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**Metabolically healthy and unhealthy obesity: differential effects on myocardial function according to metabolic syndrome, rather than obesity.**

**Running title:** Metabolically healthy obesity and preserved cardiac function

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myocardial function.

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**Abstract**

**Background** The term “metabolically healthy obesity (MHO)” is distinguished using body mass index (BMI), yet BMI is a poor index of adiposity. Some epidemiological data suggest that MHO carries a lower risk of cardiovascular disease (CVD) or mortality than being normal weight yet metabolically unhealthy.

**Objectives** We aimed to undertake a detailed phenotyping of individuals with MHO by using imaging techniques to examine ectopic fat (visceral and liver fat deposition) and myocardial function. We hypothesised that metabolically unhealthy individuals (irrespective of BMI) would have adverse levels of ectopic fat and myocardial dysfunction compared to MHO individuals.

**Subjects** Individuals were categorised as non-obese or obese ( $\text{BMI} \geq 30 \text{ kg.m}^{-2}$ ) and as metabolically healthy or unhealthy according to the presence or absence of metabolic syndrome.

**Methods** 67 individuals (mean $\pm$ SD: age  $49\pm 11$  years) underwent measurement of i) visceral, subcutaneous and liver fat using magnetic resonance imaging and proton magnetic resonance spectroscopy, ii) components of metabolic syndrome, iii) cardiorespiratory fitness, and iv) indices of systolic and diastolic function using tissue Doppler echocardiography.

**Results** Cardiorespiratory fitness was similar between all groups; abdominal and visceral fat was highest in the obese groups. Compared with age- and BMI-matched metabolically healthy counterparts, the unhealthy (lean or obese) individuals had higher liver fat and decreased early diastolic strain rate, early diastolic tissue velocity and systolic strain indicative of subclinical systolic and diastolic dysfunction. The magnitude of dysfunction correlated with the number of components of metabolic syndrome but not with BMI or with the degree of ectopic (visceral or liver) fat deposition.

**Conclusions** Myocardial dysfunction appears to be related to poor metabolic health rather than simply BMI or fat mass. These data may partly explain the epidemiological evidence on CVD risk relating to the different obesity phenotypes.

## Introduction

Obesity has been considered to confer an increased risk of cardiovascular disease (CVD) and mortality<sup>1,2</sup>. However, there is increasing recognition of a subgroup of obese patients with the mechanical complications of obesity but without the associated metabolic complications: ‘metabolically healthy obesity’ (MHO)<sup>3</sup>.

There is conflicting data in the literature regarding metabolic risk and obesity, and whether metabolic risk is determined more by the relative distribution than the absolute volume of fat. Metabolically healthy individuals have increased subcutaneous fat relative to visceral fat and lower cellular fat in liver and skeletal muscle in comparison to metabolically unhealthy individuals<sup>4</sup>. They are also more insulin-sensitive and have a better inflammatory status<sup>5,6</sup>. Several studies have demonstrated that the quantity of liver fat is more closely linked to the metabolic complications of obesity than that of visceral fat<sup>7-9</sup>. However, there is conflicting evidence that suggests excess visceral fat and insulin resistance, but not general adiposity are associated with incident pre-diabetes and type 2 diabetes in obese individuals<sup>10</sup>. A recent study of almost 30,000 individuals demonstrated that metabolically unhealthy obese individuals have a greater risk of developing diabetes than the metabolically healthy obese<sup>11</sup>. Although this area remains contentious, a number of epidemiological studies have demonstrated that MHO individuals are also at a lower risk of CVD, and have reduced morbidity and mortality compared to the metabolically unhealthy obese, suggesting that cardio-metabolic risk factors are more strongly associated with adverse cardiovascular outcomes than obesity<sup>12-16</sup>. However, not all data suggests a protective effect. A recent study

suggested that MHO confers increased risk of heart failure, but not acute myocardial infarction<sup>17</sup>. Similarly, the risk of developing CVD in the San Antonio Heart Study was found to be increased in MHO<sup>18</sup>. A recent systematic review and meta-analysis found that, after 10 years of follow-up, MHO is associated with an increased risk of total mortality and cardiovascular events<sup>19</sup>.

Several cross-sectional studies have tried to better understand the association between metabolic health and CVD by examining the impact of components of metabolic syndrome on cardiac function. Developments in echocardiographic techniques have improved the detection of relatively subtle myocardial disease<sup>20</sup>. Strain and strain rate are sensitive measures of change of myocardial shape (i.e. deformation). Strain indicates the *amount* of myocardial deformation (negative strain means shortening and positive strain, elongation); strain rate, is a measure of the *rate* of myocardial deformation. Measurement of strain and strain rate detect pre-clinical myocardial abnormalities, which can help predict risk of cardiovascular events and mortality<sup>21</sup>. In patients with metabolic syndrome, subclinical left ventricular (LV) dysfunction has been detected (lower early diastolic and systolic tissue velocities accompanied by reduced strain and strain rates) compared to matched controls, worse in those with a greater number of components of the metabolic syndrome<sup>22, 23</sup>. Considering the influence of liver fat on metabolic health and the association of non-alcoholic fatty liver disease (NAFLD) with features of the metabolic syndrome<sup>24</sup>, others have looked at NAFLD patients and demonstrated subclinical myocardial systolic and diastolic dysfunction in the presence or absence of type 2 diabetes (T2DM)<sup>25, 26</sup>.

The main aim of this cross-sectional study was to determine whether *metabolically unhealthy* individuals irrespective of their BMI or normal weight, overweight or obese category, have evidence of increased ectopic fat (with higher visceral and liver fat deposition), combined with impaired myocardial function, compared with age- and BMI-matched metabolically

healthy individuals. Furthermore we hypothesised that MHO individuals would have little evidence of ectopic fat and preserved myocardial function similar to normal weight (metabolically healthy) individuals despite their overall higher fat mass. This would provide a mechanistic context for the epidemiological data around CVD risk and the different obesity phenotypes. We employed a combination of whole body magnetic resonance imaging (MRI) to look at ectopic fat and sensitive echocardiographic measures to measure myocardial function.

### **Subjects and Methods**

*Subjects* Patients with hyperlipidemia and suspected NAFLD were prospectively recruited from specialist lipid and hepatology outpatient clinics at University Hospital Aintree, Liverpool. To recruit metabolically healthy lean and obese individuals we relied on local advertisements. As the association of BMI and metabolic health with cardiac outcomes was a novel investigation, the effect size of interest was unknown. No formal sample size was calculated and recruitment of patients was based on availability with the hope that the estimates obtained will inform future studies. The study conformed to the Declaration of Helsinki and Liverpool Research Ethics Committee approved the study (Ethics Reference 09/H1005/7). All participants gave written informed consent.

Individuals aged  $>21$  years with a body mass index (BMI)  $< 40 \text{ kg/m}^2$  who were able to walk on a treadmill and undergo MRI scanning were recruited. Those with a history of CVD (including atrial fibrillation, ischemic heart disease, heart failure or valvulopathy), type 1 or type 2 diabetes mellitus or chronic liver disease (other than NAFLD) were excluded. Individuals with a BMI  $> 40 \text{ kg/m}^2$  were excluded due to the technical challenges of performing high quality echocardiographic studies on those with morbid obesity. All females

and males consumed <14 and <21 units of alcohol per week respectively. We recorded the following medications: diuretics, statins, ezetimibe, ACE inhibitors, calcium channel antagonists and beta-blockers in all participants.

*Anthropometry* Age, gender, smoking status, past medical history, drug history and family history of CVD were established using a series of questionnaires. Smoking status was defined as never, former or current smoker. Physical activity levels were determined using the long format International Physical Activity Questionnaire <sup>27</sup>. Alcohol intake was determined using the Alcohol Use Disorders Identification Test questionnaire <sup>28</sup>. Blood pressure was measured on at least two separate occasions, with the patient sitting for at least 10 minutes. Body mass was measured after an overnight fast, without shoes, using a Tanita bioimpedance analyser, which also determined fat-free mass and fat percentage (Tanita BC420, Dolby Medical, Stirling, UK). Height was measured with a stadiometer to the nearest 0.5 cm (Seca, Birmingham, UK). BMI was calculated as weight in kilograms divided by height in metres, squared. Waist circumference was measured at the midpoint between the anterior superior iliac spine and the lower edge of the ribcage. The same individual undertook all anthropometric measurements.

*Determinations of metabolic syndrome (MS)* Participants were classified as obese (BMI  $\geq$  30 kg/m<sup>2</sup>) or non-obese (BMI < 30kg/m<sup>2</sup>), and with or without the metabolic syndrome (MS+ vs MS-). MS was defined by the Adult Treatment Panel III <sup>29</sup> as three or more of the following: waist circumference >102 cm (male) or >88 cm (female), triglycerides >1.7 mmol/L (or treatment for hyperlipidemia), HDL cholesterol <1mmol/L (male) or <1.3 mmol/L (female), systolic blood pressure >130mmHg, diastolic blood pressure >85 mmHg (or treatment for

hypertension) and fasting glucose > 6.1 mmol/L. Ten year risk of first atherosclerotic cardiovascular event was calculated using the Pooled Cohort Risk Assessment Equation<sup>30</sup>.

*Magnetic resonance imaging and proton magnetic resonance spectroscopy* Participants underwent magnetic resonance (MR) scanning using a 1.5T Siemens Symphony scanner (Siemens Medical Solutions, Erlangen, Germany) at the University of Liverpool Magnetic Resonance and Image Analysis Research Centre. A single experienced radiographer performed all of the scans.

Abdominal axial T1-weighted fast spin echo scans (axial scans, 10 mm slice thickness followed by a 10 mm gap using the integrated body coil) were used to calculate abdominal visceral and subcutaneous adipose tissue<sup>31</sup>. A blinded researcher performed all analyses of visceral and subcutaneous fat centrally.

Liver lipid was measured non-invasively using proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) as previously described<sup>31-33</sup>. NAFLD was defined as intrahepatocellular lipid content (IHCL) > 5.5% measured by <sup>1</sup>H-MRS. Three voxels of interest were identified in the liver avoiding ducts and vasculature and the mean value taken after data were processed independently. <sup>1</sup>H MR spectra from liver was quantified using the AMARES algorithm in the software package jMRUI-3.0. Intrahepatocellular lipid is expressed as percentage of CH<sub>2</sub> lipid signal amplitude relative to water signal amplitude after correcting for T<sub>1</sub> and T<sub>2</sub>. Fat quantification by <sup>1</sup>H-MRS has been validated against gold standard biochemical measurements<sup>34</sup>.



*Biochemical markers and assays* Venous blood samples were obtained from participants after an overnight fast (minimum 8 hours). Serum lipid profiles, liver function and glucose were measured using standard proprietary agents using the Olympus AU2700 analyser (Beckman Coulter (UK) Ltd). Plasma insulin and adiponectin were measured by radioimmunoassay using commercially available kits (Millipore Corporation, Billerica, MA; intra-assay CV 6% and 5% respectively). All patients with NAFLD underwent a routine liver screen (antinuclear, parietal cell, mitochondrial, smooth muscle, reticulin, liver kidney microsomal type 1 and anti-centromere antibodies, ceruloplasmin and ferritin levels and hepatitis serology).

*Cardiopulmonary exercise testing* Incremental cardiopulmonary exercise testing was performed on a treadmill (Model 770CE, RAM Medisoft Group) using a modified Bruce protocol. Breath-by-breath expiratory gases and ventilation analysis were performed (Love Medical Cardiopulmonary Diagnostics, Cheshire). Following a 3-minute warm-up at 2.8 km/h with no gradient, the initial workload was set at 2.8 km/h with a 5% gradient. Thereafter, stepwise increments were made in gradient and/or speed every 3 minutes as per the Modified Bruce Protocol. Peak patient effort was defined by any of i) a respiratory exchange ratio > 1.1, ii) heart rate > 90% of predicted maximum, iii) a plateau in  $\text{VO}_2$ , or iv) patient exhaustion<sup>35</sup>. Continuous electrocardiographic monitoring was used and all tests were physician-supervised.

*Trans-thoracic echocardiography image acquisition and interpretation* All echocardiograms were performed using a GE Vivid 7 or E9 machine with a 2.5 MHz phased array transducer and the patient in the left lateral position on a reclining couch. A combination of 2D, M-mode, pulsed wave and continuous wave Doppler and tissue Doppler was used. Conventional

echocardiographic views were obtained (parasternal long axis, parasternal short axis, apical 4 chamber, apical long axis, apical 2 chamber and subcostal).

LV diameter and wall thicknesses were measured in the parasternal long axis view using 2D or M-mode measurements. LV mass was calculated using Devereux's formula and was indexed to body surface area<sup>36</sup>. Modified Simpson's biplane method was used to determine LV ejection fraction. Mitral inflow velocities and deceleration times were measured using pulsed wave Doppler in the apical 4 chamber view. Isovolumetric relaxation time was calculated using continuous wave Doppler, with the cursor midway between left ventricular outflow and mitral inflow. For tissue Doppler imaging, colour tissue Doppler loops were recorded using a frame rate >100 frames/sec. Myocardial longitudinal function was assessed from three consecutive cycles of tissue Doppler imaging in the apical 4 chamber, apical 2 chamber and apical long axis views.

Echocardiographic data was analysed using Echopac V9.01, GE, Horten, Norway. Peak systolic and early and late diastolic myocardial tissue velocities were obtained from the basal segment of all 6 LV walls. Myocardial deformation curves were obtained from the basal segment of all 6 LV walls. Wall motion was manually tracked throughout the cardiac cycle to maintain continuity of the sampling area. Data were excluded if a smooth curve was unobtainable, or if the angle between the ventricular wall and the scan line was  $>20^{\circ}$ . From these curves, peak systolic strain, systolic and early and late diastolic strain rates were obtained. Using data from each of the 3 cardiac cycles, the values from each wall were averaged to give a mean value.

*Statistical analysis* Continuous variables are presented as mean±standard deviation or median (interquartile range) if non-normally distributed; categorical variables are presented as frequencies and percentages. Univariate statistical comparisons of patient demographics between groups were conducted; for continuous variables using a one-way analysis of variance or a Kruskal-Wallis test when non-normal; for categorical variables, using a Chi-Squared test or a Fisher's Exact test when cell frequencies were insufficient. *P*-values were corrected for multiple comparisons using Sime's procedure and declared as significant if a *P*-value <0.017 was achieved.

A multiple linear regression model was fitted to investigate the association of BMI and metabolic syndrome on cardiac function. A two-way interaction of the main effects was investigated and retained if a *p*-value < 0.1 was obtained. Sensitivity analysis was conducted, adjusting the final model for age and gender. To explore the association between cardiac function and the number of metabolic syndrome components a univariate regression model was fitted. To investigate the relative strength of each metabolic syndrome component on cardiac function, all variables were standardised. A multivariable linear regression model was fitted including the metabolic syndrome components as main effects and then adjusted for age and gender. No interactions were investigated and results are expressed as the SD change in cardiac function associated with a one SD increment in the independent variable.

The association of liver fat with cardiac function was analysed using Spearman's correlation coefficient.

Model fit was assessed using QQ plots and standardised residuals against predicted means plots. Results were declared as significant if *P*<0.05. All statistical analyses were conducted

using Stata IC 13 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP).

Accepted manuscript

## 1 Results

2 *Clinical characteristics* (Table 1): 67 participants were recruited, of whom 2 were excluded  
3 (1 with left bundle branch block on electrocardiography, 1 unable to tolerate MR scanning).  
4 22 participants had  $\geq 3$  components of the metabolic syndrome: 21 (96%) had the waist  
5 circumference component, 18 (82%) the triglyceride component, 16 (73%) the blood pressure  
6 component, 12 (55%) the HDL cholesterol component and 8 (36%) the glucose component.  
7 Tables 1 & 2 and Figure 1 show mean results in the 4 groups formed by the two  
8 classifications, obese *vs.* non-obese and with (MS+) *vs.* without (MS-) metabolic syndrome.  
9 There were no significant differences between the four groups in terms of age, gender,  
10 systolic blood pressure, smoking status or cardiorespiratory fitness (Table 1). 10 year  
11 cardiovascular risk was highest in the MS+ groups.

12  
13 Higher proportions of those in the MS+ groups were taking medication for hypertension or  
14 hyperlipidaemia, reflecting the higher prevalence of these conditions. In *non-obese MS-* 1  
15 patient was taking an ACE inhibitor, 2 calcium channel antagonists and 6 statins; in *non-*  
16 *obese MS+* 2 were taking calcium channel antagonists, 3 statins, 1 diuretics and 1 ezetimibe;  
17 in *obese MS-* 1 patient was taking a statin; in *obese MS+*, 5 were taking ACE inhibitors, 1  
18 calcium channel antagonists, 2 beta-blockers, 4 statins and 1 ezetimibe.

19  
20 *Metabolic and biochemical data* (Table 1): There were significant differences in fasting  
21 triglyceride, HDL cholesterol and glucose concentrations between the four groups. HbA1C  
22 was  $<4.5$  in all individuals. Insulin concentrations were significantly different between  
23 groups ( $P=0.002$ ) with higher levels observed in the obese groups. There were no significant  
24 differences in adiponectin concentrations between the groups. The liver screen was negative  
25 for all patients included in the study.

26 *Body composition data* (Table 1): As expected, there was a significant difference in BMI  
27 across the four groups ( $P<0.001$ ). The obese groups had greater subcutaneous adipose tissue  
28 and visceral adipose tissue than the non-obese groups, although there was no significant  
29 difference in their relative proportion (VAT:SAT ratio). The intrahepatocellular lipid content  
30 was up to four times higher in the metabolically unhealthy patients in comparison to the  
31 metabolically healthy, however the difference across groups did not reach statistical  
32 significance ( $P=0.055$ ).

33

34 *Echocardiographic data*: In terms of left ventricular (LV) morphology, there were no  
35 significant differences across the groups in LV mass, posterior wall thickness, LV mass  
36 indexed to body surface area, LV septal wall thickness or LV internal diastolic diameter  
37 (Table 2).

38

39 In terms of LV systolic function, LV ejection fraction, systolic tissue velocity ( $S'$ ) and peak  
40 systolic longitudinal strain rate did not differ significantly across the groups, however, there  
41 was a significant difference in peak systolic (longitudinal) strain ( $P=0.001$ ) (Table 2 and  
42 Figure 1A). Linear regression showed that patients with MS had significantly lower peak  
43 systolic strain than those without ( $\Delta = -2.45$ ; 95% CI -3.74, -1.15;  $P<0.001$ ), with a  
44 progressive reduction in peak systolic strain as the number of MS components increased  
45 (Figure 2A, Table 4A). Of the metabolic syndrome components, triglycerides were  
46 significantly associated with peak systolic strain (for a one SD increase: -0.29; 95% CI -0.53,  
47 -0.04;  $P=0.021$ ) (Table 4B).

48

49 A higher BMI was associated with a lower peak systolic strain (for a unit increase in BMI,  
50  $\Delta = -0.12$ ; 95% CI -0.24, -0.004;  $P=0.043$ ) (Table 3). No significant interaction was found

51 between BMI and metabolic health. Peak systolic strain was significantly inversely correlated  
52 with liver fat ( $\rho = -0.35$ ;  $p=0.0042$ ) but not with VAT:SAT ratio ( $\rho = -0.12$ ;  $P=0.36$ ).

53

54 In terms of LV diastolic function, left atrial size and E/E' ratio were not significantly  
55 different across the groups (Table 2). Early (E') and late (A') diastolic tissue velocity and  
56 early diastolic strain rate (EDSR) were significantly different between groups (all  $P<0.01$ )  
57 with lower levels observed for the metabolically unhealthy. Linear regression showed that  
58 patients with metabolic syndrome had significantly lower E' (-1.89: 95% CI -2.73, -1.06;  
59  $P<0.001$ ) and EDSR (-0.28: 95% CI: -0.41, -0.15;  $P<0.001$ ) with a progressive reduction  
60 occurring in both cardiac parameters as the number of metabolic syndrome components  
61 increased (Figures 2B and 2C, Table 4). Of the metabolic syndrome components, glucose  
62 was significantly associated with early diastolic tissue velocity (for a one SD increase: -0.30:  
63 95% CI -0.54, -0.07;  $P=0.012$ )(Table 4) and systolic BP was significantly associated with  
64 EDSR (for a one SD increase: -0.44: 95% CI -0.76, -0.11;  $P=0.01$ )(Table 4).

65

66 As to the feasibility and reproducibility of echocardiography, for tissue velocity, global peak  
67 systolic strain (%) and strain rate ( $s^{-1}$ ), the intra-observer and inter-observer variability was  
68  $0.3 \pm 0.3$ ,  $1.3 \pm 0.5$  %,  $0.1 \pm 0.1 s^{-1}$  and  $0.4 \pm 0.3$ ,  $1.2 \pm 0.8$  % and  $0.3 \pm 0.2 s^{-1}$  respectively.  
69 Despite care taken during image acquisition, it was not possible to analyse 8% of left  
70 ventricular segments due to artefact and signal noise.

71

## 72 **Discussion**

73 This integration of detailed MRI and MRS analysis of body composition, echocardiographic  
74 assessment of myocardial function and evaluation of metabolic health has provided an  
75 opportunity to compare the determinants of myocardial function in four distinct phenotypes:

76 non-obese, mean age and BMI-matched metabolically healthy and metabolically unhealthy  
77 individuals (*non-obese MS-* and *MS+*, respectively) and obese, mean age and BMI-matched  
78 metabolically healthy and metabolically unhealthy individuals (*obese MS-* and *MS+*,  
79 respectively). We find that subclinical impairment of myocardial function is more closely  
80 associated with adverse metabolic health than with either obesity or BMI. Echocardiographic  
81 measures of both diastolic and systolic myocardial function were reduced in the  
82 metabolically unhealthy *vs* healthy groups, irrespective of obesity. We also find that  
83 metabolic health is more closely associated with increased liver fat than visceral fat  
84 deposition suggesting fat distribution is pivotal. These observational data help provide  
85 mechanistic insight into the pathophysiology of (obesity-related) CVD and potentially  
86 support the epidemiological observations of differential cardiovascular outcomes among  
87 metabolically healthy and unhealthy, lean and obese individuals.

88  
89 There is evidence from transgenic animal and human models that the capacity of SAT to  
90 expand with over-feeding determines to what extent excess lipids ‘spill over’ into ectopic  
91 sites (e.g. skeletal muscle, liver and cardiac muscle), and therefore whether obesity is  
92 metabolically healthy or unhealthy<sup>37,38</sup>. However, there is currently no consensus on how to  
93 define metabolic health or metabolically healthy obesity except for the inclusion of obesity  
94 ( $\text{BMI} > 30 \text{ kg/m}^2$ ) as a criterion. Some studies use the number of components of the MS,  
95 although with different diagnostic criteria<sup>39</sup>. Others have used measures of insulin resistance,  
96 with inconsistencies in definitions or diagnostic criteria (use of HOMA-IR, Matsuda index  
97 derived from an oral glucose tolerance test or from the glucose disposal rate). Several studies  
98 have used measures of inflammation including C-reactive protein measurements. Large  
99 variations in reported prevalence of MHO (i.e. our *obese MS-* group) are a function of the  
100 varied criteria used to define this phenotype<sup>39</sup>. Examining the impact of metabolic health on



101 myocardial function, Seo *et al* and Wong *et al* have demonstrated subclinical left ventricular  
102 dysfunction in patients with an increased metabolic burden compared to control groups<sup>22, 23</sup>.  
103 However these studies provide no data on different body composition phenotypes.

104

105 Our findings are consistent with several large-scale epidemiological studies suggesting that  
106 metabolically healthy (obese and non-obese) individuals have a lower risk of CVD than  
107 metabolically unhealthy (obese and non-obese) individuals<sup>12, 13, 15</sup> although this is not  
108 universally agreed<sup>18, 19</sup>. Morkedal *et al.* have demonstrated disparate effects on coronary  
109 heart disease and heart failure, which may account for the contrasting conclusions<sup>17, 40</sup>.

110

111 There are several potential mechanisms whereby metabolically unhealthy individuals may  
112 have impaired myocardial performance. The metabolically unhealthy group tended to have  
113 higher IHCL. Previous studies demonstrated that NAFLD is associated with increased levels  
114 of both intra-pericardial and extra-pericardial fat, which may adversely influence cardiac  
115 metabolism<sup>41-43</sup>. Thus metabolically unhealthy patients may also have increased liver and  
116 intra-myocardial triglyceride, leading to lipotoxicity and apoptosis of cardiac myocytes,  
117 potentially contributing to myocardial dysfunction<sup>44</sup>. However, in our study the difference in  
118 liver triglyceride did not reach statistical significance and we did not measure cardiac  
119 triglyceride. Metabolically unhealthy individuals have increased levels of inflammatory  
120 markers (interleukins, tumour necrosis factor-alpha and high sensitivity CRP), which can  
121 result in cardiac fibrosis and myocardial stiffening<sup>45, 46</sup> and increased circulating levels of the  
122 liver-secreted glycoprotein fetuin A which further induces subclinical inflammation and  
123 perturbs lipid and glucose metabolism<sup>6, 47</sup>. Hyperinsulinaemia and/or insulin resistance may  
124 also be implicated through abnormal LV energy metabolism<sup>41</sup>; indeed the cardiovascular  
125 benefit derived from bariatric surgery was strongly associated with reduced fasting insulin

126 concentration<sup>48</sup>. It has been suggested the protective effect of MHO on outcomes may be due  
127 to higher levels of cardiorespiratory fitness but we found no evidence to support this<sup>49</sup>.

128

129 These findings may have therapeutic implications. Diastolic myocardial abnormalities are  
130 associated with an increased risk of cardiovascular events and cardiac and all-cause mortality  
131<sup>50, 51</sup>. Early detection of myocardial dysfunction may provide an opportunity for patients to  
132 modify their lifestyles, thereby affording the opportunity for primary prevention and reducing  
133 mortality risk. In the SOS (Swedish Obese Subjects) trial, a prospective, controlled, long-  
134 term study of bariatric surgery in morbidly obese people, weight loss significantly improved  
135 cardiovascular outcomes<sup>48</sup>. Obesity is of course associated with a variety of other medical  
136 complications besides T2DM and CVD, not related to metabolic health but caused by the  
137 mechanical consequences of obesity e.g. obstructive sleep apnoea or lower limb osteoarthritis  
138 or by so far unknown mechanisms e.g. the association with certain types of cancer.  
139 Furthermore, there are significant functional and psychological sequelae of obesity, which are  
140 again unrelated to metabolic health. However these findings would indicate that preventative  
141 strategies in obesity must be driven by disease-specific end-points (metabolic, mechanical  
142 and functional end points) rather than simply driven quantitatively by weight loss.

143 The study has several limitations. Due to the small group sizes for the metabolic analysis, it  
144 was not possible to calculate robust estimates. Furthermore, the small sample size also led to  
145 large variability within the measurements. However the sample size is large enough to  
146 observe clinically and statistically significant differences between the groups. It must be  
147 noted that our cohort sampling strategy may have led to a bias in the metabolically unhealthy  
148 cohort toward an abnormal phenotype that is predisposed to myocardial functional  
149 abnormalities as these patients required specialist care. Likewise, recruitment of "healthy  
150 participants" from the community may have exaggerated the bias away from the null with

151 "healthy volunteer" bias. A further limitation is the possibility that a proportion of our  
152 participants may have undiagnosed atherosclerotic disease, contributing to the apparent  
153 myocardial abnormalities. However, it was not feasible to undertake coronary angiography or  
154 myocardial functional imaging in our participants to completely exclude silent coronary  
155 artery disease but all patients were screened with clinical assessment, echocardiography and  
156 exercise electrocardiography for evidence of coronary artery disease. We were also unable to  
157 measure serum fetuin A concentrations or markers of inflammation due to sample volume  
158 limitations.

159  
160 *Conclusion* The metabolic sequelae associated with unhealthy obesity appear to underlie the  
161 functional abnormalities in myocardial systolic and diastolic function, so metabolically  
162 healthy obese subjects have normal myocardial performance. This finding may help explain  
163 the epidemiological associations of metabolically healthy obesity with lower cardiovascular  
164 morbidity and mortality.

165

166 **Author contribution:**

167 All authors assisted in the writing of the manuscript. In addition, Rebecca Dobson collected  
168 and analysed the demographic, echocardiographic and cardiorespiratory exercise data. Julia  
169 Jones cross-checked the echocardiographic data acquisition and analysis. Victoria Sprung  
170 assisted with demographic and biochemical data collection and analysis. Andrew Irwin  
171 performed the anthropometric measurements and assisted with the biochemical data  
172 collection. Valerie Adams collected the magnetic resonance imaging data. Graham Kemp  
173 analysed the magnetic resonance imaging data. Mark Hamer and Christina Daousi assisted in  
174 the characterisation of the patient groups. Fariba Shojaee-Moradie and Margot Umpleby  
175 processed and analysed all of the biochemical samples. Malcolm Burgess and Daniel

176 Cuthbertson devised the protocol for the study, and oversaw all aspects of data acquisition  
177 and analysis.

178

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182 input.

183

184 **Conflict of interest** The authors do not have any conflict of interest to declare.

185

186 **Summary statement:**

187 The metabolic sequelae associated with unhealthy obesity appear to underlie the functional  
188 abnormalities in myocardial systolic and diastolic function, so metabolically healthy obese  
189 subjects have normal myocardial performance.

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**Figure 1** Influence of body mass index and metabolic syndrome on indices of myocardial function (**A** Peak systolic strain, PSS; **B** Early diastolic tissue velocity, E'; **C** Early diastolic strain rate, EDSR).

**Figure 2** Progressive effect of the number of components of metabolic syndrome on indices of myocardial function (**A**, peak systolic strain, PSS; **B** E' early diastolic tissue velocity; **C**, early diastolic strain rate).

Accepted manuscript

**Table 1** Clinical, biochemical, metabolic, and body composition characteristics of study participants.

	<b>Non-obese, MS- (n=28)</b>	<b>Non-obese, MS+ (n=10)</b>	<b>Obese, MS - (n=15)</b>	<b>Obese, MS+ (n=12)</b>	<b>P value</b>
Age (years)	50.0 ± 7.4	53.2 ± 13.2	45.7 ± 10.3	52.9 ± 5.9	0.127
Male gender (no. %)	14 (50%)	5 (50%)	6 (40%)	6 (50%)	0.939
Body mass index (kg m <sup>-2</sup> )	25.9 ± 2.3	26.8 ± 2.0	35.3 ± 4.1	33.6 ± 3.8	<b>&lt;0.001</b>
Fat free mass (kg)	52.8 (40.9-67.0)	55.8 (41.1-63.0)	54.3 (51.9-66.6)	62.0 (47.2-67.5)	0.957
Waist circumference (cm)	92.9 ± 8.2	98.8 ± 5.3	115.7 ± 10.9	108.3 ± 9.9	<b>&lt; 0.001</b>
Systolic blood pressure (mmHg) *	121 (118-132)	118 (119-143)	125 (119-130)	135 (123-144)	0.515
10 year risk of CV event (%)*	2.5 (1.0-4.9)	5.1 (2.7-8.8)	1.8 (1.4-3.4)	6.6 (3.5-9.1)	0.038
VO <sub>2</sub> max * (ml/fat free mass/min)	43.9 (40.7-49.4)	38.4 (32.5-42.5)	43.6 (40.3-45.9)	42.7 (36.2-45.7)	0.475
Current smoker (no. %)	3 (12%)	2 (20%)	1 (7%)	2 (17%)	0.724
History of hypertension (no. %)	3 (11%)	1 (10%)	0	6 (50%)	<b>0.004</b>
Total cholesterol (mmol/L)	5.7 ± 1.1	6.8 ± 2.3	5.1 ± 1.1	6.1 ± 2.3	0.098
HDL cholesterol (mmol/L)	1.8 ± 0.6	1.4 ± 0.5	1.3 ± 0.3	1.2 ± 0.3	<b>0.001</b>
LDL cholesterol (mmol/L)	3.2 ± 1.5	3.7 ± 3.0	3.2 ± 0.9	2.9 ± 2.9	0.820
Triglycerides (mmol/L)	1.4 ± 0.9	2.9 ± 1.7	1.5 ± 0.7	4.6 ± 5.2	<b>0.001</b>
Glucose (mmol/L)	4.7 ± 0.4	5.3 ± 0.5	4.9 ± 0.5	5.5 ± 0.7	<b>&lt; 0.001</b>
Gamma-glutamyl transpeptidase *	27 (16-48)	35 (27-81)	27 (19-46)	79 (37-98)	0.062
Aspartate aminotransferase *	24 (20-27)	24 (19-33)	18 (16-24)	25 (19-43)	0.521
Alanine aminotransferase *	24 (18-39)	28 (19-45)	20 (16-34)	40 (18-73)	0.322
Insulin (pmol/l) *	75.1 (58-96)	84.4 (54-111)	107.7 (91-130)	122.6 (103-139)	<b>0.002</b>
Adiponectin (ng/ml) *	8027 (6300-16240)	11869 (7246-4417)	7416 (6095-8600)	6344 (5044-1085)	0.300
Abdominal sub-cutaneous fat (SAT) (L)	6.1 ± 2.7	6.7 ± 1.3	12.9 ± 5.3	10.0 ± 3.2	<b>&lt; 0.001</b>
Abdominal visceral fat (VAT) (L)	3.6 ± 1.7	4.9 ± 1.5	5.8 ± 1.9	5.5 ± 2.3	<b>0.001</b>
VAT:SAT *	0.6 (0.4-0.8)	0.7 (0.5-1.1)	0.4 (0.4-0.6)	0.6 (0.3-1.0)	0.2845
Hepatic triglyceride level (%) *	2.0 (1.1-12.8)	12.4 (1.6-33.2)	3.3 (2.4-7.7)	11.8 (6.1-18.0)	0.055

Values quoted are mean ± standard deviation or as median with interquartile range (\*). P-values have been corrected for multiple comparisons using Sime's procedure and declared as significant if a p<0.017 was achieved.

**Table 2** Echocardiographic (M-mode, Doppler and tissue Doppler) characteristics of study participants.

	<b>Non-obese, MS- (n=28)</b>	<b>Non-obese, MS+ (n=10)</b>	<b>Obese, MS- (n=15)</b>	<b>Obese, MS+ (n=12)</b>	<b>P value</b>
LV mass (g)	137 ± 40	131 ± 29	166 ± 35	162 ± 18	0.029
LV mass index (g/m <sup>2</sup> )	72 ± 15	70 ± 16	74 ± 14	77 ± 9	0.675
LV septal wall thickness (cm)	1.0 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	0.427
LV posterior wall thickness (cm)	1.0 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	1.1 ± 0.2	0.047
LV internal diameter (cm)	4.1 ± 0.5	4.2 ± 0.6	4.7 ± 0.5	4.3 ± 0.5	0.061
LVEF (%)	65 ± 7	61 ± 9	62 ± 7	64 ± 7	0.577
LA area (cm <sup>2</sup> )	16 ± 4	14 ± 3	17 ± 3	17 ± 3	0.282
E (m/s)	0.68 ± 0.16	0.57 ± 0.10	0.67 ± 0.14	0.60 ± 0.14	0.141
A (m/s)	0.71 ± 0.14	0.74 ± 0.25	0.67 ± 0.15	0.76 ± 0.14	0.454
E/A ratio	1.1 ± 0.3	0.9 ± 0.2	1.2 ± 0.3	1.0 ± 0.3	0.021
E/E' ratio	11.4 ± 2.5	12.4 ± 2.5	10.1 ± 2.0	12.0 ± 2.3	0.079
IVRT (ms)	69 ± 20	72 ± 33	61 ± 28	61 ± 27	0.129
Peak global S' (cm/s)	6.1 ± 1.3	5.3 ± 0.9	5.5 ± 1.0	5.2 ± 0.7	0.040
Peak global E' (cm/s)	7.2 ± 1.7	4.7 ± 0.9	7.23 ± 1.5	5.9 ± 1.6	<b>&lt; 0.001</b>
Peak global A' (cm/s)	6.2 ± 1.6	7.4 ± 1.6	5.6 ± 1.6	7.0 ± 1.1	<b>0.015</b>
Peak systolic longitudinal strain (%)	19.6 ± 2.6	17.1 ± 2.2	18.9 ± 2.1	16.4 ± 2.4	<b>0.001</b>
Peak systolic longitudinal strain rate (s <sup>-1</sup> )	1.2 ± 0.3	1.3 ± 0.2	1.4 ± 0.4	1.1 ± 0.2	0.255
Peak early diastolic longitudinal strain rate (s <sup>-1</sup> )	1.7 ± 0.3	1.4 ± 0.2	1.6 ± 0.3	1.4 ± 0.1	<b>0.001</b>
Peak late diastolic longitudinal strain rate (s <sup>-1</sup> )	1.5 ± 0.4	1.6 ± 0.3	1.5 ± 0.6	1.4 ± 0.3	0.780

LV left ventricular, LVEF left ventricular ejection fraction, LA left atrial, IVRT iso-volumetric relaxation time

Values quoted are mean ± standard deviation. *P*-values have been corrected for multiple comparisons using Sime's procedure and declared as significant if a *P*<0.017 was achieved.

**Table 3** Regression analyses for peak systolic strain, early diastolic tissue velocity and early diastolic strain rate according to presence or absence of metabolic syndrome. The results show both the unadjusted estimates and those adjusted for age and gender.

	<i>Peak Systolic Strain</i>			<i>Early Diastolic Tissue Velocity</i>			<i>Early Diastolic Strain Rate</i>		
	Estimate	CI	<i>P</i> -value	Estimate	CI	<i>P</i> -value	Estimate	CI	<i>P</i> -value
<b>Unadjusted</b>									
Metabolic Syndrome	-2.45	(-3.67, -1.23)	<b>&lt;0.001</b>	-1.89	(-2.73, -1.06)	<b>&lt;0.001</b>	-0.28	(-0.41, -0.15)	<b>&lt;0.001</b>
BMI	-0.12	(-0.24, -0.01)	<b>0.031</b>	0.02	(-0.06, 0.10)	0.597	-0.001	(-0.01, 0.01)	0.822
<b>Adjusted</b>									
Metabolic Syndrome	-2.45	(-3.74, -1.15)	<b>&lt;0.001</b>	-1.61	(-2.43, -0.78)	<b>&lt;0.001</b>	-0.26	(-0.40, -0.13)	<b>&lt;0.001</b>
BMI	-0.12	(-0.24, -0.004)	<b>0.043</b>	-0.01	(-0.08, 0.70)	0.863	-0.002	(-0.02, 0.01)	0.637
Age	0.003	(-0.07, 0.08)	0.915	-0.06	(-0.10, -0.01)	<b>0.013</b>	-0.003	(-0.01, 0.003)	0.331
Gender	-0.46	(-1.68, 0.77)	0.458	0.37	(-0.41, 1.15)	0.341	0.04	(-0.09, 0.17)	0.522

**Table 4 A** Univariate analyses for peak systolic strain, early diastolic tissue velocity and early diastolic strain rate according to number of metabolic syndrome (MS) components. Results are presented as the mean difference in cardiac function reference to zero components and corresponding 95% confidence interval (CI). **B** Multivariable linear regression analysis for peak systolic strain, early diastolic tissue velocity and early diastolic strain rate, adjusted for age and gender. All variables have been standardised and results are expressed as the SD change in cardiac function associated to a one SD increment in independent variable.

**A**

	<i>Peak Systolic Strain</i>			<i>Early Diastolic Tissue Velocity</i>			<i>Early Diastolic Strain Rate</i>		
	Estimate	CI	<i>P</i>	Estimate	CI	<i>P</i>	Estimate	CI	<i>P</i>
MS components									
1	-0.50	(-2.63, 1.62)	0.637	-0.52	(-2.04, 1.00)	0.499	-0.04	(-0.27, 0.19)	0.741
2	-2.39	(-4.47, -0.31)	<b>0.025</b>	-0.64	(-2.13, 0.85)	0.392	-0.14	(-0.37, 0.08)	0.214
3	-3.53	(-5.71, -1.35)	<b>0.002</b>	-2.30	(-3.87, -0.75)	<b>0.004</b>	-0.37	(-0.61, -0.13)	<b>0.003</b>
4	-4.15	(-6.63, -1.66)	<b>0.001</b>	-2.25	(-4.03, -0.47)	<b>0.014</b>	-0.34	(-0.61, -0.07)	<b>0.015</b>
5	-7.87	(-12.69, -3.04)	<b>0.002</b>	-4.08	(-7.54, -0.62)	<b>0.021</b>	-0.42	(-0.95, 0.11)	0.117

**B**

	<i>Peak Systolic Strain</i>			<i>Early Diastolic Tissue Velocity</i>			<i>Early Diastolic Strain Rate</i>		
	Estimate	CI	<i>P</i> -value	Estimate	CI	<i>P</i> -value	Estimate	CI	<i>P</i> -value
Waist Circumference	-0.07	(-0.34, 0.20)	0.615	0.09	(-0.16, 0.35)	0.469	0.01	(-0.27, 0.30)	0.921
Systolic BP	-0.10	(-0.40, 0.21)	0.535	-0.24	(-0.53, 0.06)	0.110	-0.44	(-0.76, -0.11)	<b>0.010</b>
Diastolic BP	-0.19	(-0.50, 0.12)	0.232	-0.10	(-0.39, 0.20)	0.523	0.16	(-0.18, 0.49)	0.345
HDL Cholesterol	0.31	(-0.00, 0.63)	0.050	0.23	(-0.07, 0.53)	0.129	0.21	(-0.12, 0.55)	0.209
Triglycerides	-0.29	(-0.53, 0.04)	<b>0.021</b>	0.01	(-0.22, 0.24)	0.897	-0.17	(-0.43, 0.09)	0.187
Glucose	-0.09	(-0.34, 0.16)	0.463	-0.30	(-0.54, -0.07)	<b>0.012</b>	-0.02	(-0.28, 0.24)	0.882
Age	-0.04	(-0.30, 0.22)	0.768	-0.28	(-0.53, -0.03)	<b>0.030</b>	-0.15	(-0.43, 0.14)	0.302
Gender	0.35	(-0.17, 0.86)	0.183	0.42	(-0.07, 0.91)	0.090	0.36	(-0.19, 0.91)	0.199



