participants in the morning; fasting was not required before blood collection. After collection, the blood samples were immediately placed on ice for transportation and were processed within 6 hours to obtain serum samples, which were subsequently stored at −80°C.

Serum metabolomic profiling analysis was performed in the Duke-NUS Metabolomics Facility. Thawed serum samples (100 μL) were spiked with 20-μL deuterium-labeled amino acid/acyl-carnitine mixture and diluted with 800-μL methanol. After centrifugation of the mixture at 17000*g* for 5 minutes at 20°C, the supernatant fraction was collected and divided into two parts: one (100 μL) for acyl-carnitine analysis and one (10 μL) for amino acid analysis. A pooled quality control sample was prepared by mixing equal amounts (10 μL) of each extracted serum sample. Extraction and measurement of acyl-carnitine and amino acid panels were performed as previously described.[18] Free and total L-carnitine analysis was carried out as previously described[19] with modifications. 10 μL of 250-μM $d_3$-L-carnitine was added to 50 μL of plasma. 20 μL of the mixture was removed for protein precipitation and further dilution using an acidified methanol-water mixture (0.6% HCl in 80% MeOH). Free carnitine was estimated from the supernatant by analyzing on an Agilent 6430 Triple Quadrupole liquid chromatography mass spectrometry and an Agilent XDB-C8 column (100 × 4.6 mm; particle size 1.8 μm) (Agilent Technologies, Santa Clara, California) kept at 30°C. Chromatography was performed by injecting 1 μL of the supernatant and eluting by mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The flow rate was at 0.4 mL/min at 95% A from 0 to 2 minutes, increased to 0.8 mL/min at 10% A from 2 to 2.5 minutes, held until 2.8 minutes, changed to 95% A from 2.8 to 3 minutes, and the flow rate was reduced to 0.4 mL/min at 95% A at 3.6 minutes, and kept until 5.0 minutes. Data acquisition and analysis were performed on an Agilent MassHunter Workstation B.06.00 Software (Santa Clara, CA, USA). For total carnitine analysis, 10 μL of 1-M KOH was added to the remaining $d_3$-L-carnitine and plasma mixture. Hydrolysis was performed by incubating the mixture at 65°C for 15 minutes, followed by neutralizing with 12-μL 1-M HCl. 20 μL of the supernatant was used for protein precipitation and dilution with acidified methanol-water mixture (0.6% HCl in 80% MeOH), followed by analysis with mass spectrometry as described above.

## 3 | STATISTICAL METHODOLOGY

We first examined bivariable association of subject clinical characteristics, physical activity, cardiac function, and LA function with high $VO_2$. High $VO_2$ was defined as a $VO_2$ > 37 (ml/kg/min) for men or $VO_2$ > 29 (ml/kg/min) for women[14] as mean $VO_2$ was 37 (ml/kg/min) for men and 29 (ml/kg/min) for women in our cohort.

**TABLE 1** Baseline clinical characteristics and physical activity data

| | $VO_2$ low (n = 82) | $VO_2$ high (n = 59) | Total (n = 141) | *P* value |
|---|---|---|---|---|
| Age (year) | 73.8 (3.6) | 66.1 (15.7) | 70.6 (11.2) | <0.0001 |
| Female | 36 (43.9%) | 23 (39.0%) | 59 (41.8%) | 0.56 |
| Ever smoker | 20 (24.4%) | 10 (17.0%) | 30 (21.3%) | 0.29 |
| Body mass index (kg/m$^2$) | 24.5 (3.0) | 22.1 (2.8) | 23.5 (3.1) | <0.0001 |
| Hypertension | 50 (61.0%) | 27 (45.8%) | 77 (54.6%) | 0.073 |
| Diabetes mellitus | 24 (29.3%) | 9 (15.3%) | 33 (23.4%) | 0.053 |
| Dyslipidemia | 46 (56.1%) | 25 (42.4%) | 71 (50.4%) | 0.11 |
| Heart rate (beats per minute) | 75.8 (13.2) | 70.3 (11.0) | 73.5 (12.5) | 0.010 |
| Central systolic blood pressure (mm Hg) | 141.6 (17.6) | 136.0 (17.9) | 139.3 (17.9) | 0.066 |
| Central diastolic blood pressure (mm Hg) | 76.7 (10.1) | 76.2 (11.7) | 76.5 (10.7) | 0.79 |
| Central mean arterial pressure (mm Hg) | 103.0 (12.1) | 100.4 (11.5) | 102.0 (11.9) | 0.2 |
| Central pulse pressure (mm Hg) | 64.9 (16.2) | 59.8 (18.5) | 62.8 (17.3) | 0.083 |
| Physical activity | | | | |
| Frequency | | | | 0.48 |
| Inactive | 15 (18.3%) | 9 (15.3%) | 24 (17.0%) | |
| Once a week | 1 (1.2%) | 3 (5.1%) | 4 (2.8%) | |
| 2 to 3 times a week | 10 (12.2%) | 5 (8.5%) | 15 (10.6%) | |
| Almost everyday | 56 (68.3%) | 42 (71.2%) | 98 (69.5%) | |
| Intensity | | | | 0.003 |
| Take it easy | 79 (96.3%) | 48 (81.4%) | 127 (90.1%) | |
| Heavy breath and sweat | 3 (3.7%) | 11 (18.6%) | 14 (9.9%) | |
| Duration | | | | 0.73 |
| <15 min | 20 (24.4%) | 14 (23.7%) | 34 (24.1%) | |
| 16 to <30 minutes | 24 (29.3%) | 14 (23.7%) | 38 (27.0%) | |
| 30 to 60 minutes | 17 (20.7%) | 11 (18.6%) | 28 (19.9%) | |
| >1 hour | 21 (25.6%) | 20 (33.9) | 41 (29.1) | |

Mean and SD were presented for continuous data and frequency and percentage for categorical data. We then used univariate logistic regression to assess the role of 83 metabolites including 65 acyl-carnitine metabolites, 16 amino acid metabolites, and 2 carnitine metabolites, in contributing to high $VO_2$. Metabolites with >25% of values below the lower limit of quantification were excluded from analysis (only C102 was excluded, hence a total of 83 metabolites were analyzed in the final sample). We normalized the distributions of all metabolites by logarithmic transformation.

We reduced the dimensionality of correlated metabolites (65 acyl-carnitine metabolites and 2 carnitine metabolites) using sparse principal component analysis (SPCA), which used a penalized matrix decomposition. Compared to regular principal component analysis, SPCA is capable of producing sparse loadings, which makes it more biologically interpretable. Specifically, we set the orthogonality constraint on each component and the number of components to be 10. We reported the description on each component and the proportion of variance accounted. The association of each 10 SPCA factors with high $VO_2$ instead of 65 acyl-carnitine metabolites and 2 carnitine metabolites were analyzed.

Furthermore, we used multivariable logistic regression to assess the role of amino acid metabolites and SPCA factors that show an association with $P < 0.05$ with high $VO_2$ in univariate analysis controlling for the significant clinical characteristics (age, body mass index [BMI], and diabetes). Heart rate was not included as it was already used to compute $VO_2$.

All statistical analyses were performed using STATA 13 (StataCorp, College Station, Texas), while the SPCA was performed by R. For all analysis, a two-tailed $P$ value of <0.05 was considered significant.

**TABLE 2** Cardiac functions by echocardiogram and LA function by CMR

| | $VO_2$ low (n = 82) | $VO_2$ high (n = 59) | Total (n = 141) | P value | Adjusted OR[a] | Adjusted P value |
|---|---|---|---|---|---|---|
| Interventricular septum thickness at end diastole (IVSd) (cm) | 0.86 (0.18) | 0.81 (0.13) | 0.84 (0.16) | 0.096 | | |
| Interventricular septum thickness at end systole (IVSs) (cm) | 1.30 (0.26) | 1.17 (0.24) | 1.24 (0.26) | 0.004 | 0.3 (0.1-1.8) | 0.2 |
| LVIDd (cm) | 4.50 (0.64) | 4.33 (0.74) | 4.42 (0.69) | 0.17 | | |
| Left ventricular internal diameter end systole (LVPWd) (cm) | 0.80 (0.14) | 0.74 (0.09) | 0.77 (0.12) | 0.0097 | 0.1 (0.003-5.2) | 0.28 |
| LVPWs (cm) | 1.45 (0.24) | 1.37 (0.28) | 1.41 (0.26) | 0.087 | | |
| LVOT (cm) | 2.07 (0.16) | 2.05 (0.17) | 2.06 (0.16) | 0.64 | | |
| Ao (cm) | 3.14 (0.46) | 3.04 (0.49) | 3.09 (0.47) | 0.23 | | |
| LA (cm) | 3.81 (0.52) | 3.50 (0.55) | 3.68 (0.56) | 0.0015 | 0.8 (0.3-2.1) | 0.71 |
| LVEF (%) | 74.44 (7.08) | 73.64 (7.63) | 74.10 (7.30) | 0.53 | | |
| LV FS (%) | 43.80 (6.32) | 42.97 (6.48) | 43.45 (6.38) | 0.47 | | |
| LVMI (grams/$m^2$) | 83.72 (27.27) | 75.20 (18.89) | 80.12 (24.38) | 0.051 | | |
| Ratio of *peak* velocity flow in early diastole E (MV E peak) (m/s) to *peak* velocity flow in late diastole by atrial contraction A (MV A peak) (m/s) (E/A ratio) > 0.8 | 28 (35.0%) | 45 (76.3%) | 73 (52.5%) | <0.001 | 4.1 (1.7–9.5) | 0.001 |
| DT (m/s) | 210.54 (38.69) | 205.25 (36.68) | 208.30 (37.81) | 0.42 | | |
| PASP (mm Hg) | 27.27 (6.47) | 27.06 (6.61) | 27.18 (6.50) | 0.86 | | |
| PV-S (cm/s) | 57.22 (11.50) | 58.48 (11.55) | 57.76 (11.49) | 0.54 | | |
| PV-D (cm/s) | 47.07 (15.45) | 49.02 (12.76) | 47.90 (14.34) | 0.45 | | |
| PV-Ar (ms) | 92.41 (16.63) | 92.42 (16.04) | 92.41 (16.32) | 1.00 | | |
| Mitral inflow duration at atrial contraciton (MV A duration (ms) | 118.45 (18.88) | 113.63 (16.75) | 116.40 (18.10) | 0.13 | | |
| Left atrial function | | | | | | |
| Reservoir strain ($\varepsilon_s$) % | 29.9 (7.8) | 32.8 (7.6) | 31.1 (7.8) | 0.036 | 1.0 (0.9-1.1) | 0.9 |
| Conduit strain ($\varepsilon_e$) % | 11.3 (4.0) | 15.6 (6.1) | 13.2 (5.5) | <0.0001 | 1.1 (1.002–1.3) | 0.045 |
| Booster strain ($\varepsilon_a$) % | 16.9 (5.6) | 16.3 (3.9) | 16.6 (5.0) | 0.46 | | |
| Reservoir strain rate (SRs) ($s^{-1}$) | 1.5 (0.5) | 1.6) (0.5) | 1.6 (0.5) | 0.06 | | |
| Conduit strain rate (SRe) ($s^{-1}$) | −1.2 (0.5) | −1.7 (0.8) | −1.4 (0.7) | <0.0001 | 0.6 (0.2-1.6) | 0.3 |
| Booster strain rate (Sra) ($s^{-1}$) | −2.2 (0.8) | −2.2 (0.6) | −2.2 (0.7) | 0.92 | | |
| SRe/SRa | 0.6 (0.6) | 0.8 (0.4) | 0.7 (0.5) | 0.14 | | |

Abbreviations: Ao, Aortic diameter; BMI, body mass index; CMR, XXX; DT, deceleration time; IVSd, XXX; IVSs, XXX; LA, left atrium; LVEF, left ventricular ejection fraction; LV FS, left ventricular fractional shortening; LVIDd, left ventricular internal diameter end diastole; LVMI, left ventricular mass index; LVOT, Levt ventricular outflow tract; LVPWd, XXX; LVPWs, left ventricular posterior wall end systole; MV A, XXX; MV E, XXX; OR, odd ratio; PASP, pulmonary artery systolic pressure; PV-Ar, pulmonary vein flow at atrial contraction; PV-D, pulmonary vein diastolic velocity; PV-S, pulmonary vein systolic velocity; SRa, XXX; SRe, XXX; SRs, XXX.

[a] Age, BMI, and diabetes were adjusted.

## 4 | RESULTS

We studied a total of 141 participants, of whom 82 (58.2%) had low $VO_2$, while 59 (41.8%) had high $VO_2$.

Compared to participants with high $VO_2$, participants with low $VO_2$ were older (mean age 73.8 ± 3.6 vs 66.1 ± 15.7 years, $P < 0.0001$), had a higher BMI (mean BMI 24.5 ± 3.0 vs 22.1 ± 2.8, $P < 0.0001$), and a trend toward higher likelihood of diabetes mellitus (29.3% vs 15.3%, $P = 0.053$). In addition, participants with low $VO_2$ were more likely to report doing low intensity exercise ("take it easy") (96.3% vs 81.4%) as opposed to higher intensity exercise ("heavy breath and sweat") (3.7% vs 18.6%) ($P = 0.003$) (Table 1).

Compared to participants with high $VO_2$, participants with low $VO_2$ had more adverse cardiovascular parameters, such as lower ratio of peak velocity flow in early diastole to peak velocity flow in late diastole by atrial contraction of >0.8 (76% vs 35%, adjusted odd ratio [OR] = 4.1, 95% confidence interval [CI]: [1.7-9.5], $P = 0.001$) and lower LA conduit strain (11.3 ± 4.0 vs 15.6 ± 6.1, adjusted OR = 1.1, 95% CI: [1.002-1.3], $P = 0.045$) (Table 2).

We analyzed serum samples from study subjects for 83 metabolites comprising 65 acyl-carnitine metabolites, 16 amino acid metabolites and 2 carnitine metabolites. The list of measured metabolites is presented in Supporting Information Tables S2.

SPCA identified 10 acyl-carnitine factors clustering in biologically related groupings (Table 3). Univariate association between each of the 10 SPCA factors and risk of high $VO_2$ is shown in Table 4. Factor 2, Factor 5, and Factor 8 showed significant negative association with high V02. However, after adjustment for significant clinical covariates such as age, BMI and diabetes, only Factor 8 remained predictive for high V02 (OR = 0.6, 95% CI: [0.4-0.9], $P = 0.013$).

Univariate associations between amino acids and risk of high $VO_2$ were significant for alanine, glutamine/glutamate, glycine, and ornithine (Table 4). Multivariate analysis adjusting for significant clinical covariates showed only alanine (OR = 0.1, 95% CI: [0.01-0.9], $P = 0.044$) and glutamine/glutamate (OR = 0.1, 95% CI: [0.01-0.5], $P = 0.007$) were independent predictors for high V02.

## 5 | DISCUSSION

In this cross-sectional study, low peak oxygen uptake was associated with potentially deleterious changes in cardiovascular structure and function as well as with higher accumulation of wide-spectrum acyl-carnitines and several amino acids.

Our novel study used a validated calculator of peak oxygen uptake to estimate peak oxygen uptake of a contemporary cohort of community elderly adults. We provide novel support for this method of peak oxygen uptake estimation by demonstrating that high calculated $VO_2$ was indeed associated with better cardiovascular structure and function. In fact, due to the detailed annotation of cardiovascular

**TABLE 3** Factors identified by sparse principal component analysis and the associated individual components, description and variance

| Factors | Description | Components | Proportion of variance accounted |
|---|---|---|---|
| 1 | Medium and long-chain carnitines | C8, C8-DC, C12:1, C12, C12-OH/C10-DC, C14:2, C14:1, C14, C16:3, C16:2,C16:1, C18:1 | 0.11 |
| 2 | Short-chain dicarboxyl/hydroxyl carnitines | C3, C4, C5:1, C4-OH, C6, C5OHC3DC,C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C103, C81DC, C8-DC | 0.063 |
| 3 | Medium and long-chain dicarboxyl/hydroxyl carnitines | C81OHC61DC, C122OHC102DC,C121OH,C142OH, C141OH, C163OHC143DC,C162OHC183OHC163DC, C201, C20, C201OHC181DC, C20OHC18DC, C221 C182OHC162DC, | 0.072 |
| 4 | Long-chain carnitines | C16, C183, C182, C181, C18, C204,C203, C202, C201, C202OHC182DC,C225, C224 C223 | 0.060 |
| 5 | Medium and long-chain dicarboxyl/hydroxyl carnitines | C4OH, C8OHC6DC, C8DC,C12OHC10DC,C141OH, C14OHC12DC, C162OH,C161OHC141DC,C18OHC16DC, C20, C201OHC181DC, C16OH,C181OHC161DC, C20OHC18DC | 0.074 |
| 6 | Wide-spectrum carnitines including odd short-chain carnitines | C2, C3, C51, C5OHC3DC, C101, C7DC, C121, C12, C14, C142OH, C163 | |
| 7 | Wide-spectrum carnitines including ketone-derived carnitine | C162OH, C16OH, C183, C182, C18, C183OHC163DC, C182OHC162DC, C204, C203, C202, C201, C203OHC183DC, C225, C223, C222, C22, free carnitine, Total Carnitine | 0.038 |
| 8 | Wide-spectrum carnitines including odd short-chain carnitines | C3, C51, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C7DC, C81DC, C8DC, C122, C121, C12OHC10DC, C14OHC12DC, C16, C16OH, C183OHC163DC, C18OHC16DC, C204, C201, C20OHC18DC, C224, C223, C222, C221 free Carnitine, Total Carnitine | 0.022 |
| 9 | Wide-spectum carnitines including ketone-derived carnitine | C2, C51, C4OH, C6, C5OHC3DC, C81OHC61DC, C101, C81DC, C12, C122OHC102DC, C121OH, C14, C142OH, C14OHC12DC, C162, C183, C182, C181, C183OHC163DC, C182OHC162DC, C181OHC161DC, C18OHC16DC, C204, C203, C201OHC181DC, C20OHC18DC, C225, free Carnitine, Total Carnitine | 0.023 |
| 10 | Medium and long-chain carnitines | C10, C143, C142, C14, C143OHC123DC, C142OH, C163, C16, C181, C18, C182OHC162DC, C204, C203, C201, C20, C221, C22 | 0.023 |

**TABLE 4** Differences in metabolomic patterns

| | $VO_2$ low | $VO_2$ high | Total | OR (95% CI) | *P* value | Adjusted OR[a] | *P* value |
|---|---|---|---|---|---|---|---|
| Acylcarnitines | | | | | | | |
| Factor 1 | −0.1 (2.7) | 0.1 (2.7) | 0 (2.7) | 1.0 (0.9-1.2) | 0.69 | | |
| Factor 2 | 0.3 (2.2) | −0.4 (1.8) | 0 (2.0) | 0.8 (0.7-1.0) | 0.030 | 0.9 (0.8-1.2) | 0.55 |
| Factor 3 | −0.01 (2.1) | 0.02 (2.4) | 0 (2.2) | 1.0 (0.9-1.2) | 0.94 | | |
| Factor 4 | −0.04 (2.2) | 0.05 (1.9) | 0 (2.1) | 1.0 (0.9-1.2) | 0.81 | | |
| Factor 5 | 0.3 (2.4) | −0.5 (2.1) | 0 (2.3) | 0.8 (0.7–1.0) | 0.038 | 0.9 (0.7-1.1) | 0.21 |
| Factor 6 | 0.2 (1.5) | −0.2 (1.7) | 0 (1.6) | 0.9 (0.7–1.1) | 0.22 | | |
| Factor 7 | −0.1 (1.8) | 0.1 (1.8) | 0 (1.8) | 1.1 (0.9-1.3) | 0.56 | | |
| Factor 8 | 0.2 (1.0) | −0.3 (1.4) | 0 (1.2) | 0.7 (0.5-1.0) | 0.035 | 0.6 (0.4-0.9) | 0.013 |
| Factor 9 | −0.1 (1.5) | 0.2 (1.0) | 0 (1.3) | 1.2 (0.9-1.5) | 0.25 | | |
| Factor 10 | 0.05 (1.4) | −0.1 (1.2) | 0 (1.3) | 0.9 (0.7-1.2) | 0.61 | | |
| Amino acids | | | | | | | |
| Ala | 6.2 (0.3) | 6.1 (0.2) | 6.2 (0.2) | 0.1 (0.03-0.7) | 0.018 | 0.1 (0.01-0.9) | 0.044 |
| Arg | 4.7 (0.2) | 4.8 (0.2) | 4.7 (0.2) | 2.1 (0.5-9.0) | 0.34 | | |
| Asp | 3.1 (0.3) | 3.1 (0.3) | 3.1 (0.3) | 0.5 (0.2-1.8) | 0.31 | | |
| Cit | 3.4 (0.4) | 3.5 (0.4) | 3.5 (0.4) | 1.8 (0.8-4.1) | 0.14 | | |
| Glu | 4.6 (0.2) | 4.4 (0.2) | 4.5 (0.2) | 0.03 (0.005-0.1) | <0.0001 | 0.1 (0.01-0.5) | 0.007 |
| Gly | 5.4 (0.2) | 5.5 (0.2) | 5.4 (0.2) | 10.8 (1.8-62.9) | 0.0080 | 5.8 (0.7-46.5) | 0.099 |
| His | 4.3 (0.2) | 4.3 (0.2) | 4.3 (0.2) | 1.8 (0.4-8.2) | 0.46 | | |
| IleLeu | 5.0 (0.3) | 4.9 (0.3) | 5.0 (0.3) | 0.6 (0.2-2.1) | 0.47 | | |
| Met | 3.2 (0.4) | 3.2 (0.4) | 3.2 (0.4) | 1.3 (0.5-3.3) | 0.52 | | |
| Orn | 4.5 (0.3) | 4.4 (0.3) | 4.4 (0.3) | 0.3 (0.1-1.0) | 0.049 | 0.4 (0.1-1.8) | 0.24 |
| Phe | 4.3 (0.2 | 4.3 (0.2) | 4.3 (0.2) | 0.4 (0.1-2.4) | 0.32 | | |
| Pro | 5.5 (0.2) | 5.5 (0.2) | 5.5 (0.2) | 0.3 (0.1-1.1) | 0.069 | | |
| Ser | 4.8 (0.2) | 4.8 (0.2) | 4.8 (0.2) | 1.0 (0.2-5.3) | 0.96 | | |
| Trp | 3.9 (0.2) | 4.0 (0.3) | 4.0 (0.3) | 3.3 (0.8-13.2) | 0.098 | | |
| Tyr | 4.3 (0.3) | 4.2 (0.3) | 4.2 (0.3) | 0.4 (0.1-1.2) | 0.085 | | |
| Val | 5.5 (0.3) | 5.4 (0.3) | 5.5 (0.3) | 0.7 (0.2-2.5) | 0.63 | | |

Abbreviations: BMI, body mass index; OR, odd ratio.
[a] Age, BMI, and diabetes were adjusted.

structure and function obtained from CMR imaging and echocardiogram, the association between $VO_2$ as calculated by this method could be directly linked to specific indices of CV structure and function.

In this community cohort without CVD, we provide novel evidence that $VO_2$ was associated with better LA function as assessed by CMR feature tracking. We observed a positive association between LA reservoir and conduit strain with high $VO_2$ in our study. In addition, LA conduit strain remained strongly associated with high $VO_2$ after adjustment for clinical factors. To the best of our knowledge, our study is the first to clearly demonstrate the potential clinical importance of LA strain by this sensitive method of CMR feature tracking. The association between high LA strain and high $VO_2$ suggests that impaired LA mechanics may lead to poor augmentation of cardiac output with exertion and decreased exercise tolerance.

We detected a significant negative correlation between peak oxygen uptake and metabolic profile in a cohort of aged community adults with different levels of $VO_2$. Previous studies have recognized differences in the metabolic profile of subjects with varying levels of activity[20–23] or upon exposure to different levels of exercise.[24,25] In this study, we found significant differences in a wide spectrum of acyl-carnitine species including short-chain dicarboxyl-carnitines as well as long-chain acyl-carnitines. The long-chain acyl-carnitines are derived from oxidation of fatty acid fuel. Elevations in long-chain acyl-carnitines have been previously associated with impaired mitochondrial fuel metabolism and obesity associated-insulin resistance.[26] The negative association of long-chain acyl-carnitines with peak oxygen uptake fits with the notion that decreased $VO_2$ is linked to diminished mitochondrial oxidative capacity and risk for metabolic disease. The study also demonstrated a negative association between short-chain dicarboxyl-carnitines, $VO_2$ and various measures of cardiac function. Previous studies have linked accumulation of dicarboxyl-carnitines and increased risk of recurrent cardiovascular events.[8]

We observed a negative association between glutamine/glutamate and alanine with high $VO_2$ in our study. Low alanine has previously been linked to higher levels of physical activity.[20] Both alanine and glutamine/glutamate serve as anaplerotic substrates, which can directly feed into the tricarboxylic acid (TCA) cycle. Lower levels of these amino acids may be indicative of higher TCA cycle turnover and increased overall mitochondrial activity, which is associated with increased exercise.[27]

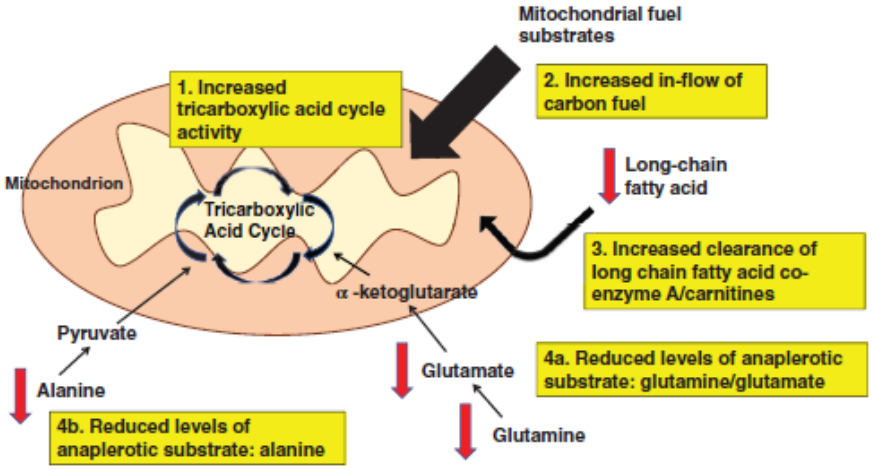


**FIGURE 1** High peak oxygen uptake ($VO_2$) may be associated with increased tricarboxylic acid cycle activity (1). This leads to increase inflow of carbon fuel into mitochondrial pathways (2). A consequence of this could include reduced accumulation of long-chain fatty acid co-enzyme A/carnitine fuel as a result of higher fuel oxidation rates (3) and reduced build-up of (4a) glutamine/glutamate and (4b) alanine due to higher anaplerorosis

As illustrated in Figure 1, a potential explanation for our combined metabolomics findings could be that higher peak oxygen uptake is associated with increased TCA cycle activity, which leads to increase inflow of carbon fuel into mitochondrial pathways. This results in reduced accumulation of long-chain fatty acids due to higher fuel oxidation rates and reduced build-up of anaplerotic substrates such as glutamine/glutamate and alanine due to higher anaplerorosis. Therefore, our study provides potential mechanistic evidence as to how increasing peak oxygen uptake, through physical activity for instance, reduces cardiovascular risk by reducing long-chain fatty acids, glutamate/glutamine and alanine.

We acknowledge limitations in our study. Sample size was relatively small although statistically significant associations between the groups could be identified. While we corrected for available clinical factors, we cannot exclude the possibility that additional factors that were not included could have influenced our findings. The serum samples were obtained in a nonfasting state, which may potentially introduce analytic differences in postabsorptive states between the subjects studied. As a community-based driven study, we recognize challenges in getting elderly community subjects to fast. Future studies comprising of fasting samples may provide additional insights as to the effect of fasting on similar analyses. One advantage of our study, however, was the prospective collection of blood samples at the same time as the cardiovascular measurements. Our study design is cross-sectional and hence we cannot infer causal relationships. Future longitudinal follow-up of these participants may provide greater insights into causality. Despite these limitations, our results highlight the clinical relevance of pursuing future clinical investigations using both a clinical imaging and a molecular approach as such an integrated approach may help identify mechanisms involved in CVDs in specific cohorts.[10]

## 6 | CONCLUSION

Community elderly adults with low peak oxygen uptake have adverse cardiovascular and metabolic parameters compared to their counterparts with high peak oxygen uptake. Combined cardiac and metabolomics phenotyping may be a promising tool to provide insights into physiological states, useful for tracking future interventions related to physical activity among community cohorts.

## CONFLICTS OF INTEREST

The authors declare no potential conflict of interests.

## ACKNOWLEDGMENT

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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**COMMENTARY**

**Main findings of this study:**

a) **Low physical activity was associated with deleterious changes in cardiovascular structure and function**
b) **Metabolomics differentiated older adults with high versus low physical activity capacities**
c) **Wide-spectrum acylcarnitines and several amino acids produced a convergent signal of impairments in cardiovascular structure associated with low physical activity capacity**

This study was designed to characterise the metabolic profile of older adults at different levels of physical activity capacities, studied in relation to their cardiovascular profiles obtained by detailed cardiovascular assessment.

Community-based older adults without physician-diagnosed heart disease, stroke or cancer underwent same-day multimodal assessment of cardiovascular function (by echocardiography and magnetic resonance feature tracking of left atrium) and aerobic capacity by peak oxygen uptake (VO2) metrics. Associations between VO2 and cardiovascular and metabolomics profiles were studied in adjusted models including standard covariates.

Based on a simple physical activity questionnaire, a validated nonexercise prediction model was used to estimate peak oxygen uptake, VO2 milliliter/kg/minute (ml/kg/min)[86, 87]. This simple physical activity questionnaire consisted of age, gender, height, weight, estimated maximum heart rate, frequency of exercise, length of time for each workout, intensity of each workout, waistline diameter, and resting heart rate.

We first examined bivariable association of subject clinical characteristics, physical activity, cardiac function, and left atrial function with high VO2. High VO2 was defined as a VO2 > 37 (ml/kg/min) for men or VO2 > 29 (ml/kg/min) for women as mean VO2 was 37 (ml/kg/min) for men and 29 (ml/kg/min) for women in our cohort. Univariate logistic regression assessed a total of 83 metabolites (65 acylcarnitine metabolites, 16 amino acid metabolites, and 2 carnitine metabolites). Metabolites with >25% of values below the lower limit of quantification were excluded from analysis (only C102 was excluded, hence a total of 83 metabolites were analysed in the final sample). We normalised the distributions of all metabolites by logarithmic transformation. We reduced the dimensionality of correlated metabolites (65 acyl-carnitine metabolites and 2 carnitine metabolites) using sparse principal component analysis (SPCA). The association of each SPCA factors with high VO2 were analysed. Multivariable logistic regression was used to assess the role of amino acid metabolites and SPCA factors that show an association with $P < 0.05$ with high VO2 in univariate analysis controlling for significant clinical characteristics (age, body mass index [BMI], and diabetes).

We studied a total of 141 participants, of whom 82 (58.2%) had low VO2, while 59 (41.8%) had high VO2. Compared to participants with high VO2, participants with low VO2 were older (mean age 73.8±3.6 vs 66.1±15.7 years, $P < 0.0001$), had a higher BMI (mean BMI 24.5±3.0 vs 22.1±2.8, $P < 0.0001$), and a trend toward higher likelihood of diabetes mellitus (29.3% vs 15.3%, $P = 0.053$). In addition, participants with low VO2 were more likely to report doing low intensity exercise (“take it easy”) (96.3% vs 81.4%) as opposed to higher intensity exercise (“heavy breath and sweat”) (3.7% vs 18.6%) ($P = 0.003$) (Table 1).

| | VO2 low (n=82) | VO2 high (n=59) | Total (n=141) | p-value |
|---|---|---|---|---|
| **Age (year)** | 73.8 (3.6) | 66.1 (15.7) | 70.6 (11.2) | <0.0001 |
| **Female** | 36 (43.9%) | 23 (39.0%) | 59 (41.8%) | 0.56 |
| **Ever smoker** | 20 (24.4%) | 10 (17.0%) | 30 (21.3%) | 0.29 |
| **Body mass index (kg/m$^2$)** | 24.5 (3.0) | 22.1 (2.8) | 23.5 (3.1) | <0.0001 |
| **Hypertension** | 50 (61.0%) | 27 (45.8%) | 77 (54.6%) | 0.073 |
| **Diabetes mellitus** | 24 (29.3%) | 9 (15.3%) | 33 (23.4%) | 0.053 |
| **Dyslipidaemia** | 46 (56.1%) | 25 (42.4%) | 71 (50.4%) | 0.11 |
| **Heart rate (beats per minute)** | 75.8 (13.2) | 70.3 (11.0) | 73.5 (12.5) | 0.010 |
| **Central systolic blood pressure (mmHg)** $^+$ | 141.6 (17.6) | 136.0 (17.9) | 139.3 (17.9) | 0.066 |
| **Central diastolic blood pressure (mmHg)** $^+$ | 76.7 (10.1) | 76.2 (11.7) | 76.5 (10.7) | 0.79 |
| **Central mean arterial pressure (mmHg)** $^+$ | 103.0 (12.1) | 100.4 (11.5) | 102.0 (11.9) | 0.20 |
| **Central pulse pressure (mmHg)** $^+$ | 64.9 (16.2) | 59.8 (18.5) | 62.8 (17.3) | 0.083 |
| | | | | |
| ***Physical activity*** | | | | |
| **Frequency** | | | | 0.48 |
| **Inactive** | 15 (18.3%) | 9 (15.3%) | 24 (17.0%) | |
| **Once a week** | 1 (1.2%) | 3 (5.1%) | 4 (2.8%) | |
| **2 to 3 times a week** | 10 (12.2%) | 5 (8.5%) | 15 (10.6%) | |
| **Almost everyday** | 56 (68.3%) | 42 (71.2%) | 98 (69.5%) | |
| **Intensity** | | | | 0.003 |
| **Take it easy** | 79 (96.3%) | 48 (81.4%) | 127 (90.1%) | |
| **Heavy breath and sweat** | 3 (3.7%) | 11 (18.6%) | 14 (9.9%) | |
| **Duration** | | | | 0.73 |
| **<15 min** | 20 (24.4%) | 14 (23.7%) | 34 (24.1%) | |
| **16 to<30min** | 24 (29.3%) | 14 (23.7%) | 38 (27.0%) | |
| **30 to 60 min** | 17 (20.7%) | 11 (18.6%) | 28 (19.9%) | |
| **>1 hour** | 21 (25.6%) | 20 (33.9) | 41 (29.1) | |

***Table 1: Baseline clinical characteristics, and physical activity data***

Compared to participants with high VO2, participants with low VO2 had more adverse cardiovascular parameters, such as lower ratio of peak velocity flow in early diastole to peak velocity flow in late diastole by atrial contraction of >0.8 (76% vs 35%, adjusted odd ratio [OR] = 4.1, 95% confidence interval [CI]: [1.7-9.5], P = 0.001) and lower LA conduit strain (11.3±4.0 vs 15.6±6.1, adjusted OR = 1.1, 95% CI: [1.002-1.3], P = 0.045) (Table 2).

| | VO2 low (n=82) | VO2 high (n=59) | Total (n=141) | p-value | *Adjusted OR | Adjusted p-value |
|---|---|---|---|---|---|---|
| **Interventricular septum thickness at end diastole (IVSd) (cm)** | 0.86 (0.18) | 0.81 (0.13) | 0.84 (0.16) | 0.096 | | |
| **Interventricular septum thickness at end systole (IVSs) (cm)** | 1.30 (0.26) | 1.17 (0.24) | 1.24 (0.26) | 0.0040 | 0.3 (0.1-1.8) | 0.20 |
| **Left ventricular internal diameter end diastole (LVIDd) (cm)** | 4.50 (0.64) | 4.33 (0.74) | 4.42 (0.69) | 0.17 | | |
| **Left ventricular internal diameter end systole (LVIDs) (cm)** | 2.52 (0.50) | 2.53 (0.44) | 2.52 (0.47) | 0.92 | | |
| **Left ventricular posterior wall end diastole (LVPWd) (cm)** | 0.80 (0.14) | 0.74 (0.09) | 0.77 (0.12) | 0.0097 | 0.1 (0.003-5.2) | 0.28 |
| **Left ventricular posterior wall end systole (LVPWs)(cm)** | 1.45 (0.24) | 1.37 (0.28) | 1.41 (0.26) | 0.087 | | |
| **Left ventricular outflow tract (LVOT) (cm)** | 2.07 (0.16) | 2.05 (0.17) | 2.06 (0.16) | 0.64 | | |
| **Aortic diameter (Ao) (cm)** | 3.14 (0.46) | 3.04 (0.49) | 3.09 (0.47) | 0.23 | | |
| **Left atrium (LA) (cm)** | 3.81 (0.52) | 3.50 (0.55) | 3.68 (0.56) | 0.0015 | 0.8 (0.3-2.1) | 0.71 |
| **Left ventricular ejection fraction (LVEF) (%)** | 74.44 (7.08) | 73.64 (7.63) | 74.10 (7.30) | 0.53 | | |
| ***Left ventricular fractional shortening (LV FS) (%)*** | 43.80 (6.32) | 42.97 (6.48) | 43.45 (6.38) | 0.47 | | |
| **Left ventricular mass index (LVMI) (grams/m$^2$)** | 83.72 (27.27) | 75.20 (18.89) | 80.12 (24.38) | 0.051 | | |
| **Ratio of *Peak* velocity flow in early diastole E (MV E Peak) (m/s) to *Peak* velocity flow in late diastole by atrial contraction A (MV A Peak) (m/s) (E/A Ratio)>0.8** | 28 (35.0%) | 45 (76.3%) | 73 (52.5%) | <0.0001 | 4.1 (1.7-9.5) | **0.001** |
| **Deceleration time (DT) (m/s)** | 210.54 (38.69) | 205.25 (36.68) | 208.30 (37.81) | 0.42 | | |
| **Pulmonary artery systolic pressure (PASP) (mmHg)** | 27.27 (6.47) | 27.06 (6.61) | 27.18 (6.50) | 0.86 | | |
| **Pulmonary vein systolic velocity (PV-S) (cm/s)** | 57.22 (11.50) | 58.48 (11.55) | 57.76 (11.49) | 0.54 | | |
| **Pulmonary vein diastolic velocity (PV-D ) (cm/s)** | 47.07 (15.45) | 49.02 (12.76) | 47.90 (14.34) | 0.45 | | |
| **Pulmonary vein flow at atrial contraction (PV-Ar) (ms)** | 92.41 (16.63) | 92.42 (16.04) | 92.41 (16.32) | 1.00 | | |
| **Mitral inflow duration at atrial contraction (MV A duration) (ms)** | 118.45 (18.88) | 113.63 (16.75) | 116.40 (18.10) | 0.13 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Left atrial function*** | | | | | | |
| **Reservoir strain ($\varepsilon_s$) %** | 29.9 (7.8) | 32.8 (7.6) | 31.1 (7.8) | 0.036 | 1.0 (0.9-1.1) | 0.90 |
| **Conduit strain ($\varepsilon_e$) %** | 11.3 (4.0) | 15.6 (6.1) | 13.2 (5.5) | <0.0001 | 1.1 (1.002-1.3) | **0.045** |
| **Booster strain ($\varepsilon_a$) %** | 16.9 (5.6) | 16.3 (3.9) | 16.6 (5.0) | 0.46 | | |
| **Reservoir strain rate (SRs) ($s^{-1}$)** | 1.5 (0.5) | 1.6 (0.5) | 1.6 (0.5) | 0.060 | | |
| **Conduit strain rate (SRe) ($s^{-1}$)** | -1.2 (0.5) | -1.7 (0.8) | -1.4 (0.7) | <0.0001 | 0.6 (0.2-1.6) | 0.30 |
| **Booster strain rate (SRa) ($s^{-1}$)** | -2.2 (0.8) | -2.2 (0.6) | -2.2 (0.7) | 0.92 | | |
| **SRe/SRa** | 0.6 (0.6) | 0.8 (0.4) | 0.7 (0.5) | 0.14 | | |

***Table 2: Cardiac Functions by echocardiogram and left atrial function by CMR*** *Age, BMI, and diabetes were adjusted.

SPCA identified 10 acyl-carnitine factors clustering in biologically related groupings (Table 3). Univariate association between each of the 10 SPCA factors and risk of high VO2 is shown in Table 4. Factor 2, Factor 5, and Factor 8 showed significant negative association with high V02. However, after adjustment for significant clinical covariates such as age, BMI and diabetes, only Factor 8 remained predictive for high V02 (OR = 0.6, 95% CI: [0.4-0.9], P = 0.013).

| **Factors** | **Description** | **Components** | **Proportion of variance accounted** |
|---|---|---|---|
| 1 | Medium and long-chain carnitines | C8, C8-DC, C12:1, C12, C12-OH/C10-DC, C14:2, C14:1, C14, C16:3, C16:2, C16:1, C18:1 | 0.11 |
| 2 | Short chain dicarboxyl/hydroxyl carnitines | C3, C4, C5:1, C5, C4-OH, C6, C5OHC3DC, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C103, C81DC, C8-DC | 0.063 |
| 3 | Medium and long chain dicarboxyl/hydroxyl carnitines | C81OHC61DC, C122OHC102DC, C121OH, C142OH, C141OH, C163OHC143DC, C162OH C183OHC163DC, C182OHC162DC, C201, C20, C202OHC182DC, C201OHC181DC, C20OHC18DC, C221 | 0.072 |
| 4 | Long chain carnitines | C16, C183, C182, C181, C18, C204, C203, C202, C201, C202OHC182DC, C225, C224 C223 | 0.060 |
| 5 | Medium and long chain dicarboxyl/hydroxyl carnitines | C4OH, C8OHC6DC, C8DC, C12OHC10DC, C141OH, C14OHC12DC, C162OH, C161OHC141DC, C16OH, C181OHC161DC, C18OHC16DC, C20, C201OHC181DC, C20OHC18DC | 0.074 |
| 6 | Wide spectrum carnitines including | C2, C3, C51, C5, C5OHC3DC, C101, C7DC, C121, C12, C14, C142OH, C163, C162OH, | 0.038 |

| | | | |
|---|---|---|---|
| | odd short chain carnitines | C16OH, C183, C182, C18, C183OHC163DC, C182OHC162DC, C204, C203, C202, C201, C203OHC183DC, C225, C223, C222, C22, Free Carnitine, Total Carnitine | |
| 7 | Wide spectrum carnitines including ketone-derived carnitine | C2, C4OH, C6, C81, C5DC, C81OHC61DC, C103, C101, C10, C81DC, C122, C143, C142, C14, C142OH, C14OHC12DC, C162, C161, C16, C162OH, C161OHC141DC, C183, C182, C183OHC163DC, C18OHC16DC, C204, C202, C201OHC181DC, C224, C222, C22 | 0.045 |
| 8 | Wide spectrum carnitines including odd short chain carnitines | C3, C51, C4DCC6OH, C5DC, C81OHC61DC, C8OHC6DC, C7DC, C81DC, C8DC, C122, C121, C12OHC10DC, C14OHC12DC, C16, C16OH, C183OHC163DC, C18OHC16DC, C204, C201, C20OHC18DC, C224, C223, C222, C221, Free Carnitine, Total Carnitine | 0.022 |
| 9 | Wide spectrum carnitines including ketone-derived carnitine | C2, C51, C4OH, C6, C5OHC3DC, C81OHC61DC, C101, C81DC, C12, C122OHC102DC, C121OH, C14, C142OH, C14OHC12DC, C162, C183, C182, C181, C183OHC163DC, C182OHC162DC, C181OHC161DC, C18OHC16DC, C204, C203, C201OHC181DC, C20OHC18DC, C225, Free Carnitine, Total Carnitine | 0.023 |
| 10 | Medium and long chain carnitines | C10, C143, C142, C14, C143OHC123DC, C142OH, C163, C16, C181, C18, C182OHC162DC, C204, C203, C201, C20, C221, C22 | 0.023 |

***Table 3. Factors identified by sparse principal component analysis and the associated individual components, description and variance.***

Univariate associations between amino acids and risk of high VO2 were significant for alanine, glutamine/glutamate, glycine, and ornithine (Table 4). Multivariate analysis adjusting for significant clinical covariates showed only alanine (OR = 0.1, 95% CI: [0.01-0.9], P = 0.044) and glutamine/glutamate (OR = 0.1, 95% CI: [0.01-0.5], P = 0.007) were independent predictors for high V02.

| | **VO2 low** | **VO2 high** | **Total** | **OR (95% CI)** | **p-value** | ***Adjusted OR** | **p-value** |
|---|---|---|---|---|---|---|---|
| ***Acylcarnitines*** | | | | | | | |
| Factor 1 | -0.1 (2.7) | 0.1 (2.7) | 0 (2.7) | 1.0 (0.9-1.2) | 0.69 | | |
| Factor 2 | 0.3 (2.2) | -0.4 (1.8) | 0 (2.0) | 0.8 (0.7-1.0) | 0.030 | 0.9 (0.8-1.2) | 0.55 |
| Factor 3 | -0.01 (2.1) | 0.02 (2.4) | 0 (2.2) | 1.0 (0.9-1.2) | 0.94 | | |
| Factor 4 | -0.04 (2.2) | 0.05 (1.9) | 0 (2.1) | 1.0 (0.9-1.2) | 0.81 | | |
| Factor 5 | 0.3 (2.4) | -0.5 (2.1) | 0 (2.3) | 0.8 (0.7-1.0) | 0.038 | 0.9 (0.7-1.1) | 0.21 |
| Factor 6 | 0.2 (1.5) | -0.2 (1.7) | 0 (1.6) | 0.9 (0.7-1.1) | 0.22 | | |
| Factor 7 | -0.1 (1.8) | 0.1 (1.8) | 0 (1.8) | 1.1 (0.9-1.3) | 0.56 | | |
| Factor 8 | 0.2 (1.0) | -0.3 (1.4) | 0 (1.2) | 0.7 (0.5-1.0) | 0.035 | **0.6 (0.4-0.9)** | **0.013** |
| Factor 9 | -0.1 (1.5) | 0.2 (1.0) | 0 (1.3) | 1.2 (0.9-1.6) | 0.25 | | |
| Factor 10 | 0.05 (1.4) | -0.1 (1.2) | 0 (1.3) | 0.9 (0.7-1.2) | 0.61 | | |
| ***Amino acids*** | | | | | | | |
| Ala | 6.2 (0.3) | 6.1 (0.2) | 6.2 (0.2) | 0.1 (0.03-0.7) | 0.018 | **0.1 (0.01-0.9)** | **0.044** |
| Arg | 4.7 (0.2) | 4.8 (0.2) | 4.7 (0.2) | 2.1 (0.5-9.0) | 0.34 | | |
| Asp | 3.1 (0.3) | 3.1 (0.3) | 3.1 (0.3) | 0.5 (0.2-1.8) | 0.31 | | |
| Cit | 3.4 (0.4) | 3.5 (0.4) | 3.5 (0.4) | 1.8 (0.8-4.1) | 0.14 | | |
| Glu | 4.6 (0.2) | 4.4 (0.2) | 4.5 (0.2) | 0.03 (0.005-0.1) | <0.0001 | **0.1 (0.01-0.5)** | **0.0070** |
| Gly | 5.4 (0.2) | 5.5 (0.2) | 5.4 (0.2) | 10.8 (1.8-62.9) | 0.0080 | 5.8 (0.7-46.5) | 0.099 |
| His | 4.3 (0.2) | 4.3 (0.2) | 4.3 (0.2) | 1.8 (0.4-8.2) | 0.46 | | |
| IleLeu | 5.0 (0.3) | 4.9 (0.3) | 5.0 (0.3) | 0.6 (0.2-2.1) | 0.47 | | |
| Met | 3.2 (0.4) | 3.2 (0.4) | 3.2 (0.4) | 1.3 (0.5-3.3) | 0.52 | | |
| Orn | 4.5 (0.3) | 4.4 (0.3) | 4.4 (0.3) | 0.3 (0.1-1.0) | 0.049 | 0.4 (0.1-1.8) | 0.24 |
| Phe | 4.3 (0.2) | 4.3 (0.2) | 4.3 (0.2) | 0.4 (0.1-2.4) | 0.32 | | |
| Pro | 5.5 (0.2) | 5.5 (0.2) | 5.5 (0.2) | 0.3 (0.1-1.1) | 0.069 | | |
| Ser | 4.8 (0.2) | 4.8 (0.2) | 4.8 (0.2) | 1.0 (0.2-5.3) | 0.96 | | |
| Trp | 3.9 (0.2) | 4.0 (0.3) | 4.0 (0.3) | 3.3 (0.8-13.2) | 0.098 | | |
| Tyr | 4.3 (0.3) | 4.2 (0.3) | 4.2 (0.3) | 0.4 (0.1-1.2) | 0.085 | | |
| Val | 5.5 (0.3) | 5.4 (0.3) | 5.5 (0.3) | 0.7 (0.2-2.5) | 0.63 | | |

***Table 4: Differences in metabolomic patterns*** *Age, BMI and diabetes were adjusted.

***Discussion***

In this study, we found significant differences in a wide spectrum of acyl-carnitine species including short-chain dicarboxyl-carnitines as well as long-chain acyl-carnitines. The long-chain acyl-carnitines are derived from oxidation of fatty acid fuel. Elevations in long-chain acylcarnitines have been previously associated with impaired mitochondrial fuel metabolism and obesity associated-insulin resistance[88]. The negative association of long-chain acyl-carnitines with peak oxygen uptake fits with the notion that decreased VO2 is linked to diminished mitochondrial oxidative capacity and risk for metabolic disease. The study also demonstrated a negative association between short-chain dicarboxyl-carnitines, VO2 and various measures of cardiac function.

Previous studies have linked accumulation of dicarboxyl-carnitines and increased risk of recurrent cardiovascular events[55]. We observed a negative association between glutamine/glutamate and alanine with high VO2 in our study. Low alanine has previously been linked to higher levels of physical activity[89]. Both alanine and glutamine/glutamate serve as anaplerotic substrates, which can directly feed into the tricarboxylic acid (TCA) cycle. Lower levels of these amino acids may be indicative of higher TCA cycle turnover and increased overall mitochondrial activity, which is associated with increased exercise[90].

The *convergence* of this metabolomics signature that is associated with exercise levels in older adults may imply that older adults with higher physical activity levels have increased TCA cycle activity which leads to increased inflow of carbon fuel into mitochondrial pathways. This results in reduced accumulation of long-chain fatty acids due to higher fuel oxidation rates and reduced build-up of anaplerotic substrates such as glutamine/glutamate and alanine due to higher anaplerorosis (Figure 1). Therefore, our study provides potential mechanistic evidence as to how increasing peak oxygen uptake, through physical activity for instance, reduces cardiovascular risk by reducing long-chain fatty acids, glutamate/glutamine and alanine.

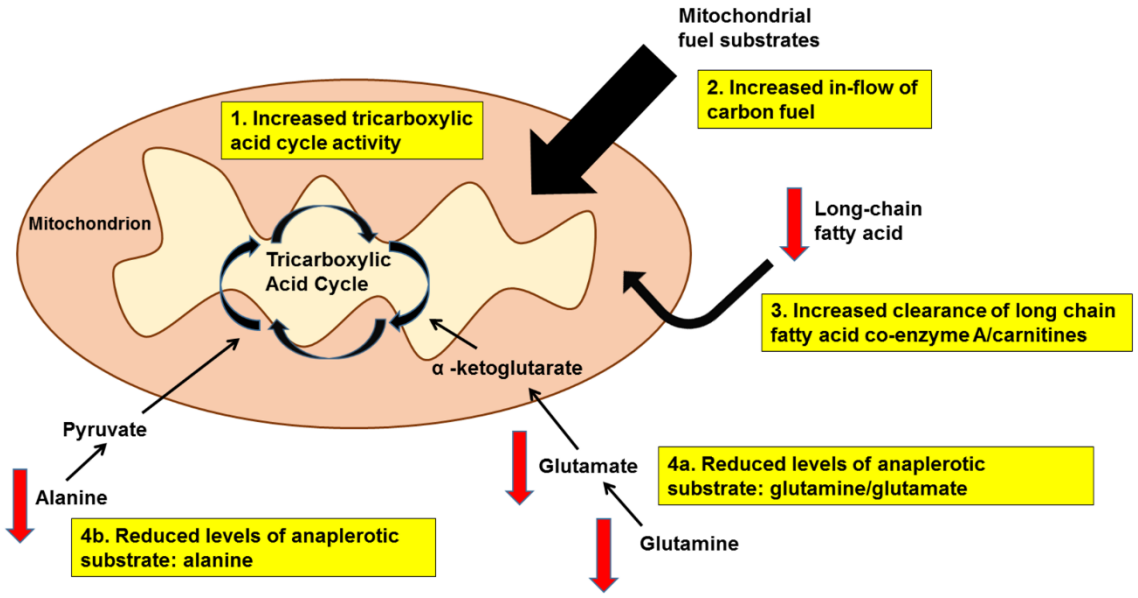


***Figure 1:*** *High peak oxygen uptake ($VO_2$) may be associated with increased tricarboxylic acid cycle activity (1). This leads to increased in-flow of carbon fuel into mitochondrial pathways (2). A consequence of this could include reduced accumulation of long chain fatty acid co-enzyme A/carnitine fuel as a result of higher fuel oxidation rates (3) and reduced build-up of (4a) glutamine/glutamate and (4b) alanine due to higher anaplerorosis.*

**Implications of our findings**

Low aerobic capacity is a strong predictor of cardiovascular disease (CVD) and all-cause mortality[91] while increases in aerobic capacity are associated with increased survival[92]. Among aged populations, poor aerobic capacity indicates closer proximity to future declines in cardiovascular and other health indices[93, 94]. Physical activity is associated with many physiologic changes including increased aerobic capacity and alterations in fuel metabolism. Animal and human model studies of heart and skeletal muscle responses to physical activity point to changes in the patterns of fuel use and mitochondrial oxidation as key components of a healthy adaptation.

Our results concur with these changes that include increased TCA cycle activity[24] and better coordination between fuel processing and TCA cycle activity[95] in the metabolome of older adults with exercise. This contrasts with studies of ageing-related frailty which have observed accumulation of fatty

acid fuel intermediates[96], mismatch between fuel supply and TCA cycle, reduced TCA cycle activity[95] and greater reliance on non-oxidative glucose metabolism.

Fatty acid oxidation, electron transport and TCA cycle genes are all up regulated by physical activity. These changes are distinct from the metabolic changes associated with heart failure which include pathophysiologic remodelling, reduced fatty acid and mitochondrial fuel oxidation and increased reliance on glucose[97]. The TCA cycle is known to be upregulated when there is a high demand for ATP. Increased energy demand stimulates regulatory enzymes of the cycle such as isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase. It has also been shown in recent studies that there is a rise in the level of TCA cycle intermediates just after an acute bout of physical activity[24]. Certain amino acids such as alanine and glutamine/glutamate can serve as metabolic fuels by feeding into the TCA cycle. The process by which amino acids are fed into the TCA cycle is known as 'anaplerosis' (or 'filling of mitochondria').

The importance of matching of carbon fuel inflow and TCA cycle activity has been replicated in a study that examined metabolic changes in the heart in response to heart failure or physical activity[95]. In heart failure there was an elevation of lactate and acylcarnitines with a reduction in TCA cycle intermediates. In contrast, exercised hearts showed decreases in both acylcarnitines as well as TCA cycle intermediates. The former result suggests accumulation of carbon fuel which is not able to be cleared by a slowing TCA cycle. The latter suggests increased consumption of carbon fuel because of higher TCA cycle activity. These findings highlight a role for increased activity of the TCA cycle brought about by sustained aerobic training, thereby improving VO2 levels and linking TCA cycle activity to cardiorespiratory fitness.

Another emerging concept is the importance of metabolic flexibility. Healthy hearts are able to switch fuel use patterns in response to available supply and immediate energy demand[98]. Reduced metabolic flexibility is associated with pathologic changes and this can be reversed with exercise interventions[99].

Future work that involves dynamic testing of heart and whole-body fuel use may be an important component of assessing cardiovascular health and response to exercise interventions. The use of metabolomics to assess these responses may represent new frontiers in this field.

Finally, this study is limited by lack of data on the association between physical activity, metabolomics, and skeletal muscle. Skeletal muscle health requires preserved mitochondrial function and energetics while physical activity prevents ageing-related muscle atrophy[100]. Furthermore, older adults who are frail or pre-frail benefit from physical activity which improves their cardiorespiratory fitness, muscle strength, function, and quality of life[101-103]. Studies that have investigated metabolism and physical training have observed changes in genes that normalised towards a younger transcriptomic signature with better mitochondrial function[104]. Therefore, future investigations into muscle metabolism would be important for understanding age-related changes related to physical activity and metabolomics.

I am the principal investigator of the study. My contribution includes obtaining grant funding for this work, setting up the study protocol, recruitment of research participants, obtaining ethical approval, data analyses, and manuscript writing.

## Research Question #3:

## Is there a better measure of cardiovascular health outcome, compared to traditional markers such as body mass index?

**PUBLICATION #5**

**Tan YH, Lim JP, Lim WS, Gao F, Teo LLY, Ewe SH, Keng BMH, Tan RS, Koh WP and Koh AS**

**Obesity in Older Adults and Associations with Cardiovascular Structure and Function.**

**Obes Facts. 2022;15:336-343[105].**

*"Introduction: Body mass index (BMI), despite being widely used as a marker of obesity, fails to fully capture cardiovascular risks as it is an insufficient biomarker of abdominal adiposity, unlike waist circumference (WC). We aimed to characterise associations between BMI and WC with cardiovascular structure and function in older adults. Methods: Among an observational cohort study of a community of older adults, transthoracic echocardiography determined cardiovascular structure and function, while aerobic capacity was determined by peak oxygen uptake (VO2) metrics. The cut-offs for obesity were 27.5kg/m2 for BMI, and >90cm for males and >80cm for females for WC. Results: 970 older adults without cardiovascular disease [mean age 73±4 years, 432 (44%) males], 124 (12.8%) were obese by BMI definition while 347 (35.7%) were obese by WC definition. Inter-definitional agreement was fair (Cohen's κ=0.345). Unlike BMI definition, participants defined as obese by WC were more likely to be women (65% vs 50%, p<.001), older (65±11 vs 63±14 years, p=.007), and had lower handgrip strength (24±0.6 vs 26±0.4 kg, p=0.022). Across BMI categories, high WC was associated with more impaired myocardial relaxation (E/A), and VO2 measurements (all p<0.05). Among those with low BMI, high WC was associated with larger left atrial volumes (p=0.003). WC, but not BMI, was independently associated with E/A (β=-0.114, SE -0.114 ± 0.024, p<0.001) in regression analysis. Conclusion: Waist circumference identified higher prevalence of obesity, possibly related to central adiposity. Across BMI categories, waist circumference identified more adverse measurements in myocardial relaxation, aerobic capacity and left atrial structure."*

Obesity Facts

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# Obesity in Older Adults and Associations with Cardiovascular Structure and Function

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**Keywords**
Obesity · Older adults · Cardiovascular disease · Ageing

**Abstract**
***Introduction:*** Body mass index (BMI), despite being widely used as a marker of obesity, fails to fully capture cardiovascular risks as it is an insufficient biomarker of abdominal adiposity, unlike waist circumference (WC). We aimed to characterize associations between BMI and WC with cardiovascular structure and function in older adults. ***Methods:*** Among an observational cohort study of a community of older adults, transthoracic echocardiography determined cardiovascular structure and function, while aerobic capacity was determined by peak oxygen uptake ($VO_2$) metrics. The cutoffs for obesity were 27.5 kg/m$^2$ for BMI, and >90 cm for males and >80 cm for females for WC. ***Results:*** Of 970 older adults without cardiovascular disease (mean age 73 ± 4 years, 432 [44%] males), 124 (12.8%) were obese by BMI definition while 347 (35.7%) were obese by WC definition. Interdefinitional agreement was fair (Cohen's κ = 0.345). Unlike the BMI definition, participants defined as obese by WC were more likely to be women (65% vs. 50%, $p < 0.001$), older (65 ± 11 vs. 63 ± 14 years, $p = 0.007$), and had lower handgrip strength (24 ± 0.6 vs. 26 ± 0.4 kg, $p = 0.022$). Across BMI categories, high WC was associated with more impaired myocardial relaxation (E/A), and $VO_2$ measurements (all $p < 0.05$). Among those with low BMI, high WC was associated with larger left atrial (LA) volumes ($p = 0.003$). WC, but not BMI, was independently associated with E/A (β = −0.114, SE −0.114 ± 0.024, $p < 0.001$) in regression analysis. ***Conclusion:*** WC identified a higher prevalence of obesity, possibly related to central adiposity. Across BMI categories, WC identified more adverse measurements in E/A, aerobic capacity, and LA structure. ***Trial Registration:*** ClinicalTrials.gov Identifier: NCT02791139.



## Introduction

Obesity and ageing are major health challenges of the 21st century. Obesity increases the risk of death from any cause and from cardiovascular disease in adults, while age is a well-established cardiovascular risk factor. Although the body of evidence indicates that obese older subjects are prone to cardiovascular morbidity [1, 2], younger adults have higher relative risks associated with obesity

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than older adults, based on body weight definitions of obesity [3]. This may lead to reduced emphasis on obesity as a risk factor among older adults [4]. However, body weight among older adults reflects a combination of overall health status and processes of aging-induced weight loss, such as sarcopenia [5]. This may explain lower relative risks associated with body mass index (BMI) definition of obesity among older adults, compared to younger adults. Therefore, the assessment of obesity based on BMI in older adults may inadequately identify older adults at risk of obesity-related cardiovascular disease.

Given the cardiometabolic effects of obesity on cardiovascular risks, waist circumference (WC) on the other hand, may enhance assessments of obesity among older adults. As a marker of central adiposity, measurement of WC is not influenced by limb sarcopenia, which is relevant among older adults with age-related sarcopenia. In addition, older adults with obesity have been recognized as a distinct metabolic phenotype (compared to older adults without obesity) that is associated with higher risks of cardiovascular disease [6]. Hypothetically, WC may have added value in identifying older adults with more adverse phenotypic alterations in cardiovascular structure and function, compared to BMI. Accordingly, we aimed to compare the relative prevalence and factors associated with obesity defined by WC vis-à-vis BMI, and to characterize their associations with cardiovascular structure and function in older adults without cardiovascular disease.

## Methods

### *Study Population*

The subjects were recruited from the Cardiac Ageing Study (CAS) [7], a prospective study initiated in 2014 that examines characteristics and determinants of cardiovascular function in elderly adults. CAS participants were recruited from the prospective, population-based cohort, the Singapore Chinese Health Study [8] and directly from the local community. The current study sample consisted of men and women who participated in the baseline CAS 2014–2017 examination who had no self-reported history of physician-diagnosed cardiovascular disease (such as coronary heart disease, atrial fibrillation), stroke, or cancer. Written informed consent was obtained from participants upon enrolment. The SingHealth Centralised Institutional Review Board (CIRC/2014/628/C) had approved the study protocol.

### *Data Acquisition*

All participants were examined and interviewed on one study visit by trained study coordinators. Participants completed a standardized questionnaire that included medical history and coronary risk factors. Hypertension was defined by current use of antihypertensive drugs or physician-diagnosed hypertension. Diabetes mellitus was defined by the current use of antidiabetic agents or physician-diagnosed diabetes mellitus. Dyslipidemia was defined by the current use of lipid-lowering agents or physician-diagnosed dyslipidemia. Smoking history was defined as ever smokers (former or current smokers) or never smokers. BMI was calculated as weight in kilograms divided by the square of height in meters. Sinus rhythm status was ascertained by resting electrocardiogram. Clinical data were obtained on the same day as assessment of echocardiography and serum collection. WC was obtained 2.5 cm above the umbilicus, an anatomical landmark associated with abdominal fat mass measured by dual-energy *X*-ray absorptiometry [9].

**Table 1.** Prevalence of obesity based on BMI versus WC

| Definition | Subjects, *n* (%) | |
|---|---|---|
| | nonobese | obese |
| BMI, ≥27.5 kg/m$^2$ | 846 (87.2) | 124 (12.8) |
| WC | | |
| >90 cm in males<br>>80 cm in females | 623 (64.3) | 347 (35.7) |

We compared two definitions of obesity, namely: (1) BMI cut-off of 27.5 kg/m$^2$ as recommended by the World Health Organization for Asian populations [10] and (2) WC cut-offs of >90 cm for males and >80 cm for females, as recommended by the International Diabetes Federation Consensus Worldwide Definition of the Metabolic Syndrome [11]. Handgrip strength was measured from each participant using the Takei hand grip dynamometer (Model TKK5401 Grip D) and following standard protocols. Participants were instructed to stand upright with their arms let down naturally. The handgrip dynamometer was held with the indicator facing outwards, and the grip width was adjusted so that the second joint of the pointing finger made a right angle at the dynamometer. Participants were then instructed to clasp the grip with full force. Measurements obtained were recorded to the nearest 0.1 kg. Two trials were performed for each hand, starting with the right hand. Only the highest value obtained from each hand was used. Overall handgrip strength was calculated as the mean of the maximum left-hand and right-hand grip strength measurements.

Echocardiography was performed using ALOKA α10 with a 3.5-MHz probe. In each subject, standard echocardiography, which included 2-D, M-mode, pulse Doppler and tissue Doppler imaging, was performed in the standard parasternal and apical (apical 4-chamber, apical 2-chamber, and apical long) views, and three cardiac cycles were recorded. Left ventricular ejection fraction, left atrial (LA) volume, and LA volume index (LAVI) were measured. The trans-mitral flow E and A waves with the sample volume position at the tip of the mitral valve leaflets from the apical 4-chamber view were recorded by Doppler echocardiography. Myocardial relaxation (E/A) ratio was computed as a ratio of peak velocity flow in early diastole E (MV E) (m/s) to peak velocity flow in late diastole by atrial contraction A (MV A) (m/s). Pulsed wave tissue Doppler imaging was performed with the sample volume at the septal and lateral annulus from the apical 4-chamber view. The frame rate was between 80 and 100 frames per second. The tissue velocity patterns

**Table 2.** Baseline characteristics based on different definitions of obesity

| Variable | BMI ≥27.5 kg/m² | | | WC >90 cm in males, >80 cm in females | | |
|---|---|---|---|---|---|---|
| | nonobese (*n* = 846) | obese (*n* = 124) | *p* value | nonobese (*n* = 624) | obese (*n* = 347) | *p* value |
| Demographics | | | | | | |
| Age, years | 63.6±13.0 | 62.6±12.3 | 0.404 | 62.7±13.7 | 65.0±11.2 | 0.007 |
| Male, *n* (%) | 374 (44.2) | 58 (46.8) | 0.591 | 312 (50.0) | 120 (34.6) | <0.001 |
| Comorbidities, *n* (%) | | | | | | |
| Ever smoked | 100 (13.2) | 16 (15.5) | 0.521 | 78 (14.3) | 38 (12.1) | 0.352 |
| Hypertension | 280 (33.1) | 67 (54.0) | <0.001 | 196 (31.4) | 151 (43.5) | <0.001 |
| Dyslipidemia | 312 (36.9) | 57 (46.0) | 0.052 | 212 (34.0) | 158 (45.5) | <0.001 |
| Diabetes mellitus | 111 (13.1) | 36 (29.0) | <0.001 | 78 (12.5) | 70 (20.2) | 0.001 |
| Blood pressure and pulse | | | | | | |
| Systolic blood pressure, mm Hg | 137±24 | 140±17 | 0.114 | 135±24 | 140±21 | 0.002 |
| Diastolic blood pressure, mm Hg | 74±12 | 78±13 | <0.001 | 74±11 | 76±13 | 0.004 |
| Pulse, beats per minute | 71±12 | 73±12 | 0.207 | 72±12 | 71±12 | 0.229 |
| Physical function: handgrip strength, kg | 25±0.4 | 26±1 | 0.263 | 26±0.4 | 24±0.6 | 0.0218 |
| $VO_2$, mL/kg/min | 36±0.2 | 32±0.6 | <0.0001 | 38±0.2 | 31±0.3 | <0.0001 |

were recorded and expressed as E', and A'. All measurements were measured by the same operator and the measurements were averaged over three cardiac cycles and adjusted by the RR interval. The specific cardiovascular function of interest in this cohort of older adults was E/A properties, for which impairments in E/A, would suggest myocardial ageing [12]. E/A was defined by ratio of peak velocity flow in MV E to peak velocity flow in late diastole by MV A, also referred to as the E/A ratio. MV E refers to the peak velocity of blood flow during early diastole from the left atrium into the left ventricle, where blood flows passively into the left ventricle during relaxation. MV A refers to the peak velocity of blood flow into the left ventricle in late diastole due to contraction of the left atrium. The echocardiography readers were blinded to the obesity status of the participants. We used a validated non-exercise prediction model comprising of physical activity questionnaire to estimate peak oxygen uptake ($VO_2$) milliliter/kilogram/minute (mL/kg/min) [13, 14], also previously used in this cohort [15].

### Statistics

Clinical characteristics are presented as means and standard deviations for continuous data and frequency and percentage for categorical data. We determined agreement between BMI and WC definitions using Cohen's kappa. We compared demographics, clinical characteristics, and echocardiographic characteristics between nonobese and obese subjects based on either BMI or WC definitions. The Student's *t* test was used for continuous data and the $\chi^2$ test was used for categorical data. Multiple linear regression analysis was subsequently performed to ascertain the relationship of cardiovascular structure and function to BMI and WC definitions, respectively. Variability of cardiovascular structure and function across BMI group and WC group were displayed in the error bar charts with standard error.

All statistical analyses were performed using STATA 15 (College Station, TX, USA). For all analyses, a two-tailed *p* value of <0.05 was considered statistically significant.

## Results

### *Obesity Definitions*

Among 970 participants, 124 (12.8%) were defined as obese by the BMI definition, while 347 (35.7%) were defined as obese by the WC definition (Table 1). Inter-definitional agreement was fair between BMI and WC (Cohen's $\kappa = 0.345$).

Based on both definitions of BMI and WC, hypertension (54% vs. 33%; $p < 0.001$ and 44% vs. 31%; $p < 0.001$) and diabetes mellitus (29% vs. 13%; $p < 0.001$ and 20% vs. 13%; $p = 0.001$) were more prevalent among those defined as obese (Table 2). However, WC identified more women (65% vs. 50%; $p < 0.001$), older participants (65 ± 11 vs. 63 ± 14 years; $p = 0.007$) and dyslipidemic (46% vs. 34%; $p < 0.001$) participants as obese. Systolic blood pressure was also significantly higher (140 ± 21 vs. 135 ± 24 mm Hg; $p = 0.002$) in obese versus nonobese participants defined by WC. Based on BMI, gender, age, dyslipidemia, and systolic blood pressure were not significantly different between obese and nonobese participants (Table 2). Participants defined as obese by WC definition had lower hand grip strength (24.2 vs. 25.9, $p = 0.022$) compared to nonobese. On the other hand, participants defined as obese by

**Table 3.** Key echocardiographic characteristics

| | Nonobese | | Obese | | *p* value |
|---|---|---|---|---|---|
| | mean ± SD | 95% conf. interval | mean ± SD | 95% conf. interval | |
| BMI definition | | | | | |
| Left atrial diameter, cm | 3.50±0.55 | 3.46–3.53 | 3.94±0.53 | 3.84–4.04 | <0.001 |
| LAVI (mL/m²) | 20.5±7.43 | 20.0–21.1 | 22.3±7.90 | 20.8–23.8 | 0.020 |
| LVEF (%) | 72.6±8.3 | 72.0–73.1 | 71.1±9.7 | 69.3–72.8 | 0.074 |
| Peak velocity flow in MV E peak (m/s) | 0.74±0.17 | 0.73–0.75 | 0.73±0.18 | 0.70–0.76 | 0.591 |
| Peak velocity flow in late diastole by MV A peak (m/s) | 0.72±0.21 | 0.71–0.73 | 0.78±0.19 | 0.75–0.82 | 0.003 |
| Ratio MV E peak: MV A peak | 1.13±0.46 | 1.09–1.16 | 0.98±0.35 | 0.91–1.04 | <0.001 |
| WC definition | | | | | |
| Left atrial diameter, cm | 3.46±0.54 | 3.42–3.50 | 3.73±0.58 | 3.66–3.79 | <0.001 |
| LAVI (mL/m²) | 20.0±6.9 | 19.5–20.6 | 22.1±8.3 | 21.1–23.0 | <0.001 |
| LVEF (%) | 72.4±8.4 | 71.7–73.0 | 72.3±8.7 | 71.4–73.3 | 0.935 |
| Peak velocity flow in MV E peak (m/s) | 0.74±0.17 | 0.73–0.76 | 0.73±0.17 | 0.71–0.75 | 0.279 |
| Peak velocity flow in late diastole by MV A peak (m/s) | 0.70±0.21 | 0.68–0.72 | 0.77±0.19 | 0.75–0.79 | <0.001 |
| Ratio MV E peak: MV A peak (E/A) | 1.17±0.49 | 1.13–1.21 | 1.00±0.37 | 0.96–1.04 | <0.001 |

LVEF, left ventricular ejection fraction; SD, standard deviation.

**Table 4.** Multivariate regression model for E/A ratio

| Variables | Obesity based on BMI | | | | Obesity based on WC | | | |
|---|---|---|---|---|---|---|---|---|
| | adjusted $R^2$ | standard coefficient (β) | std. Error | *p* value | adjusted $R^2$ | standard coefficient (β) | std. Error | *p* value |
| Hypertension | 0.139 | −0.288 | −0.288±0.031 | <0.001 | 0.455 | −0.036 | −0.036±0.028 | 0.198 |
| Diabetes mellitus | | −0.156 | −0.156±0.042 | <0.001 | | −0.032 | −0.032±0.034 | 0.035 |
| BMI, kg/m² | | −0.059 | −0.059±0.043 | 0.168 | | | | |
| WC, cm | | | | | | −0.114 | −0.114±0.024 | <0.001 |
| Dyslipidemia | | | | | | −0.032 | −0.032±0.027 | 0.230 |
| Age, years | | | | | | −0.021 | −0.021±0.0001 | <0.001 |
| Female | | | | | | 0.037 | 0.037±0.023 | 0.107 |

BMI definition had similar hand grip strength (26.4 vs. 25.2, $p = 0.26$) compared to nonobese. Participants defined as obese by either WC or BMI definitions, had lower $VO_2$, compared to nonobese participants (Table 2).

*Cardiovascular Structure and Function Based on Obesity Definitions*

In general, participants who were defined by both BMI and WC as obese had larger left ventricular dimensions (online suppl. Table A; for all online suppl. material, see www.karger.com/doi/10.1159/000521729). Participants who were defined by BMI as obese had significantly lower E/A ratio compared to those who were not obese (1.13 ± 0.46 vs. 0.98 ± 0.35; $p < 0.001$). Similarly, participants who were defined by WC as obese had significantly lower E/A ratio compared to those who were not obese (1.17 ± 0.49 vs. 1.00 ± 0.37; $p < 0.001$) (Table 3). Left atrial size was also significantly larger in obese individuals in both the BMI group (3.94 ± 0.53 vs. 3.50 ± 0.55; $p < 0.001$) and WC group (3.73 ± 0.58 vs. 3.46 ± 0.54; $p < 0.001$). The LAVI was also found to be significantly higher in obese individuals in both the BMI (22.3 ± 7.90 vs. 20.5 ± 7.43; $p = 0.020$) and WC (22.1 ± 8.3 vs. 20.0 ± 6.9; $p < 0.001$) groups. Left ventricular ejection fraction percentage was over 70% and not significantly different in both obese and nonobese for both BMI (72.6 ± 8.3 vs. 71.1 ± 9.7; $p = 0.074$) and WC (72.4 ± 8.4 vs. 72.3 ± 8.7; $p = 0.935$) (Table 3) (online suppl. Table A).

However, across high or low BMI categories, high WC was associated with more adverse mean E/A and $VO_2$

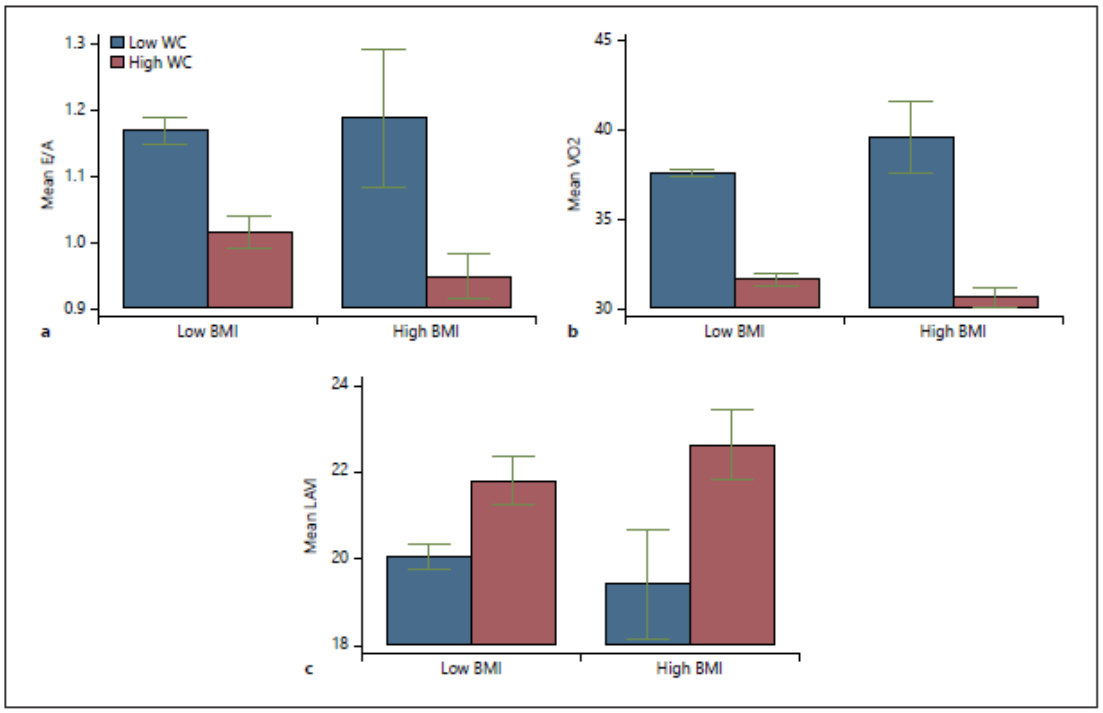


**Fig. 1.** Cardiovascular function and structure by BMI and WC. **a** E/A ratio (mean and standard error) by BMI and WC: across BMI categories, mean E/A was lower among those with high WC. *blue (mean E/A 1.17) versus red (mean E/A 1.01) (low BMI); $p < 0.0001$; *blue (mean E/A 1.19) versus red (mean E/A 0.95) (high BMI); $p = 0.019$. **b** $VO_2$ (mean and standard error) by BMI and WC. Mean $VO_2$ was lower among those with high WC *blue (mean $VO_2$ 37.7) versus red (mean $VO_2$ 31.7) (low BMI); $p < 0.0001$; *blue (mean $VO_2$ 39.7) versus red (mean $VO_2$ 30.6) (high BMI); $p < 0.0001$. **c** LAVI (mean and standard error) by BMI and WC. Mean LAVI was larger among those with high WC despite low BMI. *blue (mean LAVI 20.0) versus red (mean LAVI 21.8) (low BMI); $p = 0.003$; *blue (mean LAVI 19.4) versus red (mean LAVI 22.6) (high BMI); $p = 0.18$.

measurements (Fig. 1a, b). Among those low BMI, high WC was associated with more adverse mean LAVI (Fig. 1c).

Multiple linear regression analysis was performed in BMI and WC groups to assess association of the E/A ratio with obesity status after adjustment for significant covariates (Table 4). Adjusted $R^2$ value was 13.9% and 45.5% for BMI and WC groups, respectively. When adjusted for hypertension and diabetes mellitus, BMI was not associated with cardiovascular function. In contrast, WC was associated with E/A ($\beta = -0.114$, SE $-0.114 \pm 0.024$, $p < 0.001$), independent of age and diabetes mellitus. With each 1 cm increase in WC, E/A ratio declined by 0.114.

## Discussion

Based on a cohort of older adults, the prevalence of obesity varied depending on the definition used. The prevalence of obesity was higher at 35.7% based on WC, and only 12.8% based on BMI. Although both definitions identified more adverse alterations in cardiovascular structure and function, only WC was independently associated with impaired E/A. Importantly, even within nonobese BMI category, high WC was associated with impairments in E/A, aerobic capacity, and LA structure.

Comparing between definitions of BMI versus WC, WC identified the presence of obesity in adults who were older in age, whereas BMI did not differentiate between

adults with older age. Although both definitions are intrinsically different and are not interchangeable, these prevalence rates highlight the importance of using appropriate definitions of obesity, particularly among older adults with aged biology.

The limited ability of BMI to identify obesity among older adults have been previously appreciated [16]. At extremes of age and weight, BMI has limited utility [17, 18]. Apart from age, older adults have fluctuating body weights, related to ageing or accumulation of systemic illnesses [19, 20]. We observed that those defined as obese by WC had lower hand grip strength, a possible reflection of concomitant muscle sarcopenia. Our findings concur with studies that have found associations between abdominal adiposity and poorer physical outcomes in sarcopenic adults [21]. This adds to the body of evidence that shows inverse associations between muscle strength and adiposity-related obesity markers, particularly among older adults [22–24].

Our findings are novel because they depict distinct associations between WC and cardiovascular structure and function among older adults. WC was linearly associated with impairments in myocardial function, namely E/A, a common early manifestation of myocardial ageing. Left atrial size was larger among the obese as defined by both BMI and WC definitions, a well-recognized risk factor for atrial fibrillation development [25, 26]. While cardiometabolic complications of central adiposity are well established in current literature [27–29], our data provide a clue as to how obesity contributes to cardiovascular dysfunctions that may herald the onset and lead towards cardiovascular disease development.

The obesity-related risks of cardiovascular disease are well established [2, 30]. While our cross-sectional study precludes causal inferences, the alterations in E/A and LA structure, point to specific key alterations in the cardiovascular system that are commonly involved in obesity-related heart failure and ageing, such as heart failure with preserved ejection fraction or atrial fibrillation among older adults. Importantly, we observed adverse alterations in E/A, LA structure, and aerobic capacity, among those with nonobese BMI but defined as obese by WC. Our observations are supported by recent studies that also reported metabolic abnormalities among individuals deemed to have normal BMI [18]. In a small study, women with normal BMI and high body fat percentage had lower resting metabolic rate and oxygen consumption, when compared to women with normal BMI and no excess in body fat percentage [31]. In a clinical study of heart failure patients, lean-fat patients with high waist to hip ratio and low BMI, had the worst outcomes at 1-year for heart failure hospitalization or mortality [32]. These observations should prompt intense efforts to address the early subclinical risks of atrial or ventricular dysfunction in older adults defined as obese by WC but lean by BMI.

From a clinical perspective, these results serve to emphasize the use of WC in addition to BMI, as a routine practice, particularly among older adults as well as among those defined as nonobese by BMI definition [23]. This may imply greater search or attention for cardiovascular dysfunction among those with high WC, in appropriate healthcare settings. In addition, therapeutic strategies against obesity may use these cardiovascular structural and functional features as targets useful for monitoring response to therapies, to reduce burdens of central obesity-related cardiovascular disease [33–35].

We acknowledge limitations in our study. In the absence of body fat measurements, the use of WC may only represent an incomplete measure of body fat composition. However, WC is not interchangeable with body fat [5]. Furthermore, for purposes of cardiovascular risk assessment, WC is an accepted marker of central adiposity [36]. Our findings are based on Asian older adults, utilizing obesity cut-offs based on prior Asian data. Hence our findings may not be extrapolated to cohorts of non-Asian descent. Importantly, we recognize that BMI may not be an accurate measure of obesity for Asians. The Asian phenotype of obesity comprises of higher proportions of visceral fat in the central abdominal regions ("central obesity") compared to Western populations [37–40]. Similar studies from other cohorts would be necessary to confirm our observations and improve generalizability. The observational study design does not imply causality between markers of obesity and cardiovascular function. Adaptive versus pathogenic responses cannot be differentiated based on this clinical study design. We did not correct for details such as medication data hence effects arising from medication treatment are unknown. This is a low-risk community cohort, hence marginal values in some of the observed measurements may reflect underestimation rather than overestimation of clinical significance. Even so, the large study sample provided reasonable sample power.

## Conclusion

The prevalence of obesity varied depending on the definition used. WC identified higher prevalence of obesity, possibly related to central adiposity. Across BMI catego-

ries, WC identified more adverse measurements in E/A, aerobic capacity, and LA structure. WC may better characterize the impact of obesity on cardiovascular ageing.


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## Statement of Ethics

Written informed consent was obtained from the participants upon enrolment. The SingHealth Centralised Institutional Review Board (CIRC/2014/628/C) had approved the study protocol.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.


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## Author Contributions

A.S. Koh, J.P. Lim, and W.S. Lim conceptualized and designed the study. Y.H. Tan, B.M.H. Keng, F. Gao, L.L.Y. Teo, R.S. Tan, S.H. Ewe, and W.P. Koh performed data acquisition, analysis, and interpretation. Y.H. Tan, A.S. Koh, F. Gao, and W.S. Lim prepared the manuscript and figures.

## Data Availability Statement

The data generated or analyzed are included in this article and or online supplementary files. Further inquiries can be directed to the corresponding author.

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**Commentary:**

1. **Body mass index is an imperfect metric of cardiovascular health measurement for older adults**

We undertook this work because the traditional anthropometric metric of cardiovascular health, body mass index (BMI) is imperfect for older adults. The limited ability of BMI to identify obesity among older adults have been previously appreciated[106]. At extremes of age and weight, BMI has limited utility[107, 108]. Apart from age, older adults have fluctuating body weights, related to ageing or accumulation of systemic illnesses[109, 110].

Furthermore, numerous physiological changes in muscle, fat and bone occurs during ageing. A key geriatric syndrome that occurs with ageing is sarcopenia, which is the progressive and generalised loss of muscle mass and function with advancing age[111]. With progressive ageing, skeletal muscle fibre size and number decreases linearly at a rate of 3-5% per decade, accelerating up to 30-40% after the fifth decade of life[112]. Sarcopenia leads to poor muscle strength, aerobic capacity, and adverse outcomes such as falls, disability, reductions in quality of life, and higher mortality[113, 114].

Therefore, body weight among older adults reflects a combination of overall health status and processes of ageing-induced weight loss, such as sarcopenia. This may inadvertently be reflected as lower relative risks associated with body mass index definition of obesity among older adults, compared to the effect of BMI on younger adults[115, 116]. The assessment of obesity based on BMI in older adults may inadequately identify older adults at risk of obesity-related cardiovascular disease. Given the cardiometabolic effects of obesity on cardiovascular risks, waist circumference (WC) on the other hand, may enhance assessments of obesity among older adults. As a marker of central adiposity, measurement of WC is not influenced by limb sarcopenia, which is relevant among older adults with age-related sarcopenia. In addition, older adults with obesity have been recognised as a distinct metabolic phenotype (compared to older adults without obesity) that is associated with higher risks of

cardiovascular disease[117]. Hypothetically, WC may have added value in identifying older adults with more adverse phenotypic alterations in cardiovascular structure and function, compared to BMI.

In this study, we compared how WC differed from BMI in characterizing cardiovascular structure and function in older adults without cardiovascular disease. Subjects were prospectively recruited from the local community and consisted of men and women who had no self-reported history of physician-diagnosed cardiovascular disease (such as coronary heart disease, atrial fibrillation), stroke, or cancer. All participants were examined and interviewed on one study visit by trained study coordinators. Participants completed a standardised questionnaire that included medical history and coronary risk factors. Hypertension was defined by current use of antihypertensive drugs or physician-diagnosed hypertension. Diabetes mellitus was defined by the current use of antidiabetic agents or physician-diagnosed diabetes mellitus. Dyslipidaemia was defined by the current use of lipid-lowering agents or physician-diagnosed dyslipidaemia. Smoking history was defined as ever smokers (former or current smokers) or never smokers. BMI was calculated as weight in kilograms divided by the square of height in meters. Sinus rhythm status was ascertained by resting electrocardiogram.

Clinical data were obtained on the same day as assessment of echocardiography. WC was obtained 2.5 cm above the umbilicus, an anatomical landmark associated with abdominal fat mass measured by dual-energy X-ray absorptiometry[118]. We compared two definitions of obesity, namely: (a) BMI cutoff of 27.5 $kg/m^2$ as recommended by the World Health Organization for Asian populations[119] and (b) WC cut-offs of >90 cm for males and >80 cm for females, as recommended by the International Diabetes Federation Consensus Worldwide Definition of the Metabolic Syndrome[120]. Handgrip strength was measured from each participant using the Takei hand grip dynamometer (Model TKK5401 Grip D) and following standard protocols. Participants were instructed to stand upright with their arms let down naturally. The handgrip dynamometer was held with the indicator facing outwards, and the grip width was adjusted so that the second joint of the pointing finger made a right angle at the dynamometer. Participants were then instructed to clasp the grip with full force. Measurements obtained were recorded to the nearest 0.1 kg. Two trials were performed for each hand, starting with the right hand. Only the

highest value obtained from each hand was used. Overall handgrip strength was calculated as the mean of the maximum lefthand and right-hand grip strength measurements.

Echocardiography was performed using ALOKA α10 with a 3.5- MHz probe. In each subject, standard echocardiography, which included 2-D, M-mode, pulse Doppler and tissue Doppler imaging, was performed in the standard parasternal and apical (apical 4-chamber, apical 2-chamber, and apical long) views, and three cardiac cycles were recorded. Left ventricular ejection fraction, left atrial (LA) volume, and LA volume index (LAVI) were measured. The trans-mitral flow E and A waves with the sample volume position at the tip of the mitral valve leaflets from the apical 4-chamber view were recorded by Doppler echocardiography. Myocardial relaxation (E/A) ratio was computed as a ratio of peak velocity flow in early diastole E (MV E) (m/s) to peak velocity flow in late diastole by atrial contraction A (MV A) (m/s). Pulsed wave tissue Doppler imaging was performed with the sample volume at the septal and lateral annulus from the apical 4-chamber view. The frame rate was between 80 and 100 frames per second. The tissue velocity patterns were recorded and expressed as E', and A'. All measurements were measured by the same operator and the measurements were averaged over three cardiac cycles and adjusted by the RR interval. The specific cardiovascular function of interest in this cohort of older adults was E/A properties, for which impairments in E/A, would suggest adverse myocardial ageing[121]. E/A was defined by ratio of peak velocity flow in MV E to peak velocity flow in late diastole by MV A, also referred to as the E/A ratio. MV E refers to the peak velocity of blood flow during early diastole from the left atrium into the left ventricle, where blood flows passively into the left ventricle during relaxation. MV A refers to the peak velocity of blood flow into the left ventricle in late diastole due to contraction of the left atrium. The echocardiography readers were blinded to the obesity status of the participants.

We used a validated non-exercise prediction model comprising of physical activity questionnaire to estimate peak oxygen uptake (VO2) millilitre/kilogram/minute (mL/kg/min)[86], also previously used in this cohort[85].

Clinical characteristics are presented as means and standard deviations for continuous data and frequency and percentage for categorical data. We determined agreement between BMI and WC definitions using Cohen's kappa. We compared demographics, clinical characteristics, and echocardiographic characteristics between non-obese and obese subjects based on either BMI or WC definitions. The student's t test was used for continuous data and the $\chi 2$ test was used for categorical data. Multiple linear regression analysis was subsequently performed to ascertain the relationship of cardiovascular structure and function to BMI and WC definitions, respectively. Variability of cardiovascular structure and function across BMI group and WC group were displayed in the error bar charts with standard error. All statistical analyses were performed using STATA 15 (College Station, TX, USA). For all analyses, a two-tailed p value of <0.05 was considered statistically significant.

Among 970 participants, 124 (12.8%) were defined as obese by the BMI definition, while 347 (35.7%) were defined as obese by the WC definition (Table 1). Inter-definitional agreement was fair between BMI and WC (Cohen's $\kappa = 0.345$).

| **Definition** | **Number of subjects, n (%)** | |
|---|---|---|
| | **Non-obese** | **Obese** |
| Body Mass Index $\geq 27.5 kg/m^2$ | 846 (87.2%) | 124 (12.8%) |
| Waist circumference<br>>90cm in males<br>>80cm in females | 623 (64.3%) | 347 (35.7%) |

***Table 1: Prevalence of Obesity based on Body mass index versus Waist circumference***

Based on both definitions of BMI and WC, hypertension (54% vs. 33%; $p < 0.001$ and 44% vs. 31%; $p < 0.001$) and diabetes mellitus (29% vs. 13%; $p < 0.001$ and 20% vs. 13%; $p = 0.001$) were more prevalent among those defined as obese (Table 2). However, WC identified more women (65% vs. 50%; $p < 0.001$), older participants (65 ± 11 vs. 63 ± 14 years; $p = 0.007$) and dyslipidaemia (46% vs. 34%; $p < 0.001$) participants as obese. Systolic blood pressure was also significantly higher (140 ± 21 vs. 135 ± 24 mm Hg; $p = 0.002$) in obese versus nonobese participants defined by WC. Based on BMI,

gender, age, dyslipidaemia, and systolic blood pressure were not significantly different between obese and nonobese participants. Participants defined as obese by WC definition had lower hand grip strength (24.2 vs. 25.9, p = 0.022) compared to nonobese. On the other hand, participants defined as obese by BMI definition had similar hand grip strength (26.4 vs 25.2, p=0.26) compared to non-obese. Participants defined as obese by either WC or BMI definitions, had lower peak oxygen uptake, compared to non-obese participants. (Table 2).

| **Variable** | **Body mass index ≥27.5 kg/m$^2$** | | | **Waist circumference >90cm in males, >80cm in females** | | |
|---|---|---|---|---|---|---|
| | **Non-obese (n=846)** | **Obese (n=124)** | **p-value** | **Non-obese (n= 624)** | **Obese (n=347)** | **p-value** |
| Demographics | | | | | | |
| Age, years | 63.6 ± 13.0 | 62.6 ± 12.3 | 0.404 | 62.7 ± 13.7 | 65.0 ± 11.2 | 0.007 |
| Male, n (%) | 374 (44.2%) | 58 (46.8%) | 0.591 | 312 (50.0%) | 120 (34.6%) | <0.001 |
| Co-morbidities, n (%) | | | | | | |
| Ever smoked | 100 (13.2%) | 16 (15.5%) | 0.521 | 78 (14.3%) | 38 (12.1%) | 0.352 |
| Hypertension | 280 (33.1%) | 67 (54.0%) | <0.001 | 196 (31.4%) | 151 (43.5%) | <0.001 |
| Dyslipidaemia | 312 (36.9%) | 57 (46.0%) | 0.052 | 212 (34.0%) | 158 (45.5%) | <0.001 |
| Diabetes mellitus | 111 (13.1%) | 36 (29.0%) | <0.001 | 78 (12.5%) | 70 (20.2%) | 0.001 |
| Blood pressure and pulse | | | | | | |
| Systolic blood pressure, mmHg | 137 ± 24 | 140 ± 17 | 0.114 | 135 ± 24 | 140 ± 21 | 0.002 |
| Diastolic blood pressure, mmHg | 74 ± 12 | 78 ± 13 | <0.001 | 74 ± 11 | 76 ± 13 | 0.004 |
| Pulse, beats per minute | 71 ± 12 | 73 ± 12 | 0.207 | 72 ± 12 | 71 ± 12 | 0.229 |
| Physical function: Handgrip strength, kg | 25 ± 0.4 | 26± 1 | 0.263 | 26±0.4 | 24±0.6 | 0.0218 |
| Peak oxygen uptake, ml/kg/min ($V0_2$) | 36±0.2 | 32±0.6 | <0.0001 | 38±0.2 | 31±0.3 | <0.0001 |

***Table 2: Baseline characteristics based on different definitions of obesity***

*Cardiovascular structure and function based on obesity definitions*

In general, participants who were defined by both BMI and WC as obese had larger left ventricular dimensions. Participants who were defined by BMI as obese had significantly lower E/A ratio compared to those who were not obese (1.13 ± 0.46 vs 0.98 ± 0.35; P<0.001). Similarly, participants who were defined by WC as obese had significantly lower E/A ratio compared to those who were not obese (1.17 ± 0.49 vs 1.00 ± 0.37; P<0.001). (Table 3). Left atrial size was also significantly larger in obese individuals in both the BMI group (3.94 ± 0.53 vs 3.50 ± 0.55; P<0.001) and WC group (3.73 ± 0.58 vs 3.46 ± 0.54; P<0.001). The left atrial volume index (LAVI) was also found to be significantly higher in obese individuals in both the BMI (22.3 ± 7.90 vs 20.5 ± 7.43; P=0.020) and WC (22.1 ± 8.3 vs 20.0 ± 6.9; P<0.001) groups. Left ventricular ejection fraction percentage was over 70% and not significantly different in both obese and non-obese for both BMI (72.6 ± 8.3 vs 71.1 ± 9.7; P=0.074) and WC (72.4 ± 8.4 vs 72.3 ± 8.7; P=0.935) (Table 3).

**Body mass index definition**

| | Non-obese | | Obese | | |
|---|---|---|---|---|---|
| | **Mean ± SD** | **95% conf. interval** | **Mean ± SD** | **95% conf. interval** | **p-value** |
| Left atrial diameter, cm | 3.50 ± 0.55 | 3.46-3.53 | 3.94 ± 0.53 | 3.84-4.04 | <0.001 |
| Left atrial volume index (ml/m$^2$) | 20.5 ± 7.43 | 20.0-21.1 | 22.3 ± 7.90 | 20.8-23.8 | 0.020 |
| Left ventricular ejection fraction (LVEF) (%) | 72.6 ± 8.3 | 72.0-73.1 | 71.1 ± 9.7 | 69.3-72.8 | 0.074 |
| Peak velocity flow in early diastole E (MV E peak), m/s | 0.74 ± 0.17 | 0.73-0.75 | 0.73 ± 0.18 | 0.70-0.76 | 0.591 |
| Peak velocity flow in late diastole by atrial contraction A (MV A peak), m/s | 0.72 ± 0.21 | 0.71-0.73 | 0.78 ± 0.19 | 0.75-0.82 | 0.003 |
| Ratio MV E peak: MV A peak | 1.13 ± 0.46 | 1.09-1.16 | 0.98 ± 0.35 | 0.91-1.04 | <0.001 |

**Waist circumference definition**

| | Non-obese | | Obese | | |
|---|---|---|---|---|---|
| | **Mean ± SD** | **95% conf. interval** | **Mean ± SD** | **95% conf. interval** | **p-value** |

| | | | | | |
|---|---|---|---|---|---|
| Left atrial diameter, cm | 3.46 ± 0.54 | 3.42-3.50 | 3.73 ± 0.58 | 3.66-3.79 | <0.001 |
| Left atrial volume index (ml/m$^2$) | 20.0 ± 6.9 | 19.5-20.6 | 22.1 ± 8.3 | 21.1-23.0 | <0.001 |
| Left ventricular ejection fraction (LVEF) (%) | 72.4 ± 8.4 | 71.7-73.0 | 72.3 ± 8.7 | 71.4-73.3 | 0.935 |
| Peak velocity flow in early diastole E (MV E peak), m/s | 0.74 ± 0.17 | 0.73-0.76 | 0.73 ± 0.17 | 0.71-0.75 | 0.279 |
| Peak velocity flow in late diastole by atrial contraction A (MV A peak), m/s | 0.70 ± 0.21 | 0.68-0.72 | 0.77 ± 0.19 | 0.75-0.79 | <0.001 |
| Ratio MV E peak: MV A peak (E/A) | 1.17 ± 0.49 | 1.13-1.21 | 1.00 ± 0.37 | 0.96-1.04 | <0.001 |

***Table 3: Key echocardiographic characteristics***

However, across high or low BMI categories, high WC was associated with more adverse mean E/A and V02 measurements. (Figure 1a, 1b). Among those low BMI, high WC was associated with more adverse mean LAVI (Figure 1c).

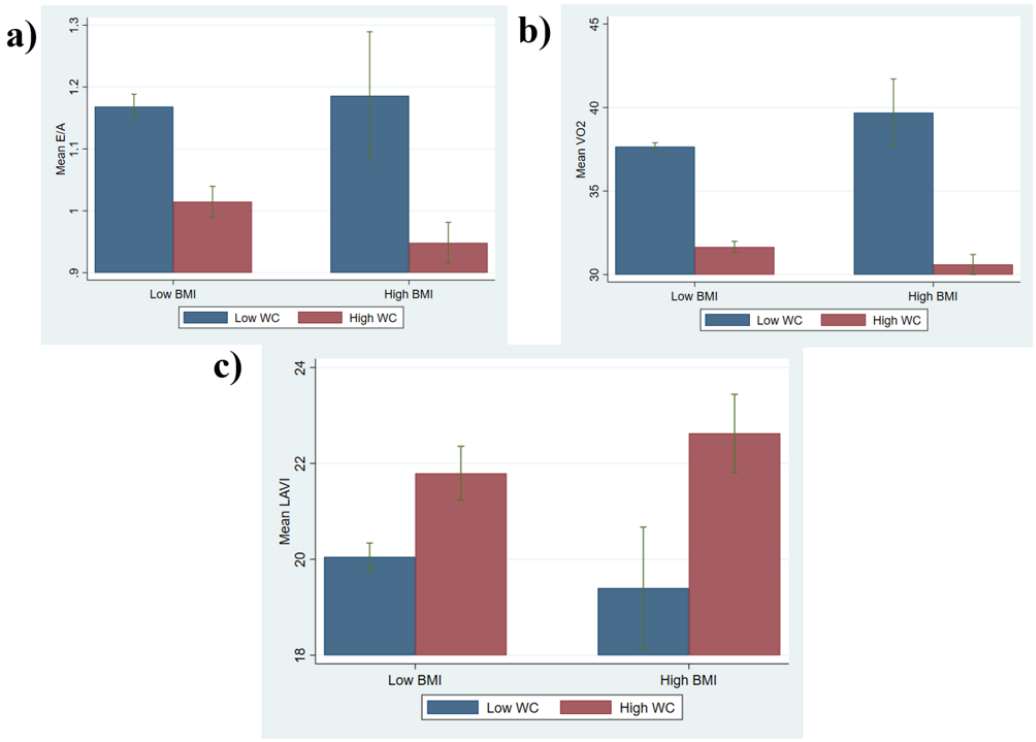


***Figure 1a,b,c: Distribution of myocardial relaxation (E/A), aerobic capacity (VO2) and left atrial volume index (LAVI) based on BMI and WC.***

Multivariable linear regression analysis was performed in BMI and WC groups to assess association of the E/A ratio with obesity status after adjustment for significant co-variates (Table 4). Adjusted $R^2$ value was 13.9% and 45.5% for BMI and WC groups, respectively. When adjusted for hypertension and diabetes mellitus, BMI was not associated with cardiovascular function. In contrast, WC was associated with E/A (β=-0.114, SE -0.114 ± 0.024, p<0.001), independent of age and diabetes mellitus. With each one-centimetre increase in WC, E/A ratio declined by 0.114.

| | **Obesity based on body mass index** | | | | **Obesity based on waist circumference** | | | |
|---|---|---|---|---|---|---|---|---|
| **Variables** | **Adjusted $R^2$** | **Standard coefficient (β)** | **Std. Error** | **p-value** | **Adjusted $R^2$** | **Standard coefficient (β)** | **Std. Error** | **p-value** |
| Hypertension | 0.139 | -0.288 | -0.288 ± 0.031 | <0.001 | 0.455 | -0.036 | -0.036 ± 0.028 | 0.198 |
| Diabetes mellitus | | -0.156 | -0.156 ± 0.042 | <0.001 | | -0.032 | -0.032 ± 0.034 | 0.035 |
| Body mass index, kg/m$^2$ | | -0.059 | -0.059 ± 0.043 | 0.168 | | | | |
| Waist circumference, cm | | | | | | -0.114 | -0.114 ± 0.024 | <0.001 |
| Dyslipidaemia | | | | | | -0.032 | -0.032 ±0.027 | 0.230 |
| Age, years | | | | | | -0.021 | -0.021 ± 0.0001 | <0.001 |
| Female | | | | | | 0.037 | 0.037 ± 0.023 | 0.107 |

***Table 4: Multivariate regression model for E/A ratio***

**2) Waist circumference, rather than body mass index, better characterises the impact of obesity on cardiac ageing**

WC identified higher prevalence of obesity, possibly related to central adiposity. Across BMI categories, WC identified more adverse measurements in myocardial relaxation, aerobic capacity and left atrial structure.

The prevalence of obesity was higher at 35.7% based on WC, and only 12.8% based on BMI. Comparing between definitions of BMI versus WC, WC identified the presence of obesity in adults who were older in age, whereas BMI did not differentiate between adults with older age. Although both definitions are intrinsically different, and are not interchangeable, these prevalence rates highlight the importance of using appropriate definitions of obesity, particularly among older adults with aged biology.

Although both definitions identified more adverse alterations in cardiovascular structure and function, only WC was independently associated with impaired myocardial relaxation. Importantly, even within non-obese BMI category, high WC was associated with impairments in myocardial relaxation, aerobic capacity and left atrial structure.

The limited ability of BMI to identify obesity among older adults have been previously appreciated[106]. At extremes of age and weight, BMI has limited utility. Apart from age, older adults have fluctuating body weights, related to ageing or accumulation of systemic illnesses[107]. We observed that those defined as obese by WC had lower hand grip strength, a possible reflection of concomitant muscle sarcopenia. Our findings concur with studies that have found associations between abdominal adiposity and poorer physical outcomes in sarcopenic adults[122]. This adds to the body of evidence that shows inverse associations between muscle strength and adiposity-related obesity markers, particularly among older adults[123, 124].

Our findings are novel because they depict distinct associations between WC and cardiovascular structure and function among older adults. WC was linearly associated with impairments in myocardial function, namely myocardial relaxation (E/A), a common early manifestation of myocardial ageing. Left atrial size was larger among the obese, a well-recognised risk factor for atrial fibrillation development[125]. While cardiometabolic complications of central adiposity are well established in current literature[126, 127], our data provide a clue as to how obesity contributes to cardiovascular dysfunctions that may herald the onset and lead towards cardiovascular disease development.

The obesity-related risks of cardiovascular disease are well-established[128, 129]. While our cross-sectional study precludes causal inferences, the alterations in myocardial relaxation and left atrial structure, point to specific key alterations in the cardiovascular system that are commonly involved in obesity-related heart failure and ageing, such as heart failure with preserved ejection fraction or atrial fibrillation among older adults. Importantly, we observed adverse alterations in myocardial relaxation, left atrial structure and aerobic capacity, among those with non-obese BMI but defined as obese by WC.

**3) Obesity defined by waist circumference as a marker of cardiometabolic risk**

Our observations are supported by recent studies that also reported metabolic abnormalities among individuals deemed to have normal BMI[108]. In a small study, women with normal BMI and high body fat percentage had lower resting metabolic rate and oxygen consumption, when compared to women with normal BMI and no excess in body fat percentage[130]. In a clinical study of heart failure patients, lean-fat patients with high waist to hip ratio and low BMI, had the worst outcomes at one-year for heart failure hospitalization or mortality[131]. These observations should prompt intense efforts to address the early subclinical risks of atrial or ventricular dysfunction in older adults defined as obese by waist circumference but lean by BMI.

In our earlier study, metabolites associated with poorer cardiorespiratory function in ageing were independent of the effect of body mass index.

| | VO2 low | VO2 high | Total | OR (95% CI) | p-value | *Adjusted OR | p-value |
|---|---|---|---|---|---|---|---|
| ***Amino acids*** | | | | | | | |
| **Ala** | 6.2 (0.3) | 6.1 (0.2) | 6.2 (0.2) | 0.1 (0.03-0.7) | 0.018 | 0.1 (0.01-0.9) | 0.044 |
| **Arg** | 4.7 (0.2) | 4.8 (0.2) | 4.7 (0.2) | 2.1 (0.5-9.0) | 0.34 | | |
| **Asp** | 3.1 (0.3) | 3.1 (0.3) | 3.1 (0.3) | 0.5 (0.2-1.8) | 0.31 | | |
| **Cit** | 3.4 (0.4) | 3.5 (0.4) | 3.5 (0.4) | 1.8 (0.8-4.1) | 0.14 | | |
| **Glu** | 4.6 (0.2) | 4.4 (0.2) | 4.5 (0.2) | 0.03 (0.005-0.1) | <0.0001 | 0.1 (0.01-0.5) | 0.0070 |
| **Gly** | 5.4 (0.2) | 5.5 (0.2) | 5.4 (0.2) | 10.8 (1.8-62.9) | 0.0080 | 5.8 (0.7-46.5) | 0.099 |
| **His** | 4.3 (0.2) | 4.3 (0.2) | 4.3 (0.2) | 1.8 (0.4-8.2) | 0.46 | | |
| **IleLeu** | 5.0 (0.3) | 4.9 (0.3) | 5.0 (0.3) | 0.6 (0.2-2.1) | 0.47 | | |
| **Met** | 3.2 (0.4) | 3.2 (0.4) | 3.2 (0.4) | 1.3 (0.5-3.3) | 0.52 | | |
| **Orn** | 4.5 (0.3) | 4.4 (0.3) | 4.4 (0.3) | 0.3 (0.1-1.0) | 0.049 | 0.4 (0.1-1.8) | 0.24 |
| **Phe** | 4.3 (0.2) | 4.3 (0.2) | 4.3 (0.2) | 0.4 (0.1-2.4) | 0.32 | | |
| **Pro** | 5.5 (0.2) | 5.5 (0.2) | 5.5 (0.2) | 0.3 (0.1-1.1) | 0.069 | | |
| **Ser** | 4.8 (0.2) | 4.8 (0.2) | 4.8 (0.2) | 1.0 (0.2-5.3) | 0.96 | | |
| **Trp** | 3.9 (0.2) | 4.0 (0.3) | 4.0 (0.3) | 3.3 (0.8-13.2) | 0.098 | | |
| **Tyr** | 4.3 (0.3) | 4.2 (0.3) | 4.2 (0.3) | 0.4 (0.1-1.2) | 0.085 | | |
| **Val** | 5.5 (0.3) | 5.4 (0.3) | 5.5 (0.3) | 0.7 (0.2-2.5) | 0.63 | | |

***Table 4: Metabolomic patterns associated with peak oxygen capacity, independent of BMI*** **Age, BMI and diabetes were adjusted.*

In a separate cohort that had investigated associations between waist circumference and metabolic profiles, visceral adiposity was significantly associated with amino acids such as glutamate, glycine, methionine, isoleucine and proline[132]. These metabolites were similarly observed in our cohort (Table 4), associated with poorer peak oxygen uptake in older adults.

I am the principal investigator of the study. My contribution includes obtaining grant funding for this work, setting up the study protocol, recruitment of research participants, obtaining ethical approval, data analyses, and manuscript review.

# RESEARCH QUESTION #4:

## Recognising the need to incorporate multiple biological inputs, would an expansive machine learning (ML) approach help rank key factors that determine healthy cardiovascular health in ageing?

**PUBLICATION #6**

**Loh DR, Yeo SY, Tan RS, Gao F, Koh AS.**

**Explainable Machine-Learning Predictions To Support Personalised Cardiology Strategies. *Eur Heart J Digit Health*. 2022;3:49-55**

*"Aims: A widely practiced intervention to modify cardiac health, the effect of physical activity on older adults is likely heterogeneous. While machine learning (ML) models that combine various systemic signals may aid in predictive modelling, the inability to rationalise predictions at a patient personalised level is a major shortcoming in the current field of ML. Methods and Results: We applied a novel methodology, Shapley Additive Explanations (SHAP), on a dataset of older adults n = 86 (mean age 72±4 years) whose physical activity levels were studied alongside changes in their left ventricular (LV) structure. SHAP was tested to provide intelligible visualization on the magnitude of the impact of the features in their physical activity levels on their LV structure. As proof of concept, using repeated K-cross validation on the train set (n = 68), we found the Random Forest Regressor with the most optimal hyperparameters, which achieved the lowest mean squared error. With the trained model, we evaluated its performance by reporting its mean absolute error and plotting the correlation on the test set (n = 18). Based on collective force plot, individually numbered patients are indicated on the horizontal axis, and each bandwidth implies the magnitude (i.e., effect) of physical parameters (higher in red; lower in blue) towards prediction of their LV structure. Conclusions: As a tool that identified specific features in physical activity that predicted cardiac structure on a per patient level, our findings support a role for explainable ML to be incorporated into personalised cardiology strategies."*

ESC European Society of Cardiology
European Heart Journal Digital Health
https://doi.org/10.1093/ehjdh/ztab096

ORIGINAL ARTICLE

# Explainable machine learning predictions to support personalized cardiology strategies

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**Aims** A widely practiced intervention to modify cardiac health, the effect of physical activity on older adults is likely heterogeneous. While machine learning (ML) models that combine various systemic signals may aid in predictive modelling, the inability to rationalize predictions at a patient personalized level is a major shortcoming in the current field of ML.

**Methods and results** We applied a novel methodology, SHapley Additive exPlanations (SHAP), on a dataset of older adults $n = 86$ (mean age 72 ± 4 years) whose physical activity levels were studied alongside changes in their left ventricular (LV) structure. SHAP was tested to provide intelligible visualization on the magnitude of the impact of the features in their physical activity levels on their LV structure. As proof of concept, using repeated K-cross-validation on the train set ($n = 68$), we found the Random Forest Regressor with the most optimal hyperparameters, which achieved the lowest mean squared error. With the trained model, we evaluated its performance by reporting its mean absolute error and plotting the correlation on the test set ($n = 18$). Based on collective force plot, individually numbered patients are indicated on the horizontal axis, and each bandwidth implies the magnitude (i.e. effect) of physical parameters (higher in red; lower in blue) towards prediction of their LV structure.

**Conclusions** As a tool that identified specific features in physical activity that predicted cardiac structure on a per patient level, our findings support a role for explainable ML to be incorporated into personalized cardiology strategies.



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## Graphical Abstract

**Key question(s)**

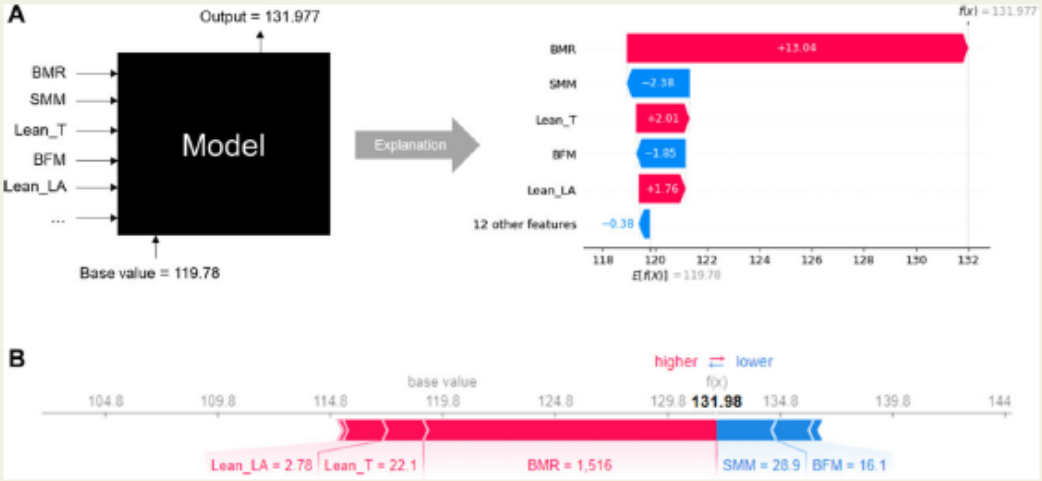
This study explored whether explainable machine learning techniques could identify and explain features in physical activity that predicted cardiac structure on a per patient level for older adults.

**Key finding(s)**

In a proof-of-concept study that included 86 older adults, a novel approach using Shapley Additive Explanations (SHAP) methodology provided intelligible visualization on the magnitude of the impact of the features in their physical activity levels on their cardiac structure.

**Take-home message**

There is practical clinical value in incorporating explainability tools such as SHAP into machine learning prediction.
Interpretability may have a role in enhancing personalized medicine strategies.




**Keywords** Artificial intelligence • Cardiology • Machine learning • Ageing • Physical activity • Explainable • SHAP



## Introduction

Currently, multiple groups are working on developing machine learning (ML) techniques for cardiovascular disease.[1–3] A common theme across this rapidly burgeoning field is the experimental use of heterogeneous methodologies. While the pursuit to fine-tune ML models in disease prediction is an ongoing one, there is far less work on operationalizing these models for future clinical translation.

Backed by power in large datasets present in population-based healthcare, we anticipate immense potential for ML to influence healthcare goals of interest to large population sets. The field of physical activity is a prime example, where strategies personalized to individuals will likely have widespread healthcare impact. Physical activity has an important role in modulating the impact of population ageing on cardiovascular disease as well as ageing-related declines in muscle mass and overall function.[4] However, there is wide interindividual variation in responses to physical activity.[5]

As physical activity is a major modifiable lifestyle factor that can mitigate ageing-related changes in cardiovascular function in conjunction to sarcopenia and frailty, focusing work from ML to personalize physical activity strategies is likely impactful.

In this work, we applied the SHapley Additive exPlanations (SHAP) methodology on a dataset of older adults whose physical activity levels were studied in conjunction with changes in their left ventricular (LV) structure. We hypothesize that intelligent visualization

of physical factors of greatest impact on LV structure by the SHAP approach would identify unique features on a per patient level.

# Materials and methods

## Study population

We studied data from a random pilot sample of human subjects recruited from the Cardiac Ageing Study (CAS),[6] a prospective study initiated in 2014 that examines characteristics and determinants of cardiovascular function in elderly adults. The current study sample consisted of men and women who participated in the baseline CAS 2014–2017 examination who had no self-reported history of physician-diagnosed cardiovascular disease (such as coronary heart disease, atrial fibrillation), stroke, or cancer. Written informed consent was obtained from participants upon enrolment. The SingHealth Centralised Institutional Review Board (CIRC/2014/628/C) had approved the study protocol.

Subjects underwent transthoracic echocardiography. Briefly, echocardiography was performed using ALOKA α10 with a 3.5 MHz probe. In each subject, standard echocardiography, which included 2D, M-mode, pulse Doppler, and tissue Doppler imaging, was performed in the standard parasternal and apical (apical four-chamber, apical two-chamber, and apical long) views, and three cardiac cycles were recorded. Left ventricular ejection fraction and LV mass were measured. From the parasternal long-axis view, LV dimensions were assessed and LV mass was calculated using the Devereux's formula.[7] All measurements were measured by the same operator, and the measurements were averaged over three cardiac cycles and adjusted by the RR interval.

## Machine learning

With the collected data, the participants' physical functional parameters were identified and grouped together as features (Supplementary material online, *Appendix SA*). They were then used to predict the target variable, LV mass. The dataset was randomly divided, with 80% used for training ($n = 68$) and 20% used for testing ($n = 18$). Missing feature data were also replaced with mean values.

The Random Forest (RF) is an ML technique based on a collection of decision trees.[8] Given our small dataset, RF is a suitable choice of model because it can handle large numbers of variables with relatively small numbers of observations.[9] The RF does this by including many trees, in which each tree is generated for a portion of the data which is randomly sampled with replacement. Each tree generates an output and the RF inference is determined according to the aggregate of the output from the different trees. The ability of the RF to deal with a non-linear boundary and the combination of outputs from multiple trees allows the technique to give an accurate output.[8]

In our approach, we used grid search and four-fold cross-validation on the train set to find the optimal RF Regressor, which had the lowest mean validation mean squared error. The final tuned parameters were listed in Supplementary material online, *Table S1*. With the trained model, we evaluated its performance by reporting its mean absolute error and plotting the correlation on the test set.

## Using SHAP to interpret model

SHAP was used as a unified framework to interpret model predictions. Specifically, we used Tree SHAP, a variant of SHAP to provide explanations for the individual predictions made by RF. We created waterfall and individual force plots, where each feature value was visualized as a force that either increases or decreases the base value. Shapley values were aggregated to provide global importance.

# Results

We used RF regression to analyse the dataset and complemented it with SHAP to interpret the output. The objective is to rank variables by local and global importance, for determining LV structure, among a cohort of community older adults involved in physical activity.

The baseline clinical characteristics and cardiovascular measurement of the study population are described in *Table 1*.

Based on the test set (*Figure 1*), there is an observed correlation between the predicted and actual values with $R^2$ value of 0.67. Both curves follow each other closely and an acceptable mean absolute error of 18.917 (<1 SD of 47.704 for the test set distribution). This implies that our RF model is moderately accurate at predicting the LV mass.

Based on the train set (*Figure 2*), basal metabolic rate (BMR) was the most important feature in determining the LV structure due to its greatest average impact on the model output, as indicated by the mean absolute SHAP values. Other features such as appendicular lean mass (ALM) were found to have unimportant as their mean SHAP values were zero. As a more informative alternative, *Figure 3* describes the relationship between the features and their global impact based on the computed SHAP values for each instance. For example, higher BMR contributed to a larger LV mass, showing positive correlation. This is because a high BMR feature value (in red) maps to a higher positive SHAP value, which is equivalent to the positive change in value from the expected LV mass prediction for that observation. On the other hand, a low BMR feature value (in blue) generally maps to a lower SHAP value that falls within the left distribution, where most of them correspond to a negative contribution to the expected output.

Based on the test set (*Figure 4*), the SHAP TreeExplainer visually provides local interpretability to a model's prediction for an individual patient in two related flavours. *Figure 4A* can be thought of as the decomposed version of *Figure 4B*, detailing the model's decision in a sequential manner. This is because each of the feature contribution can be independently calculated using SHAP values and then summed up to give the final prediction. For example, when predicting the LV mass for Patient #6, a BMR feature value of 1516 contributed a corresponding SHAP value of 13.04, resulting in a final predicted LV mass of 132. It can also be observed that the effect of BMR for this patient outweighs other weaker positive factors [e.g. Lean T and arm mass (Lean LA)] and negative factors [e.g. skeletal muscle mass (SMM) and body fat mass (BFM)].

Individual force plots can also be combined to produce stacked SHAP explanations, which can be arranged according to their original ordering (*Figure 5*) or clustering similarity (Supplementary material online, *Figure SA*). Based on the test set, *Figure 5* resembles the line plot for the predicted values in *Figure 1*, where the vertical axis describes the predicted LV mass by the RF Regressor while the horizontal axis shows the original patient ordering. Each band width implies the magnitude (i.e. effect) of physical parameters (higher in red; lower in blue) towards prediction of their LV structure. Again,

**Table 1 Baseline clinical characteristics and cardiovascular measurements of the study population**

| | Study population ($n = 86$) |
|---|---|
| Clinical covariates | |
| Age, years | 72 (4.2) |
| Female sex (%) | 43 (50) |
| Weight, kg | 59.6 (10.7) |
| Systolic blood pressure, mmHg | 150 (37.1) |
| Diastolic blood pressure, mmHg | 73 (10.7) |
| Pulse, beats per minute | 74 (13.0) |
| Physical functional parameters | |
| Skeletal muscle mass, kg | 22.0 (4.6) |
| Body fat mass, kg | 19.3 (6.8) |
| Percentage body fat, % | 31.4 (8.0) |
| Waist–hip ratio | 0.9 (0.06) |
| Fitness score | 66.2 (9.2) |
| Basal metabolic rate, kcal | 1255 (167.2) |
| Arm mass, kg | 2.0 (.5) |
| Trunk mass, kg | 18.3 (3.4) |
| Appendicular lean mass, kg | 16.4 (3.8) |
| Cardiac measurements by echocardiography | |
| Interventricular septum thickness at end-diastole (IVSD) (cm) | 0.8 (0.1) |
| Interventricular septum thickness at end-systole (IVSS) (cm) | 1.2 (0.2) |
| Left ventricular internal diameter end-diastole (LVIDD) (cm) | 4.4 (0.5) |
| Left ventricular internal diameter end-systole (LVIDS) (cm) | 2.4 (0.5) |
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| Aortic diameter (AO), cm | 3.0 (0.5) |
| Left atrium (LA) (cm) | 3.6 (0.6) |
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| Left ventricular fractional shortening (LVFS) (%) | 44 (6.8) |
| Left ventricular mass, g | 119 (42.7) |
| Left atrial volume, mL | 36 (12) |
| Peak velocity flow in early diastole E (MV E peak), m/s | 0.6 (0.1) |
| Peak velocity flow in late diastole by atrial contraction A (MV A peak), m/s | 0.8 (0.2) |
| Ratio MV E peak: MV A peak | 0.9 (0.3) |
| Mitral valve flow deceleration time (MV DT) (ms) | 200 (31) |
| Pulmonary artery systolic pressure (PASP) (mmHg) | 27 (6.4) |

Standard deviations are in parentheses.

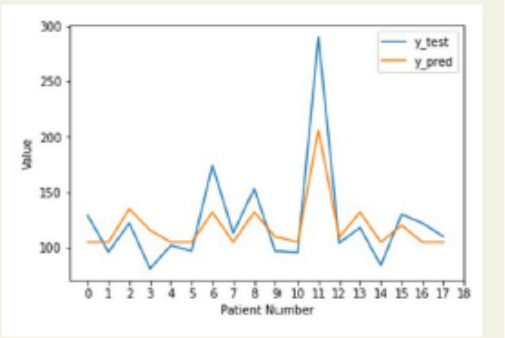


**Figure 1** Line plots comparing the true and predicted left ventricular mass by the Random Forest Regressor on the test set.

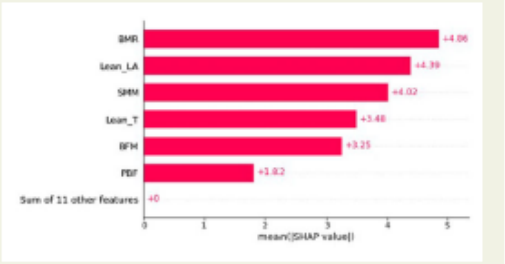


**Figure 2** Bar plot consisting of features sorted by their importance, which is measured as the mean absolute SHapley Additive exPlanations values, within the train set.

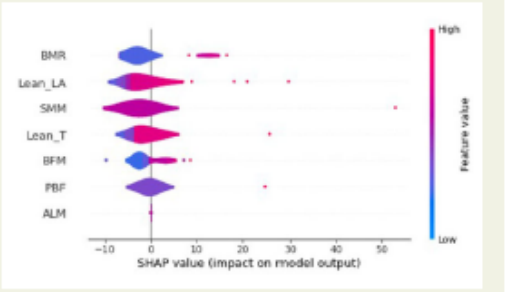


**Figure 3** Summary plot describing the relationship between the value of the feature and the impact on the prediction within the train set. Only the top seven features were displayed.

using Patient #6 as an example, BMR was observed to be the single predominant positive factor on LV mass, outweighing other weaker positive factors and negative factors. In contrast, LV mass in Patient

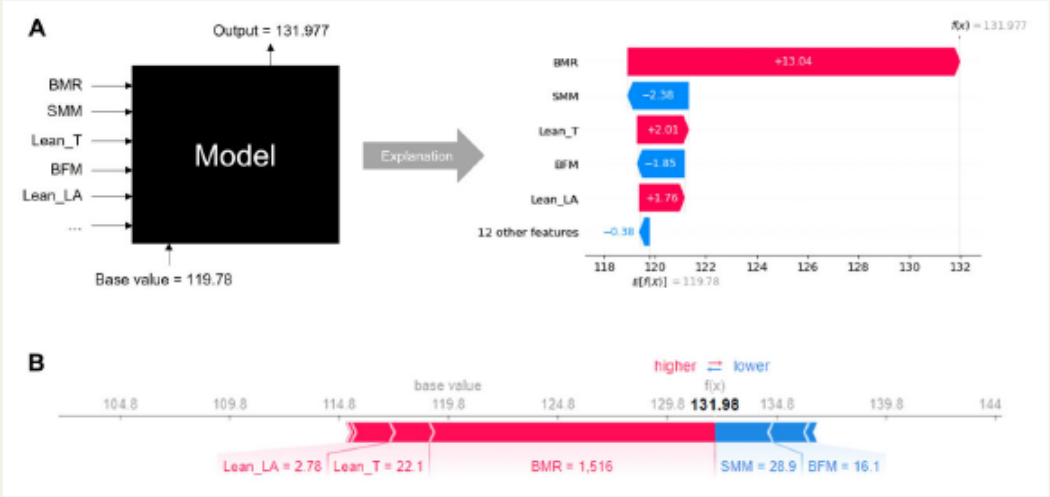


**Figure 4** SHapley Additive exPlanations provides explainability to the predicted left ventricular mass of the black box Random Forest Regressor for Patient #6 in the form of waterfall plot (*A*) and individual force plot (*B*).

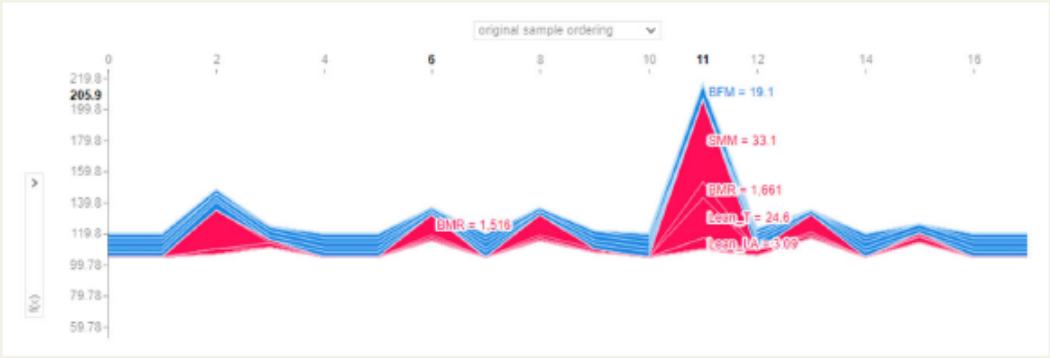


**Figure 5** Collective force plot for test set based on original patient ordering. The plot has been superimposed to show the impact of the physical functional parameters for Patients #6 and #11.

#11 was predicted jointly by several positive factors (e.g. SMM, BMR, Lean T). This suggests that intervening on these prominent physical functional parameters (in red) would more likely improve the cardiac health state of Patient #11, as opposed to Patient #6 who has less deterministic parameters.

A heatmap plot with the same clustering order, yielding the same curve can also be presented (Supplementary material online, *Figure SA*). In both figures, clinicians can see that Patients #6, #8, and #12 were grouped as similar instances (renamed as instances 1, 2, and 3, respectively) due to their comparable features after clustering. The clinicians can therefore infer that these patients in the same subgroup can be characterized as having similarly high BMR as the main contributor to their poor cardiac outcome, which also suggests activities that can lower their BMR may be effective for this group of patients.

## Discussion

In this exploratory work, we demonstrated the utility of SHAP to enhance interpretation of factors associated with physical activity and cardiac structure.

In contrast to vast volumes of work performed on optimizing model accuracy,[1–3] ML work on model interpretability is scarce. However, our work adds to recent work by a handful of others who

recognize the value of SHAP for model interpretation. In the field of cardiology, Lu *et al.*[10] used XGBoost regression in conjunction with SHAP analyses to identify heart failure clinical subtypes based on electronic health records. Their model utilized structured data from electronic health records to aid clinicians in detecting heart failure stages but did not include other clinical information. In our work, we studied clinical parameters in conjunction with patient-specific LV structure and determined the relative importance of patient-specific factors. The use of transthoracic echocardiogram[11] as an imaging test of choice for LV assessment is an added novelty of our work. Similarly, another recent study used SHAP approach to depict electrocardiographic features associated with LV geometry.[12] Taken together, innovative solutions that combine clinical parameters with detailed cardiovascular imaging may represent novel approaches for ML interpretation.

The existing gaps in ML work that are geared towards visual interpretation present fresh opportunities for this field. In a large review comprising of 103 cohorts and over 3 million individuals,[13] most studies in ML only reported the best performing models and evaluation metrics that were suited to their own dataset. While these methods should continue to form the backbone of ML work, stronger emphasis on *interpretability* could further enhance clinical applications. The clinicians also may be able to better corroborate findings across different studies despite the technical heterogeneity (e.g. hyperparameter selection, data partitioning). In this study, we showed that the RF regression model performed well in predicting the LV mass using a set of physical functional parameters, and further demonstrated the use of SHAP as a visualization tool to provide informative plots based on explanations that justify the model's decision.

As a unified framework for interpreting model predictions, SHAP is associated with three key desirable properties, namely local accuracy, missingness and consistency.[14] These properties make SHAP a superior method over other attribution methods such as Local Interpretable Model-Agnostic Explanations (LIME).[15] On a local level, individual force plot and waterfall plot can be created for every instance, where each feature value can be visualized as a force that either increases or decreases the base value (i.e. the average of all predictions). Furthermore, all the individual force plots can also be stacked horizontally to produce a collective force plot and placed side by side according to clustering similarity, allowing clinicians to easily identify groups of similar instances.

As an extension, Shapley values can also be aggregated to provide global interpretability. Global importance can be calculated by summing the absolute Shapley values per feature across the data as a way of quantifying the marginal contribution of each predictor towards the target variable. By sorting the features in decreasing order of importance, the feature importance plot allows clinicians to visualize the most important features that require more attention. It is critical to point out that the implementation of SHAP, which is based on the magnitude of feature attributions, is different from the permutation feature importance, which is based on the decrease in model performance.

SHAP also offers summary plot, which may be more informative as it combines feature importance with feature effects as well as shows the relationship between the value of a feature and its impact on the prediction from a more global perspective. Finally, a heatmap can also be plotted, which allows for data in two dimensions. The variable feature importance is sorted in descending order along the vertical axis and uses hot-to-cold scheme to reflect the features' contributions towards the predictions for the instances that lie on the horizontal axis.

The potential impact of local explanations for ML models is profound. The incorporation of an explainability tool like SHAP into clinical workflow is especially important in overcoming the resistance of adopting such black box models due to the perils of blindly trusting their outputs at face value. Understanding why these algorithms make certain predictions is just as crucial as their accuracy because it facilitates transparency and can assist the clinicians to make more informed decisions. The upshot of this implementation is that patient outcomes may improve. Further research in this area is needed.

Our exploratory work may be limited by a small dataset. However, the goal of this exploration was to determine suitable ML methods to present data in clinically useful ways, rather than on model accuracy. In the area of interpretability, we have confined our results to using SHAP methodology. We acknowledge that there may be other methodology for interpretability, such as LIME,[16] counterfactual fairness,[17] and justification narratives[18] that are available in the wider AI field. However, in our task which requires the measurement of feature importance for the clinicians to interpret, SHAP stands out as the only additive feature attribution method that satisfies the two key properties of consistency and accuracy.[14]

## Conclusion

There appears to be practical clinical value in incorporating explainability tools such as SHAP into ML prediction. Interpretability may have a role in enhancing personalized medicine strategies. With some guidance, the generated SHAP plots are easy to understand with the well-designed colour variations and intuitive labels, even for a layman without any background in ML. The SHAP API is also publicly available and well-documented,[19] hence it can be easily integrated into any user interface that supports python. We hope our work provides the motivation for the medical industry to begin incorporating such explainability tools into their workflow with the overall goal of improving personalized medicine.

## Supplementary material

Supplementary material is available at *European Heart Journal – Digital Health* online.

## Acknowledgements

We thank the staff of the laboratories involved for participating in the conduct of the study.

## Declaration of Helsinki

The authors state that their study complies with the Declaration of Helsinki, that the locally appointed ethics committee has approved

the research protocol and that informed consent has been obtained from the subjects.

## Funding

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**Conflict of interest:** none declared.

## Data availability

Data cannot be shared publicly for ethical reasons due to institutional restrictions.

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**Commentary:**

1) **Use of machine learning as a tool to identify personalised physical activity factors of impact to cardiovascular health of older adults**

This paper tested a method in Artificial Intelligence, known as Explainable Machine Learning, to identify personalised factors related to cardiovascular health state among older adults. Currently, multiple groups are working on developing machine learning techniques for cardiovascular disease[133-135]. Backed by power in large datasets present in population-based healthcare, there is immense potential for machine learning to influence healthcare goals of interest to large population sets. The field of physical activity is a prime example, where strategies personalised to individuals will likely have widespread healthcare impact. Physical activity has an important role in modulating the impact of population ageing on cardiovascular disease as well as ageing-related declines in muscle mass and overall function[136]. However, there is wide interindividual variation in responses to physical activity[137].

As physical activity is a major modifiable lifestyle factor that can mitigate ageing related changes in cardiovascular function in conjunction to sarcopenia and frailty, focusing work from machine learning to personalise physical activity strategies is likely impactful.

In this work, we applied machine learning methodology on a dataset of older adults whose physical activity levels were studied in conjunction with changes in their left ventricular (LV) structure. We hypothesise that intelligent visualization of physical factors of greatest impact on LV structure by the machine learning approach, would identify unique features on a per patient level.

Materials and Methods

*Study Population*

We studied data from a random pilot sample of human subjects recruited from the Cardiac Ageing Study (CAS)[138], a prospective study initiated in 2014 that examines characteristics and determinants of cardiovascular function in elderly adults. The current study sample consisted of men and women who participated in the baseline CAS 2014-2017 examination who had no self-reported history of physician-diagnosed cardiovascular disease (such as coronary heart disease, atrial fibrillation), stroke or cancer. Written informed consent was obtained from participants upon enrolment. The SingHealth Centralised Institutional Review Board (CIRC/2014/628/C) had approved the study protocol.

Subjects underwent transthoracic echocardiography. Briefly, echocardiography was performed using ALOKA α10 with a 3.5 MHz probe. In each subject, standard echocardiography, which included 2-D, M-mode, pulse Doppler and tissue Doppler imaging, was performed in the standard parasternal and apical (apical 4-chamber, apical 2-chamber and apical long) views, and three cardiac cycles were recorded. Left ventricular ejection fraction (LVEF) and left ventricular (LV) mass were measured. From the parasternal long-axis view, left ventricle (LV) dimensions were assessed and LV mass was calculated using the Devereux's formula [139]. All measurements were measured by the same operator, and the measurements were averaged over three cardiac cycles and adjusted by the RR interval.

*Machine Learning*

**Data pre-processing**

Categorical variables are converted into numerical form. Rows with missing data are removed.

**Model Evaluation Metric**

We performed ROC curve analysis and used mean cross-validated ROC AUC as the main metric for model evaluation.

**Comparison of classifiers**

The Random Forest Classifier (RF) is used. Initial comparisons are performed without model tuning, optimisation, and feature selection. Using the Repeated Stratified K-Fold Cross Validation strategy, we assessed the performance of models in predicting cardiac outcome.

**Reproducibility**

Wherever possible, we set all 'random_state' = 1. This ensure that the results from each cross-validation splits and tree-based models are reproducible using the same random state.

With the collected data, the participants' physical functional parameters were identified and grouped together as features (*Supplementary: Appendix A*). They were then used to predict the target variable, LV mass. The dataset was randomly divided, with 80% used for training (n = 68) and 20% used for testing (n = 18). Missing feature data were also replaced with mean values.

| Feature Name | Description |
|---|---|
| **SMM** | Skeletal Muscle Mass |
| **BFM** | Body Fat Mass |
| **PBF** | Percentage Body Fat |
| **WHR** | Waist hip ratio |
| **Fitness score** | Fitness score |
| **BMR** | Basal Metabolic Rate |
| **Lean LA** | Arm mass |
| **Lean T** | Trunk mass |
| **ALM** | Appendicular lean mass |

***Appendix A: Relevant Physical Functional Parameters of the Participants***

The Random Forest (RF) is a machine learning technique based on a collection of decision trees[140]. Given our small dataset, RF is a suitable choice of model because it can handle large numbers of variables with relatively small numbers of observations[141]. The random forest does this by including many trees, in which each tree is generated for a portion of the data which is randomly sampled with replacement. Each tree generates an output, and the random forest inference is determined according to the aggregate of the output from the different trees. The ability of the RF to deal with a non-linear boundary and the combination of outputs from multiple trees allows the technique to give an accurate output[140].

In our approach, we used grid search and four-fold cross validation on the train set to find the optimal RF Regressor, which had the lowest mean validation mean squared error. The final tuned parameters were listed in *Supplementary Table 1*. With the trained model, we evaluated its performance by reporting its mean absolute error and plotting the correlation on the test set.

| Parameter | Value |
|---|---|
| **n_estimators (# of trees)** | 5 |
| **max_depth** | 2 |
| **min_samples_split** | 3 |
| **min_samples_leaf** | 1 |

***Table 1: RF Regressor fine-tuned parameters***

*Using SHAP to Interpret Model*

SHAP was used as a unified framework to interpret model predictions. Specifically, we used Tree SHAP, a variant of SHAP to provide explanations for the individual predictions made by RF. We created waterfall and individual force plots, where each feature value was visualised as a force that either increases or decreases the base value. Shapley values were aggregated to provide global importance.

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| **Female sex (%)** | 43 (50) |
| **Weight, kg** | 59.6 (10.7) |
| **Systolic blood pressure, mm Hg** | 150 (37.1) |

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| **Left ventricular outflow tract (LVOT) (cm)** | 2.1 (0.3) |
| **Aortic Diameter (AO), cm** | 3.0 (0.5) |
| **Left atrium (LA) (cm)** | 3.6 (0.6) |
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| **Left ventricular mass, g** | 119 (42.7) |
| **Left atrial volume, ml** | 36 (12) |
| **Peak velocity flow in early diastole E (MV E peak), m/s** | 0.6 (0.1) |
| **Peak velocity flow in late diastole by atrial contraction A (MV A peak), m/s** | 0.8 (0.2) |
| **Ratio MV E peak: MV A peak** | 0.9 (0.3) |
| **Mitral valve flow deceleration time (MV DT) (ms)** | 200 (31) |
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**Table 1: Baseline clinical characteristics and cardiovascular measurements of the study population.** *Standard deviations are in parentheses.*

Based on the test set (Figure 1), there is an observed correlation between the predicted and actual values with $R^2$ value of 0.67. Both curves follow each other closely and an acceptable mean absolute error of

18.917 (less than one standard deviation of 47.704 for the test set distribution). This implies that our RF model is moderately accurate at predicting the LV mass.

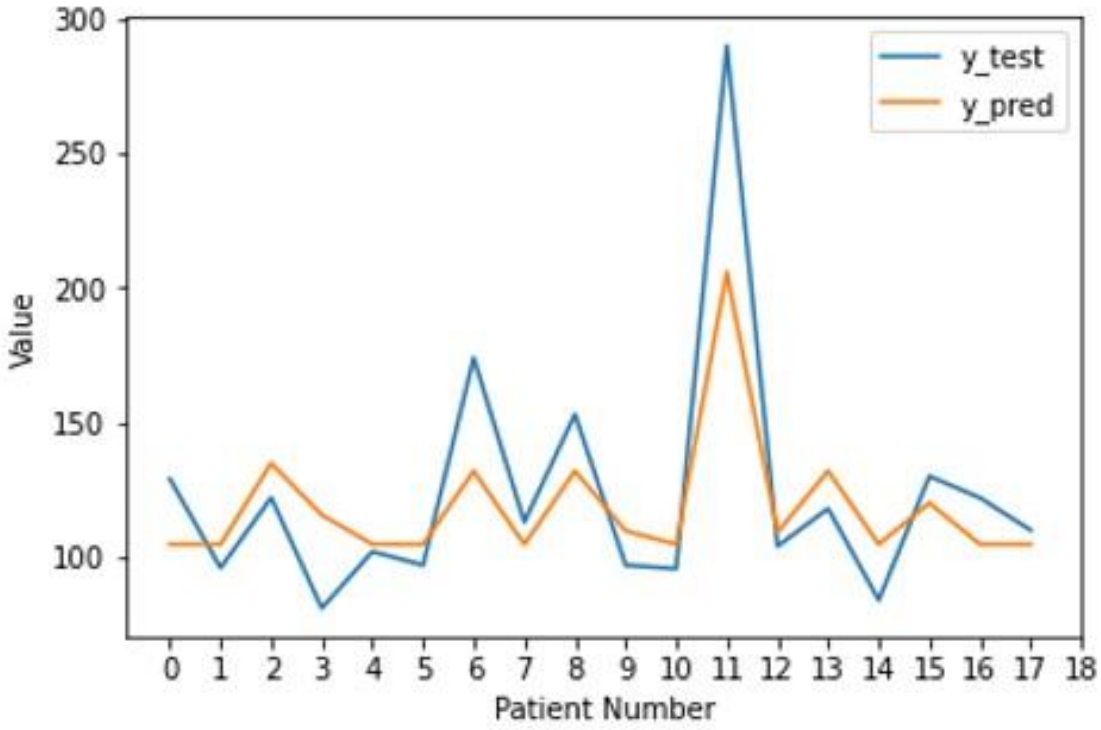



***Figure 1: Line plots comparing the true and predicted LV mass by the RF Regressor on the test set.***

Based on the train set (Figure 2), BMR was the most important feature in determining the LV structure due to its greatest average impact on the model output, as indicated by the mean absolute SHAP values.

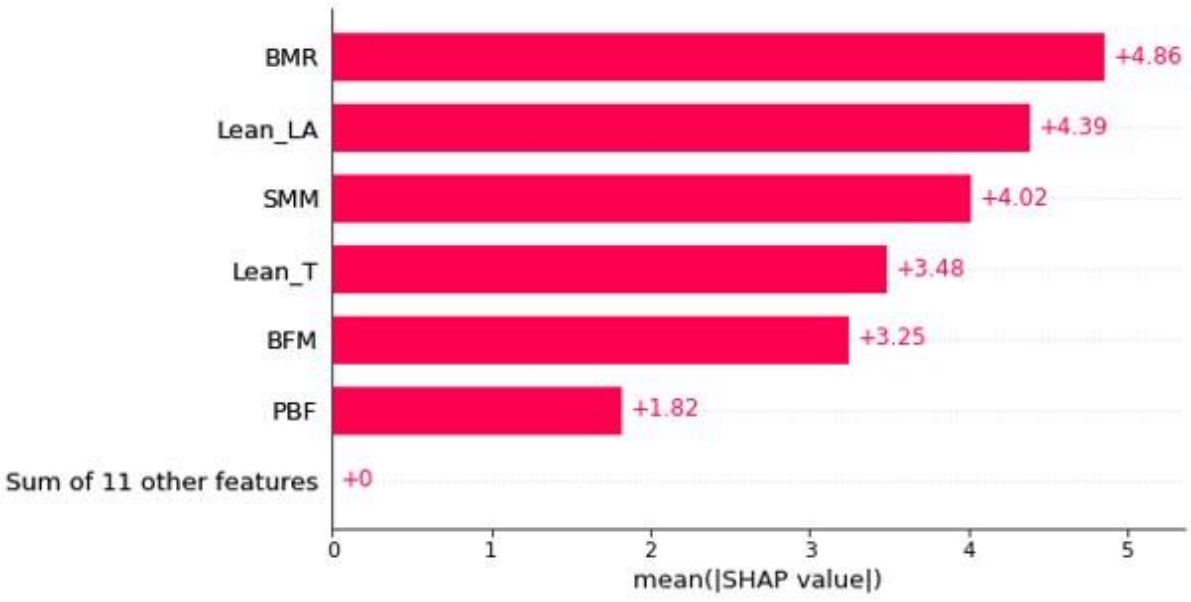


***Figure 2: Bar plot consisting of features sorted by their importance, which is measured as the mean absolute SHAP values, within the train set.***

Other features such as ALM were found to be less important as their mean SHAP values were zero. As a more informative alternative, Figure 3 describes the relationship between the features and their global impact based on the computed SHAP values for each instance. For example, higher BMR contributed to a larger LV mass, showing positive correlation. This is because a high BMR feature value (in red) maps to a higher positive SHAP value, which is equivalent to the positive change in value from the expected LV mass prediction for that observation. On the other hand, a low BMR feature value (in blue) generally maps to a lower SHAP value that falls within the left distribution, where most of them correspond to a negative contribution to the expected output.

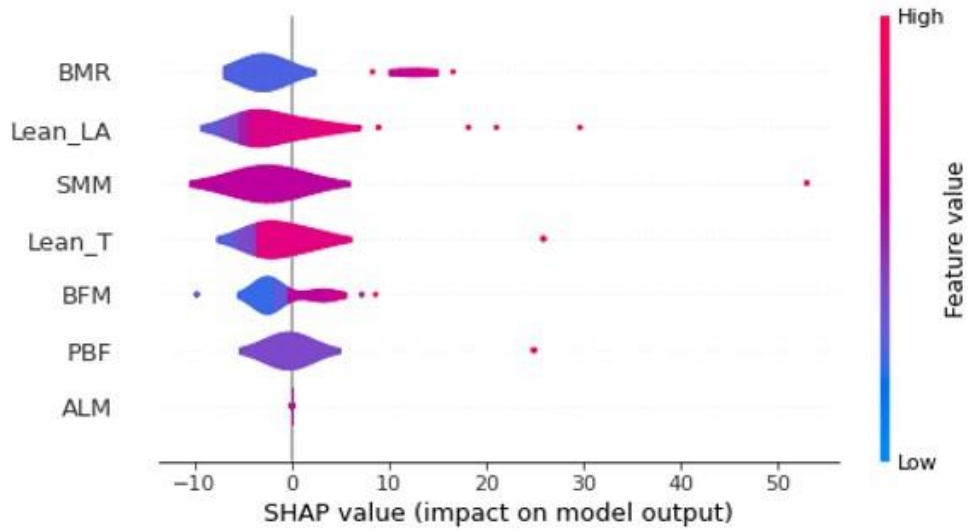



***Figure 3: Summary plot describing the relationship between the value of the feature and the impact on the prediction within the train set. Only the top 7 features were displayed.***

Based on the test set (Figure 4), the SHAP TreeExplainer visually provides local interpretability to a model's prediction for an individual patient in two related flavours. Figure 4A can be thought of as the decomposed version of Figure 4B, detailing the model's decision in a sequential manner. This is because each of the feature contribution can be independently calculated using SHAP values and then summed up to give the final prediction. For example, when predicting the LV mass for patient #6, a BMR feature value of 1516 contributed a corresponding SHAP value of 13.04, resulting in a final predicted LV mass of 132. It can also be observed that the effect of BMR for this patient outweighs other weaker positive factors (e.g., Lean T and Lean LA) and negative factors (e.g., SMM and BFM).

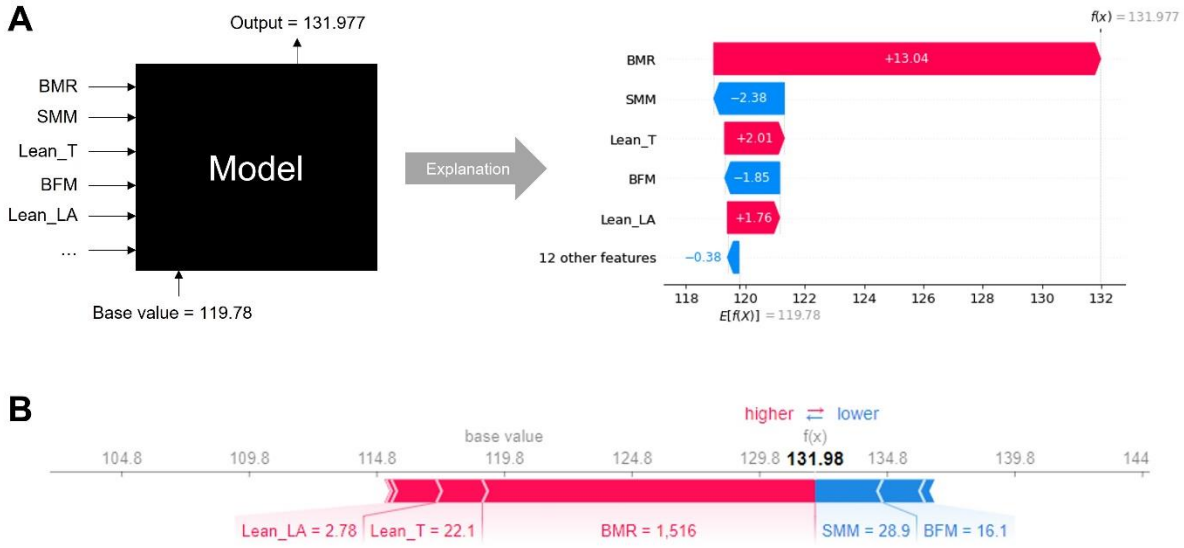



***Figure 4: SHAP provides explainability to the predicted LV mass of the black box RF Regressor for patient #6 in the form of waterfall plot (A) and individual force plot (B).***

Individual force plots can also be combined to produce stacked SHAP explanations, which can be arranged according to their original ordering (Figure 5) or clustering similarity (*Supplementary Figure A*). Based on the test set, Figure 5 resembles the line plot for the predicted values in Figure 1, where the vertical axis describes the predicted LV mass by the RF Regressor while the horizontal axis shows the original patient ordering. Each band width implies the magnitude (i.e., effect) of physical parameters (higher in red; lower in blue) towards prediction of their LV structure. Again, using patient #6 as an example, BMR was observed to be the single predominant positive factor on LV mass, outweighing other weaker positive factors and negative factors. In contrast, LV mass in patient #11 was predicted jointly by several positive factors (e.g., SMM, BMR, Lean T). This suggests that intervening on these prominent physical functional parameters (in red) would more likely improve the cardiac health state of patient #11, as opposed to patient #6 who has less deterministic parameters.

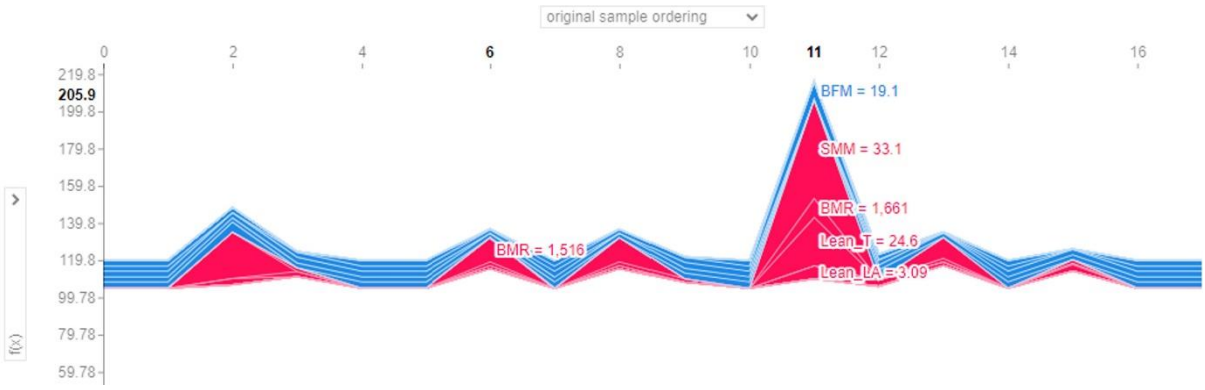
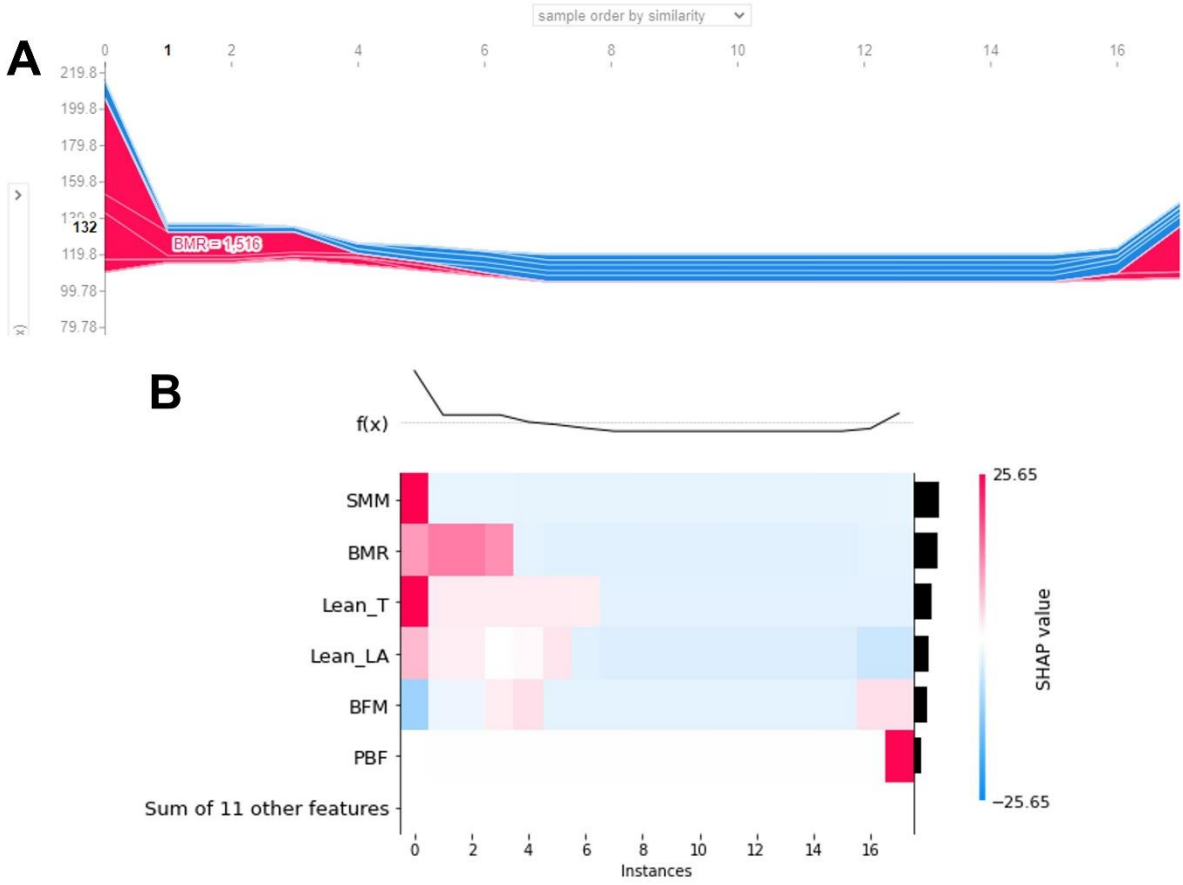



***Figure 5: Collective force plot for test set based on original patient ordering. The plot has been superimposed to show the impact of the physical functional parameters for patients #6 and #11.***

A heatmap plot with the same clustering order, yielding the same curve can also be presented (*Supplementary Figure A*). In both figures, clinicians can see that the patients #6, #8 and #12 were grouped as similar instances (renamed as instances 1, 2 and 3 respectively) due to their comparable features after clustering. The clinicians can therefore infer that these patients in the same subgroup can be characterised as having similarly high BMR as the main contributor to their poor cardiac outcome, which also suggests activities that can lower their BMR may be effective for this group of patients.




***Supplementary Figure A: Using hierarchical agglomerative clustering to order instances within the test set by explanation similarity, presented in the form of a (A) Collective Force Plot and (B) Heatmap Plot.***

### 2) Use of machine learning for 'Interpretation' in personalised medicine: a new frontier

In this exploratory work, we demonstrated the utility of SHAP to enhance interpretation of factors associated with physical activity and cardiac structure.

In contrast to vast volumes of work performed on optimizing model accuracy[133-135], machine learning work on model interpretability is scarce. However, our work adds to recent work by a handful of others who recognise the value of SHAP for model interpretation. In the field of cardiology, Lu et al[142] used XGBoost regression in conjunction with SHAP analyses to identify heart failure clinical subtypes based on electronic health records. Their model utilised structured data from electronic health records to aid clinicians in detecting heart failure stages but did not include other clinical information. In our work, we studied clinical parameters in conjunction with patient-specific LV structure and determined the relative importance of patient specific factors. The use of transthoracic echocardiogram[143] as an imaging test of choice for LV assessment, is an added novelty of our work. Similarly, another recent study used SHAP approach to depict electrocardiographic features associated with left ventricular geometry[144]. Taken together, innovative solutions that combine clinical parameters with detailed cardiovascular imaging may represent novel approaches for machine learning interpretation.

The existing gaps in machine learning work that are geared towards visual interpretation present fresh opportunities for this field. In a large review comprising of 103 cohorts and over 3 million individuals[145], most studies in machine learning only reported the best performing models and evaluation metrics that were suited to their own dataset. While these methods should continue to form the backbone of ML work, stronger emphasis on interpretability could further enhance clinical applications. The clinicians also may be able to better corroborate findings across different studies despite the technical heterogeneity (e.g., hyperparameter selection, data partitioning). In this study, we showed that the RF regression model performed well in predicting the LV mass using a set of physical functional parameters, and further demonstrated the use of SHAP as a visualization tool to provide informative plots based on explanations that justify the model's decision.

As a unified framework for interpreting model predictions, SHAP is associated with three key desirable properties, namely local accuracy, missingness and consistency[146]. These properties make SHAP a superior method over other attribution methods such as Local Interpretable Model-Agnostic Explanations (LIME)[147]. On a local level, individual force plot and waterfall plot can be created for every instance, where each feature value can be visualised as a force that either increases or decreases the base value (i.e., the average of all predictions). Furthermore, all the individual force plots can also be stacked horizontally to produce a collective force plot and placed side by side according to clustering similarity, allowing clinicians to easily identify groups of similar instances.

As an extension, Shapley values can also be aggregated to provide global interpretability. Global importance can be calculated by summing the absolute Shapley values per feature across the data as a way of quantifying the marginal contribution of each predictor towards the target variable. By sorting the features in decreasing order of importance, the feature importance plot allows clinicians to visualise the most important features that require more attention. It is critical to point out that the implementation of SHAP, which is based on the magnitude of feature attributions, is different from the permutation feature importance, which is based on the decrease in model performance.

SHAP also offers summary plot, which may be more informative as it combines feature importance with feature effects as well as shows the relationship between the value of a feature and its impact on the prediction from a more global perspective. Finally, a heatmap can also be plotted, which allows for data in two dimensions. The variable feature importance is sorted in descending order along the vertical axis and uses hot-to-cold scheme to reflect the features' contributions towards the predictions for the instances that lie on the horizontal axis.

The potential impact of local explanations for ML models is profound. The incorporation of an explainability tool like SHAP into clinical workflow is especially important in overcoming the resistance of adopting such black box models due to the perils of blindly trusting their outputs at face

value. Understanding why these algorithms make certain predictions is just as crucial as their accuracy because it facilitates transparency and can assist the clinicians to make more informed decisions. The upshot of this implementation is that patient outcomes may improve. Further research in this area is needed.

### 3) Machine learning to converge heterogenous features, including metabolomics and physical activity and its effects on cardiovascular health

We used machine learning to study multidomain features**.** The model identified key physical functional parameters that identified cardiac function (E/A ratio) at the individual patient level. The data—clinical data, medical images, biological factors, physical function data, socioeconomic data, etc.—were used to train the model using AI methods that included pre-processing of each cross-validation fold, identification and aggregation of significant features, and optimization of each feature set with scaling. Extracted feature sets were input to derive optimal pipelines to yield a final tuned model (Figure A)

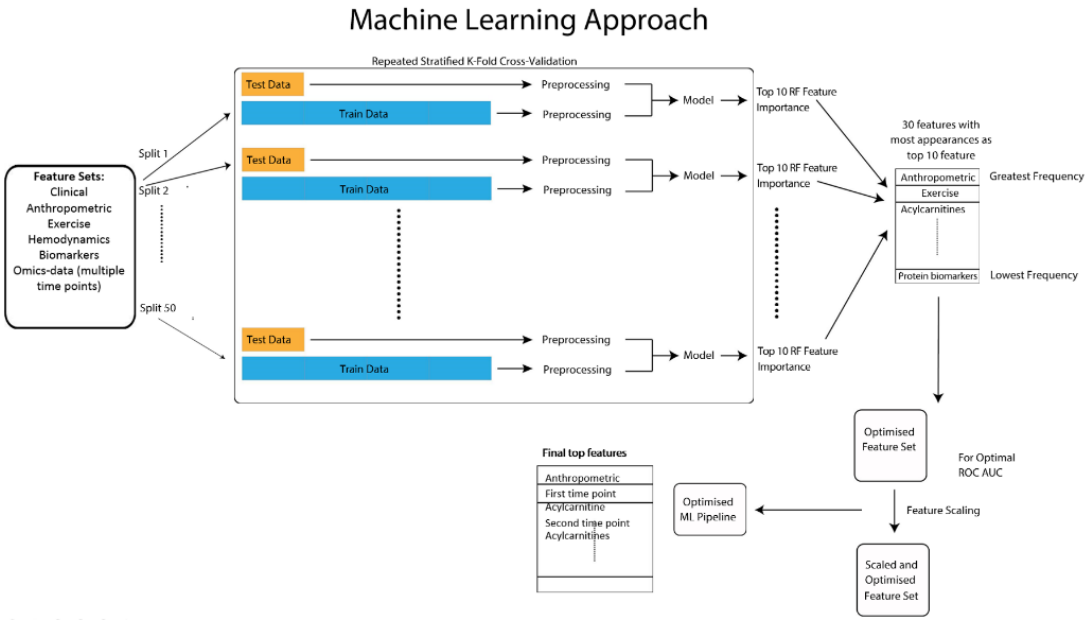



***Figure A: Machine Learning Approach to Conglomerate Multiple Datasets for cardiovascular ageing***

In total, 226 features across 6 feature sets. Subsequently, the model extracted top 30 features (body size, multiple timepoint acyl carnitines, amino acids, natriuretic peptides and hemodynamics) across all cross-validation splits, increasing ROC AUC to 0.791. Elimination of lowest ranked features resulted on ROC peaking to 0.825 (top features: body size, multiple timepoint acylcarnitines). Tree-based pipeline optimization model tuning improved final AUC to 0.875.

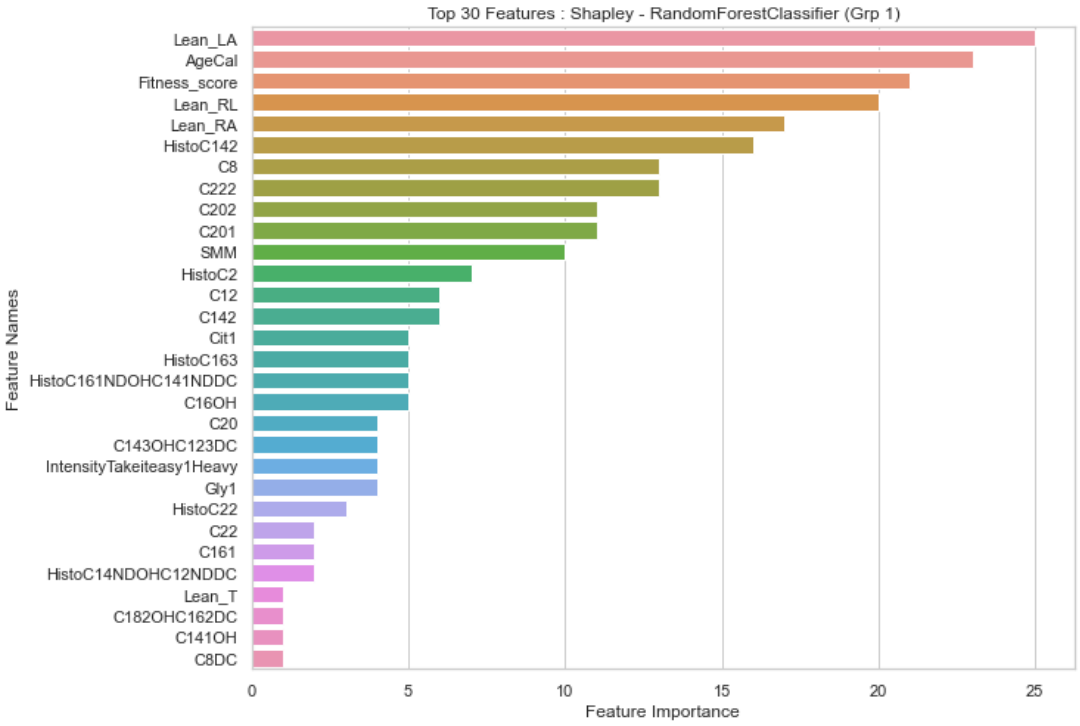



***Figure B: Top ranked features among older adults predictive of cardiac ageing indicates high importance of acylcarnitines (denoted as C14:2, C8, C22:2, C20:1) in addition to conventional factors such as physical fitness score.***

**Clinical Implications of this machine learning approach**

Machine learning conglomerates multidomain feature sets to predict cardiovascular health states and may be used to follow age- and inter-related factors. Identified significant features may elucidate modifiable risks or prioritise candidate therapeutic targets.

Our approach builds upon those performed by existing studies of applied machine learning in metabolomics and cardiovascular disease, where machine learning techniques have been used to conglomerate large health datasets. For instance, random forest feature importance have been used to identify both weight gain[148] and heart failure biomarkers[149] from metabolomics and clinical data. Similar techniques have been applied to both create a predictive model through evaluation of ROC AUC using an ensemble of machine learning models to identify predictors of cardiovascular disease using random forest feature importance[150].

**Limitations**

Our exploratory work may be limited by a small dataset. However, the goal of this exploration was to determine suitable machine learning methods to present data in clinically useful ways, rather than on model accuracy. In the area of interpretability, we have confined our results to using SHAP methodology. We acknowledge that there may be other methodology for interpretability, such as LIME[151], counterfactual fairness[152] and justification narratives[153] that are available in the wider artificial intelligence field. However, in our task which requires the measurement of feature importance for the clinicians to interpret, SHAP stands out as the only additive feature attribution method that satisfies the two key properties of consistency and accuracy[146].

While there are no specific guidelines on minimum sample sizes in machine learning, the consensus is that the more features there are, the greater the sample size should be[154]. The smaller the sample size, the higher the resulting variance which will be further exacerbated by greater number of features. The

consequence is overfitting models that might have overestimated scores, which perform poorly in real-world scenarios. For investigations that involve multiple domains such as ageing, and lifestyle factors such as physical activity, this may be evident from high variance seen in the ROC curves.

The field also recognises that ethical issues may arise with AI-driven medical judgements. Patients may find it challenging to understand how they work, and physicians may find it difficult to accept and confirm recommendations made by AI. Patients and physicians may subconsciously lose some of their autonomy in treatment decision-making. While AI systems may produce precise predictions, understanding the reasoning behind the treatment recommendations is essential for making an educated choice, and establishing comprehensible and transparent AI algorithms are important steps. While AI algorithms may make treatment suggestions, physicians must still be ultimately responsible for making medical decisions in alignment with patient preferences and informed consent.

**Future directions**

Artificial intelligence has great capacity to include many diverse concepts in clinical research that has evaded traditional statistical methods of analysis. Softer clinical characteristics, such as ethnicity, socioeconomic status, and healthcare financing systems, could be included in future AI models to derive more relevant real-world interpretations. Clinical studies may also benefit from having more liberal inclusion criteria and better recruitment prospects with a broader-based analysis method using AI. Using AI permits the inclusion of more extensive datasets and more variables to examine complex intervariable interactions and feature weightage without dropping data. Extending this application by embedding AI algorithms in live datasets or registries to perform dynamic real-time analysis adaptive to changes in global trends or novel therapies may be next steps.

I am the principal investigator of the study. My contribution includes obtaining grant funding for this work, setting up the study protocol, recruitment of research participants, obtaining ethical approval, data analyses, and manuscript review.

# THESIS CONCLUSION

Ageing-related changes in cardiac structure and function are linked to physical activity. Our work has observed relationships between physical activity and fatty oxidation pathways, linked to aerobic capacity[155] and mitochondrial fuel metabolism pathways. These findings suggest metabolic underpinnings between physical activity and cardiovascular health in ageing.

Given the importance of physical activity as an essential modifiable lifestyle factor in mitigating ageing-related cardiovascular risk, a better understanding of the effect of physical activity on underlying cellular metabolic processes has improved our understanding of disease pathophysiology and highlights new potential targets for disease prevention. We acknowledge that our work has focused on leisure time physical activity rather than exercise training. While our findings may not be easily extrapolated to more extreme forms of exercise training, leisure time physical activity is prognostically related to major adverse cardiac events[156], and thus relevant to population-based cohort studies.

Much work remains ahead of us. There are limitations within omics technology such as metabolomics that the field should be cognisant of.

First, observed effect changes are sensitive to cohort sizes and prevalence of disease in the studied population. Therefore, magnitude of associations between metabolites and cardiac risks may not be generalisable to other cohorts. However, broadly speaking, interpretation may be extrapolated to similar cohorts based upon context, such as community versus hospital cohorts. Our work reflect data from community cohorts and would reflect general population across the life course of ageing.

Secondly, sex dimorphisms in the serum metabolome have been observed. Interpretations of metabolomics and cardiac data need to be considered in future larger studies to account for sex dimorphisms in the serum metabolome[157, 158]. However, studies have shown that age and female sex are

associated with greater ventricular stiffness in community adults without cardiovascular disease. Thus, our findings are relevant to real-world scenarios [159, 160].

Thirdly, dietary, and other lifestyle factors pertinent to metabolomic perturbations were not adjusted. No restrictions on diet and the fasting state of blood tests were not specified in this real-world investigation of older adults [10]. While diet and exercise levels may affect metabolites in the blood [161], fasting has not been a major source of variability in most metabolites although acylcarnitines may demonstrate some variability based on fasting status [162, 163]. For future studies, correlations between hexoses and essential amino acids with markers of mitochondrial metabolism may provide insights into the nutritional status of participants [164].

Fourth, using targeted metabolomic profiling allowed for quantifying absolute metabolite concentrations but resulted in a limited breadth of analysis. However, targeted profiling allowed us to demonstrate a quantifiable extent in relation to cardiovascular endpoints. Although these metabolites represent a small portion of the human metabolome, they report on critical pathways for cellular metabolism.

Fifth, skeletal muscle mass, a relevant variable of interest in ageing studies [165] was not investigated in these studies. Circulating long chain acylcarnitines and alanine levels may help track changes in metabolic pathways common to cardiac and skeletal muscle. Future studies that include biomarkers, such as muscle mass-derived cystatin C or nitric oxide-mediated epithelial signalling citrate/arginine ratio, may provide further mechanistic insights [166, 167].

Sixth, these data are observational in nature and preclude causal inferences. Proper randomised control trials are necessary to provide robust evidence for the role of physical activity in altering metabolomics, in addition to how these changes will impact cardiovascular health in ageing.

Seventh, we did not collect data on endothelial function of the participants. We recognise the importance of endothelial function in population-based studies of cardiovascular health.

Despite these limitations, our findings deepen our understanding of the mechanistic effects of physical activity on cardiovascular health in ageing. Further studies with larger cohorts or longer follow-ups may better depict the clinical impact of these mechanisms and could include multiple-omics techniques that combine genomics, transcriptomics, and proteomics.

With regards to artificial intelligence, it has transformed the way data aids in medical diagnosis and delivery of medical care. Healthcare is rapidly evolving, and the strength of artificial intelligence lies in its capacity to rapidly analyse numerous patient traits and large volumes of data to deliver personalised medicine. In treatment decision-making, artificial intelligence methods may suggest customised interventions by comparing each patient with instances like their own, and identify subtle patterns not readily seen using conventional methods, while providing patient-centred care accounting for each person's particular requirements and traits. Previously, physicians had made treatment recommendations guided by their own knowledge, clinical guidelines, and experiences, in discussion with patient's preferences. However, the complexity of contemporary healthcare and rapidly growing collection of medical data, including omics, has led to a need for more sophisticated decision-making tools.

Overall, the omics explosion represented in this thesis via metabolomics will require concomitant deep analysis alongside numerous patient traits and lifestyle habits such as physical activity. By drawing in upon all these complexities, artificial intelligence represents a new tool to provide recommendations customised to individual patient profiles, maximizing the chances of favourable outcomes in complex life course like ageing, in contrast to a one-size-fits-all strategy faced commonly in clinical guidance.

## Future Directions

The role of the metabolome in cardiovascular health prediction and risk stratification would be expanded in the future, to include pre-specified analyses in specific patient subsets such as between women versus men. More rigorous evaluation of the observed metabolic signals could also be performed within prospective randomised trial settings, including response of the metabolome to cardiac treatments or interventions. Interrogation of the metabolome in relation to more diverse lifestyle factors such as diet and microbiome would also be useful.

# APPENDIX

## CITATION METRICS

### Publication #1

## Dissecting clinical and metabolomics associations of left atrial phasic function by cardiac magnetic resonance feature tracking

[HTML] from nature.com
Full View

| | |
|---|---|
| Authors | Angela S Koh, Fei Gao, Shuang Leng, Jean-Paul Kovalik, Xiaodan Zhao, Ru San Tan, Kevin Timothy Fridianto, Jianhong Ching, Serene JM Chua, Jian-Min Yuan, Woon-Puay Koh, Liang Zhong |
| Publication date | 2018/5/25 |
| Journal | Scientific Reports |
| Volume | 8 |
| Issue | 1 |
| Pages | 8138 |
| Publisher | Nature Publishing Group UK |
| Description | Among community cohorts, associations between clinical and metabolite factors and complex left atrial (LA) phasic function assessed by cardiac magnetic resonance (CMR) feature tracking (FT) are unknown. Longitudinal LA strain comprising reservoir strain (εs), conduit strain (εe) and booster strain (εa) and their corresponding peak strain rates (SRs, SRe, SRa) were measured using CMR FT. Targeted mass spectrometry measured 83 circulating metabolites in serum. Sparse Principal Component Analysis was used for data reduction. Among community adults (n = 128, 41% female) (mean age: 70.5 ± 11.6 years), age was significantly associated with εs (β = −0.30, p < 0.0001), εe (β = −0.3, p < 0.0001), SRs (β = −0.02, p < 0.0001), SRe (β = 0.04, p < 0.0001) and SRe/SRa (β = −0.01, p = 0.012). In contrast, heart rate was significantly associated with εa (β = 0.1, p = 0.001) and SRa (β ... |
| Total citations | Cited by 26 |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|---|---|
| Scholar articles | Dissecting clinical and metabolomics associations of left atrial phasic function by cardiac magnetic resonance feature tracking<br>AS Koh, F Gao, S Leng, JP Kovalik, X Zhao, RS Tan... - Scientific Reports, 2018<br>Cited by 26 Related articles All 8 versions |

**Publication #2**

## Exacerbation of cardiovascular ageing by diabetes mellitus and its associations with acyl-carnitines

[HTML] from nih.gov

| | |
|---|---|
| Authors | Fei Gao, Jean-Paul Kovalik, Xiaodan Zhao, Vivian JM Chow, Hannah Chew, Louis LY Teo, Ru San Tan, Shuang Leng, See Hooi Ewe, Hong Chang Tan, Tsze Yin Tan, Lye Siang Lee, Jianhong Ching, Bryan MH Keng, Liang Zhong, Woon-Puay Koh, Angela S Koh |
| Publication date | 2021/6/6 |
| Journal | Aging (Albany NY) |
| Volume | 13 |
| Issue | 11 |
| Pages | 14785 |
| Publisher | Impact Journals, LLC |
| Description | Objective: To demonstrate differences in cardiovascular structure and function between diabetic and non-diabetic older adults. To investigate associations between acyl-carnitines and cardiovascular function as indexed by imaging measurements.<br><br>Methods: A community-based cohort of older adults without cardiovascular disease underwent current cardiovascular imaging and metabolomics acyl-carnitines profiling based on current and archived sera obtained fifteen years prior to examination.<br><br>Results: A total of 933 participants (women 56%, n= 521) with a mean age 63±13 years were studied. Old diabetics compared to old non-diabetics had lower myocardial relaxation (0.8±0.2 vs 0.9±0.3, p= 0.0039); lower left atrial conduit strain (12±4.3 vs 14±4.1, p= 0.045), lower left atrial conduit strain rate (-1.2±0.4 vs-1.3±0.5, p= 0.042) and lower ratio of left atrial conduit strain to left atrial booster strain (0.5±0.2 vs 0.7±0.3, p= 0 … |
| Total citations | Cited by 9 |


Scholar articles Exacerbation of cardiovascular ageing by diabetes mellitus and its associations with acyl-carnitines
F Gao, JP Kovalik, X Zhao, VJM Chow, H Chew… - Aging (Albany NY), 2021
Cited by 9 Related articles All 7 versions

**Publication #3**

## Amino acid differences between diabetic older adults and non-diabetic older adults and their associations with cardiovascular function

Authors Jean-Paul Kovalik, Xiaodan Zhao, Fei Gao, Shuang Leng, Vivian Chow, Hannah Chew, Louis LY Teo, Ru San Tan, See Hooi Ewe, Hong Chang Tan, Hai Ning Wee, Lye Siang Lee, Jianhong Ching, Bryan MH Keng, Woon-Puay Koh, Liang Zhong, Angela S Koh

Publication date 2021/9/1

Journal Journal of Molecular and Cellular Cardiology

Volume 158

Pages 63-71

Publisher Academic Press

Description Background

Ageing and insulin resistant states such as diabetes mellitus frequently coexist and increase the risk of cardiovascular disease development among older adults. Here we investigate metabolic differences in amino acid profiles between ageing and diabetes mellitus, and their associations with cardiovascular function.

Methods

In a group of community older adults we performed echocardiography, cardiac magnetic resonance imaging as well as cross sectional and longitudinal metabolomics profiling based on current and archived sera obtained fifteen years prior to examination.

Results

We studied a total of 515 participants (women 50%, $n = 255$) with a mean age 73 (SD = 4.3) years. Diabetics had higher alanine (562 vs 448, $p < 0.0001$), higher glutamate (107 vs 95, $p = 0.016$), higher proline (264 vs 231, $p = 0.008$) and lower arginine (107 vs 117, $p = 0.043$), lower citrulline (30 vs 38, $p = 0.006$) levels (μM ...

**Publication #4**

## Metabolomic correlates of aerobic capacity among elderly adults

[PDF] from wiley.com
Full View

Authors: Angela S Koh, Fei Gao, Ru S Tan, Liang Zhong, Shuang Leng, Xiaodan Zhao, Kevin T Fridianto, Jianhong Ching, Si Y Lee, Bryan MH Keng, Tee Joo Yeo, Shu Y Tan, Hong C Tan, Chin T Lim, Woon-Puay Koh, Jean-Paul Kovalik

Publication date: 2018/10

Journal: Clinical cardiology

Volume: 41

Issue: 10

Pages: 1300-1307

Publisher: Wiley Periodicals, Inc.

Description:

Background

Aerobic capacity is a powerful predictor of cardiovascular disease and all-cause mortality, and it declines with advancing age.

Hypothesis

Since physical activity alters body metabolism, metabolism markers will likely differ between subjects with high vs low aerobic capacities.

Methods

Community-based participants without physician-diagnosed heart disease, stroke or cancer underwent same-day multimodal assessment of cardiovascular function (by echocardiography and magnetic resonance feature tracking of left atrium) and aerobic capacity by peak oxygen uptake ($VO_2$) metrics. Associations between $VO_2$ and cardiovascular and metabolomics profiles were studied in adjusted models including standard covariates.

Results

We studied 141 participants, of whom 82 (58.2%) had low $VO_2$, while 59 (41.8%) had high $VO_2$. Compared to participants with high $VO_2$, participants with low $VO_2$ had ...

Total citations: Cited by 18


Scholar articles: Metabolomic correlates of aerobic capacity among elderly adults
AS Koh, F Gao, RS Tan, L Zhong, S Leng, X Zhao... - Clinical cardiology, 2018
Cited by 18 Related articles All 6 versions

**Publication #5**

## Obesity in older adults and associations with cardiovascular structure and function

[HTML] from karger.com

Authors Yen How Tan, Jun Pei Lim, Wee Shiong Lim, Fei Gao, Louis LY Teo, See Hooi Ewe, Bryan MH Keng, Ru San Tan, Woon-Puay Koh, Angela S Koh

Publication date 2022/5/17

Journal Obesity Facts

Volume 15

Issue 3

Pages 336-343

Publisher S. Karger AG

Description Introduction

Body mass index (BMI), despite being widely used as a marker of obesity, fails to fully capture cardiovascular risks as it is an insufficient biomarker of abdominal adiposity, unlike waist circumference (WC). We aimed to characterize associations between BMI and WC with cardiovascular structure and function in older adults.

Methods

Among an observational cohort study of a community of older adults, transthoracic echocardiography determined cardiovascular structure and function, while aerobic capacity was determined by peak oxygen uptake (VO 2) metrics. The cut-offs for obesity were 27.5 kg/m 2 for BMI, and> 90 cm for males and> 80 cm for females for WC.

Results

Of 970 older adults without cardiovascular disease (mean age 73±4 years, 432 [44%] males), 124 (12.8%) were obese by BMI definition while 347 (35.7%) were obese by WC definition. Inter-definitional agreement was fair (Cohen's κ= 0 …

**Publication #6**

## Explainable machine learning predictions to support personalized cardiology strategies

[HTML] from oup.com
Full View

| | |
|---|---|
| Authors | De Rong Loh, Si Yong Yeo, Ru San Tan, Fei Gao, Angela S Koh |
| Publication date | 2022/3/1 |
| Journal | European Heart Journal-Digital Health |
| Volume | 3 |
| Issue | 1 |
| Pages | 49-55 |
| Publisher | Oxford University Press |

Description

Aims

A widely practiced intervention to modify cardiac health, the effect of physical activity on older adults is likely heterogeneous. While machine learning (ML) models that combine various systemic signals may aid in predictive modelling, the inability to rationalize predictions at a patient personalized level is a major shortcoming in the current field of ML.

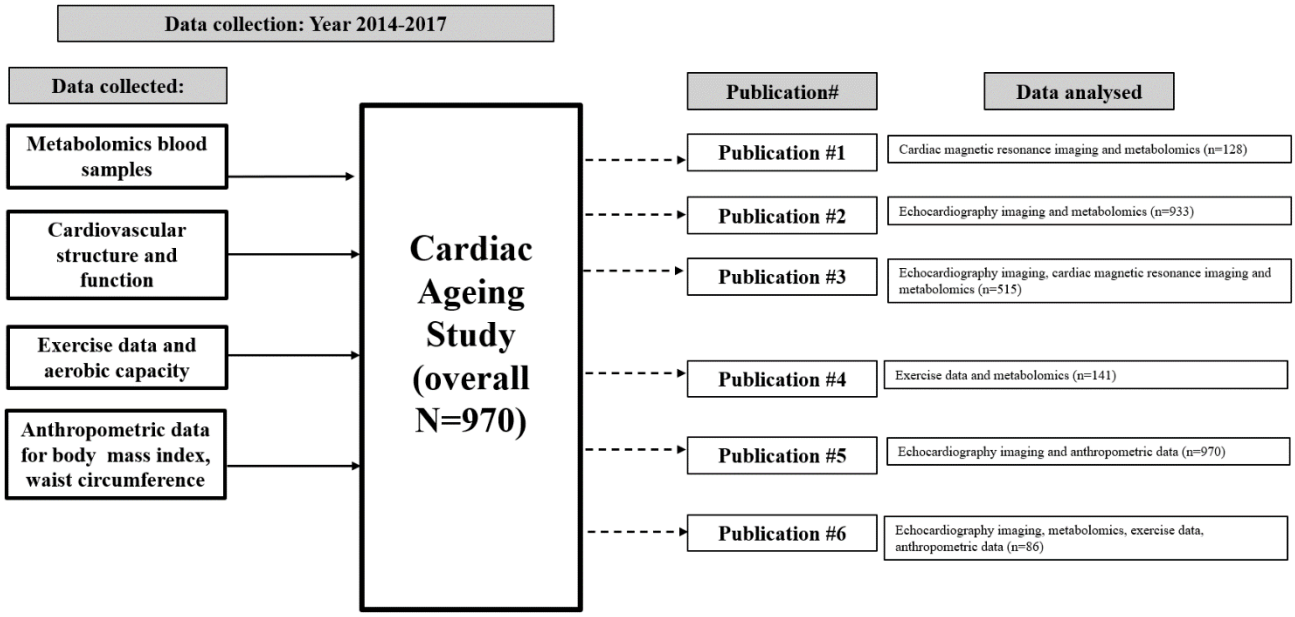
Methods and results

We applied a novel methodology, SHapley Additive exPlanations (SHAP), on a dataset of older adults $n = 86$ (mean age $72 \pm 4$ years) whose physical activity levels were studied alongside changes in their left ventricular (LV) structure. SHAP was tested to provide intelligible visualization on the magnitude of the impact of the features in their physical activity levels on their LV structure. As proof of concept, using repeated K-cross-validation on the train set ($n = 68$), we found the Random Forest ...

Total citations Cited by 3


Scholar articles Explainable machine learning predictions to support personalized cardiology strategies
DR Loh, SY Yeo, RS Tan, F Gao, AS Koh - European Heart Journal-Digital Health, 2022
Cited by 3 Related articles All 6 versions

## TIMELINE OF THE COHORT AND PUBLICATIONS



## DETAILED METHODS

We used data from a cohort study of older adults recruited from community population. The Cardiac Ageing Study (CAS) was a community-based study of middle aged to older adults (mean age 72±4 years) examined in 2014-2017 who did not have clinical cardiovascular disease (CVD) at baseline. In CAS, we characterised CV structure and function using novel cardiovascular imaging techniques such as magnetic resonance imaging and echocardiography. We used imaging markers to define individuals with worse structural and functional alterations that likely represent cardiovascular ageing. In conjunction with physical activity and circulating metabolites in this population, we performed cross-sectional analyses.

### Cardiac Magnetic Resonance Imaging

Cine cardiac magnetic resonance was performed using balanced steady state free precession sequence. All participants were imaged on a 3T magnetic resonance imaging system (Ingenia, Philips Healthcare, The Netherlands) with a dStream Torso coil (maximal number of channels 32). BFFE end-expiratory breath hold cine images were acquired in multi-planar long-axis views (2-, 3-, and 4-chamber views) and a stack of parallel short-axis views to cover the left ventricle (LV) from base to apex. Typical parameters were as follows: TR/TE 3/1 ms; flip angle, 45°; in-plane spatial resolution, 1.0 mm x 1.0 mm to 1.5 mm x 1.5 mm; slice thickness, 8 mm; pixel bandwidth, 1797 Hz; field of view, 300 mm; frame rate, 30 or 40 per cardiac cycle. We developed an in-house semi-automatic algorithm to track the distance (L) between the left atrioventricular junction and a user-defined point at the mid posterior LA wall on standard CMR 2- and 4-chamber views[35, 36]. Both 2- and 4-chamber views were used to generate the average strain and strain rate results. Longitudinal strain ($\varepsilon$) at any time point ($t$) in the cardiac cycle from end-diastole (time 0) was calculated as: $\varepsilon(t) = (L(t) - L_0)/L_0$. LA reservoir strain ($\varepsilon_s$), conduit strain ($\varepsilon_e$) and booster strain ($\varepsilon_a$) were calculated at $t$ equals left ventricular end-systole, diastasis and pre-LA systole, respectively. To derive the peak strain rate (SR) indices, peak values of the first time derivative of the strain-time curve at systole, diastasis and LA contraction were measured.

Strain and SR parameters from both 2- and 4-chamber views were averaged to obtain mean results for analysis

**Echocardiography**

Echocardiography was performed using ALOKA α10 with a 3.5- MHz probe. In each subject, standard echocardiography, which included 2-D, M-mode, pulse Doppler and tissue Doppler imaging, was performed in the standard parasternal and apical (apical 4-chamber, apical 2-chamber, and apical long) views, and three cardiac cycles were recorded. Left ventricular ejection fraction, left atrial (LA) volume, and LA volume index (LAVI) were measured. The trans-mitral flow E and A waves with the sample volume position at the tip of the mitral valve leaflets from the apical 4-chamber view were recorded by Doppler echocardiography. Myocardial relaxation (E/A) ratio was computed as a ratio of peak velocity flow in early diastole E (MV E) (m/s) to peak velocity flow in late diastole by atrial contraction A (MV A) (m/s). Pulsed wave tissue Doppler imaging was performed with the sample volume at the septal and lateral annulus from the apical 4-chamber view. The frame rate was between 80 and 100 frames per second. The tissue velocity patterns were recorded and expressed as E', and A'. All measurements were measured by the same operator and the measurements were averaged over three cardiac cycles and adjusted by the RR interval. The specific cardiovascular function of interest in this cohort of older adults was E/A properties, for which impairments in E/A, would suggest adverse myocardial ageing[121]. E/A was defined by ratio of peak velocity flow in MV E to peak velocity flow in late diastole by MV A, also referred to as the E/A ratio. MV E refers to the peak velocity of blood flow during early diastole from the left atrium into the left ventricle, where blood flows passively into the left ventricle during relaxation. MV A refers to the peak velocity of blood flow into the left ventricle in late diastole due to contraction of the left atrium.

**Metabolomics Profiling**

We used targeted metabolomics profiling for this work. Antecubital venous blood samples (20–30 ml) were taken from consenting participants in the morning. After collection, the blood samples were immediately placed on ice for transportation and were processed within 6 h to obtain serum samples,

which were subsequently stored at −80 °C. Serum metabolomic profiling analysis was performed in the Duke-NUS Metabolomics Facility. Thawed serum samples (100 µl) were spiked with 20 µl deuterium-labelled amino acid/acyl-carnitine mixture and diluted with 800 µl methanol. After centrifugation of the mixture at 17,000 g for 5 mins at 20 °C, the supernatant fraction was collected and divided into two parts: one (100 µl) for acylcarnitine analysis and one (10 µl) of each extracted serum sample. Amino acids were separated using a C8 column (Rapid Resolution HT, 4.5 × 50 mm, 1.8 µm, Zorbax SB-C8) on an Agilent 1290 Infinity LC system (Agilent Technologies, CA, USA) coupled with quadrupole-ion trap mass spectrometer (QTRAP 5500, AB Sciex, DC, USA). Mobile phase A (10/90 Water/Acetonitrile) and Mobile phase B (90/10 Water/ Acetonitrile), both containing 10 mM of Ammonium formate, were used for chromatography separation. Acylcarnitine measurements were made using flow injection tandem mass spectrometry on the Agilent 6430 Triple Quadrupole LC/MS system (Agilent Technologies, CA, USA). The sample analysis was carried out at 0.4 ml/min of 80/20 Methanol/water as mobile phase, and injection of 4 µL of sample. Data acquisition and analysis were performed on Agilent Mass Hunter Workstation B.06.00 Software.

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