Sprung, VS, Bowden Davies, KA, Norman, JA, Thompson, A, Mitchell, KL, Wilding, JPH, Kemp, GJ and Cuthbertson, DJ

Metabolic syndrome is associated with reduced flow mediated dilation independent of obesity status.

Metabolic syndrome is associated with reduced flow mediated dilation independent of obesity status

Short title: Metabolic health, obesity and FMD

Victoria S Sprung*[1,2,3], Kelly A Bowden Davies*[1,3,4], Juliette A Norman[1,3], Andrew Thompson[5], Katie L Mitchell[6], John PH Wilding[1,3], Graham J Kemp[1,7], Daniel J Cuthbertson[1,3] (* joint first authors)

[1]Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, UK;

[2]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK;

[3]Obesity and Endocrinology Research Group, Liverpool University Hospitals NHS Foundation Trust, Liverpool, UK;

[4]School of Biomedical, Nutritional and Sport Sciences, Newcastle University, Newcastle upon Tyne, UK;

[5]Institute of Translational Medicine, University of Liverpool, Liverpool, UK;

[6]Institute of Psychology Health and Society, University of Liverpool, Liverpool, UK;

[7]Liverpool Magnetic Resonance Imaging Centre (LiMRIC), University of Liverpool, Liverpool, UK.

Corresponding author and address for reprints:

Victoria S Sprung, Research Institute for Sport and Exercise Sciences, Physical Activity Exchange, 5 Primrose Hill, Liverpool, L3 2AT

E-mail: V.S.Sprung@ljmu.ac.uk

Key words: endothelial function, flow mediated dilation (FMD), metabolic syndrome, obesity

Word count: 3362

Abstract

Background: Data suggest that metabolic health status, incorporating components of metabolic syndrome (MetS), predicts cardiovascular disease (CVD) risk better than body mass index (BMI). This study aims to explore the association of MetS and obesity with endothelial function, a prognostic risk factor for incident CVD.

Methods: Forty-four participants were phenotyped according to BMI as non-obese *vs.* obese (<30 or >30 kg/m^2) and according to the International Diabetes Federation criteria of MetS: ≤2 criteria MetS (MetS-) *vs.* ≥3 criteria MetS (MetS+); **i)** *non-obese MetS- vs*. **ii)** *non-obese MetS+* and **iii)** *obese MetS- vs.* **iv)** *obese MetS+*. Flow-mediated dilation (FMD), body composition including liver fat (magnetic resonance imaging and spectroscopy), dietary intake, intensities of habitual physical activity and cardio-respiratory fitness, were determined. Variables were analysed using a one-factor between-groups analysis of variance (ANOVA) and linear regression; mean (95% CI) are presented.

Results: Individuals with MetS+ displayed lower FMD than those with MetS-. For non-obese individuals mean difference between MetS+ and MetS- was 4.1% [(1.0, 7.3); P=0.004] and obese individuals had a mean difference between MetS+ and MetS- of 6.2% [(3.1, 9.2); P<0.001]. Although there was no association between BMI and FMD (P=0.27), an increased number of MetS components was associated with a lower FMD (P=0.005), and after adjustment for age and sex, 19.7% of the variance of FMD was explained by MetS whereas only 1.1% was explained by BMI.

Conclusions: In this study cohort, components of MetS, rather than obesity *per se*, contribute to reduced FMD, which suggests a reduced bioavailability of nitric oxide and thus increased risk of CVD.

Introduction

Obesity is strongly linked with an adverse cardio-metabolic profile and a number of chronic diseases including type 2 diabetes (T2D) and cardiovascular disease (CVD) (1, 2). Body mass index (BMI) is widely used clinically to determine the risk of complications relating to an excess accumulation of fat: the higher an individual's BMI, the greater their risk of obesity-related complications (3). In contrast, some data suggest that adults with a higher BMI can have a reduced mortality risk compared to non-obese counterparts, an puzzling finding known as the 'obesity paradox', shown in T2D (4) and CVD (5). Metabolic syndrome (MetS) is defined as a cluster of risk factors including abdominal obesity, hypertension, dyslipidemia and insulin resistance. The International Diabetes Federation (IDF) report the role of MetS in the CVD epidemic, and highlight the importance of understanding the further role of vascular regulation and body fat distribution (6).

While obesity also has mechanical and psychological implications, there is a growing recognition that not all obese individuals are 'unhealthy', and not all non-obese individuals are 'healthy', with respect to their metabolic profiles. Some data suggest there is a lower T2D/CVD risk in overweight/obese people when there is an absence of Mets components but that there is a higher T2D/CVD risk in normal weight people in the presence of one/more MetS components (7). This has led to the identification of sub-phenotypes within BMI (i.e. metabolically healthy *vs.* unhealthy obesity and healthy *vs.* unhealthy normal weight), categories determined by the presence/absence of components of the MetS. There is currently no consensus on a precise definition for these terms/BMI sub-phenotypes, researchers questioning the degree of cardiovascular protection conferred by being metabolically healthy and many suggesting that metabolically healthy obesity represents a 'transient metabolic state' in a progressive and inevitable journey towards T2D and CVD (8-11).

When considering cardiovascular risk in these metabolically phenotyped groups, previous research has largely focused on the overall incidence of CVD (8, 9, 12-14). While this is important, endothelial function, an early, prognostic and reversible marker of CVD, is much less explored. The endothelium plays a pivotal role in vascular homeostasis (15), and brachial artery flow-mediated dilation (FMD) is predictive of future CVD risk (16). Endothelial dysfunction, characterised by decreased nitric oxide

(NO) bioavailability, resulting in vascular inflammation, vasoconstriction, and thrombosis (17, 18), has been mechanistically related to the greater risk of cardiovascular events in people with obesity (19, 20). To put this measurement into a pathophysiological perspective, a meta-analysis reports that a 1% increase in FMD is associated with a pooled relative risk reduction in CVD of 0.87 (95% CI, 0.83-0.91) (21). Furthermore, there is evidence that FMD has independent prognostic value to predict cardiovascular events that may better that of traditional risk factors (16). Evidence is lacking on how MetS alone, or in combination with obesity, affects FMD.

The aim of this cross-sectional study was to explore the impact of obesity and MetS on endothelial function using measurements of FMD. Careful phenotypic characterisation of participants was undertaken incorporating assessments of lifestyle (including dietary records and physical activity by objective monitoring), measurements of cardio-respiratory fitness (CRF; by $\dot{V}O_2$), obesity and body composition (liver fat determined by MR scanning) and of cardio-metabolic health (including assessment of MetS using International Diabetes Federation criteria).

Materials and Methods

Participants

Forty-four individuals (30 male, 14 female) with a mean age of 46±11 years were recruited via local advertisement across hospital departments and university campuses. Exclusions included cardiovascular, respiratory, kidney, liver and/or endocrine complications, smoking and >14 units/week of alcohol consumption; all participants were medication free. The study conformed to the *Declaration of Helsinki* and was approved by the North West Research Ethics Committee (14/NW/1145; 14/NW/1147; 15/NW/0550). All participants were informed of the protocol verbally and in writing before providing written informed consent prior to any assessments.

Study design

All participants completed habitual monitoring of physical activity (PA) and dietary consumption over a period of 4 days (including one weekend day), followed by two assessment visits. The first assessment

visit, at Aintree University Hospital, comprised anthropometry, fasting biochemistry, and cardio-respiratory fitness ($\dot{V}O_2$ peak). The second assessment at the University of Liverpool MRI Centre (LiMRiC) comprised flow mediated dilation (FMD) and proton magnetic resonance spectroscopy (^{1}H-MRS). Prior to each study visit, participants were required to fast overnight for >8 hours, abstain from alcohol and caffeine for 24 hours and from exercise for 48 hours; up to 500ml of water was permitted in the morning of a visit.

Brachial artery flow mediated dilation (FMD)

Endothelial function was assessed by measuring FMD in response to a 5 min ischaemic stimulus, induced by forearm cuff inflation placed immediately distal to the olecranon process, as previously described (22). Briefly, baseline images were recorded for 1 min prior to forearm cuff inflation (~220 mmHg) for 5 min. Artery diameter and blood flow velocity recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter. Peak brachial artery diameter and blood flow velocity, and the time taken to reach these peaks following cuff release were recorded. Post-test analysis of brachial artery diameter was undertaken using custom-designed automated edge-detection and wall-tracking software.

Cardio-respiratory fitness

$\dot{V}O_2$ peak was determined using the modified Bruce protocol on a treadmill (Model 77OCE, RAM Medisoft Group, Manchester, UK) with breath-by-breath monitoring and analysis of expiratory gases and ventilation (Love Medical Cardiopulmonary Diagnostics, Manchester, UK). The $\dot{V}O_2$ peak was determined by any of the following: respiratory exchange ratio >1.15, heart rate >90% predicted maximum, plateau in $\dot{V}O_2$, or exhaustion, data is presented relative to total body mass and lean mass determined by BIA.

Biochemical measures

Blood samples were collected and analysed using the Olympus AU2700 analyser (Beckman Coulter, High Wycombe, UK) with standard proprietary reagents as follows: glucose with hexokinase, total

cholesterol and HDL-cholesterol with cholesterol esterase/oxidase and triglyceride with glycerol kinase. LDL-cholesterol was calculated according to the Friedewald formula.

Anthropometric measures

Height was measured while participants were standing upright, with their back and head straight so that their Frankfurt plane was horizontal, to the nearest 0.5 cm using a stadiometer (Model 220, Seca, Germany). Waist circumference measurements (at the umbilicus) and hip circumference measurements (at the greater trochanter) were taken in duplicate. After 5 minutes rest, blood pressure was determined as an average of 3 measurements using an automated monitor (Dinamap, G & E Medical, USA). Bio-impedance (BIA; Tanita, BC 420, Dolby Medical Stirling, UK) was used in all participants to quantify body composition; those who were safe for MR scanning had the more detailed measures outlined below.

MR determination of adipose tissue and liver fat

Magnetic resonance methods were performed using a 1.5 T Siemens Symphony MRI scanner (Siemens Medical Solutions, Erlangen, Germany) as previously described (23-25). Volumetric analysis of adipose tissue was used to quantify regional fat; proton magnetic resonance spectroscopy (^{1}H-MRS) was used to determine intrahepatic cellular lipid (IHCL): 'liver fat' percentage relative to water.

Habitual physical activity monitoring and dietary analysis

Physical activity monitoring PA was monitored using a validated (26) SenseWear mini armband (BodyMedia Inc., Pittsburgh, PA, USA). Participants were requested to wear the armband at all possible times (except when bathing and swimming (27)), and wear time (recorded as ~98%) was monitored using SenseWear Professional software (version 8.0). Data collected from the armband included: daily average step count, total energy expenditure, active energy expenditure and time spent in different intensity levels of PA including: sleep, lying down, sedentary, light, moderate, vigorous and very vigorous (<1.5, >1.5-3, >3-6, >6-9, >9 metabolic equivalents respectively).

147 *Dietary analysis* Total energy consumption, carbohydrate, protein and fat content were determined from
148 dietary records by a registered nutritionist (KLM) using Nutritics (Nutrition Analysis Software for
149 Professionals; https://www.nutritics.com/p/home; accessed 17/07/2017).

150 **Individual phenotyping**

151 Following physiological assessment, participants were phenotyped according to obesity status and
152 presence or absence of MetS. Individuals were characterised into one of four groups based on BMI
153 (non-obese <30 *vs* obese ≥30 kg/m^2) and the presence or absence of MetS according to IDF criteria (6);
154 we refer to these groups as i) 'non-obese MetS-', ii) 'non-obese MetS+', iii) 'obese MetS-' and iv)
155 'obese MetS+.

156 **Sample size calculation**

157 The primary outcome variable was FMD. Based on previous data (22) and a two-sample t-test (post-
158 hoc comparison) with a 0.05 two-sided significance level, a sample size of 10 per group would have
159 80% power to detect a difference in means of 3.5%, assuming a common standard deviation of 2.5%
160 (G*Power 3.1.5 (28)).

161 **Statistical analysis**

162 All data were explored for normality by visual inspection. Comparisons of group demographics were
163 explored using one factor between-groups analysis of variance (ANOVA) for continuous variables and
164 chi-squared for categorical outcomes. The main outcome variables (e.g. FMD, cardio-respiratory
165 fitness, and liver fat) were analysed using a one factor between-groups ANOVA, with Bonferroni
166 correction for multiple comparisons. All FMD data were analysed, and are presented, as covariate-
167 controlled for baseline artery diameter measured prior to the introduction of hyperaemia in each test;
168 this approach is more accurate for scaling changes in artery diameter than simple percentage change
169 (29, 30). Regression models, adjusted for age and sex, were fitted to categories of BMI and number of
170 MetS components to explore the association with FMD. Finally, we estimated the amount of variance
171 explained in FMD by BMI and number of MetS components using an incremental sums of squares
172 approach. Distribution data are presented as mean±SD and outcomes of ANOVA as mean (95% CI).

The alpha level of statistical significance was set at $P<0.05$. Statistical analysis was performed using SPSS for Windows (Version 24.0, SPSS, Chicago, IL, USA).

Results

Participant characteristics

Gender, age and BMI for each of the groups are summarised in Table 1. The differences between the mean BMI and components of MetS were in line with WHO and IDF classifications, respectively. Age and gender were not significantly different between groups ($P>0.05$). Overall, habitual physical activity did not differ between BMI categories of MetS; however, sedentary behaviour was greater in both of the obese groups compared to non-obese MetS- ($P\leq0.028$) and light intensity PA was lower ($P\leq0.001$). Total energy consumption, carbohydrate, protein and fat did not differ significantly between groups ($P>0.05$) (Table 1). Macronutrient percentages of all groups combined were 53±10% carbohydrate, 26±9% protein, and 21±4% fat.

Flow mediated dilation

FMD was higher in the MetS- individuals in both the non-obese and obese groups (Figure 1A). The non-obese MetS- individuals had a greater FMD than their MetS+ counterparts [4.1% (1.0, 7.3; $P=0.004$)] and obese MetS+ [4.3% (1.3, 7.3; $P=0.002$)], with no difference compared to obese MetS-. The mean difference between the obese MetS- and obese MetS+ was 6.2% (3.1, 9.2; $P<0.0001$), and non-obese MetS+ was 6.0% (2.8, 9.2; $P<0.0001$). There was no significant difference between the MetS+ groups. An increased number of MetS components was associated with a lower FMD ($P=0.04$; Figure 2A), differences were observed from the healthy reference group (0 components) for those with 3 ($P=0.005$) or ≥4 ($P=0.023$) components of MetS. In contrast, when using a healthy BMI as a reference group (18.5-24.9 kg/m^2), none of the categories were statistically different for FMD ($P=0.27$; Figure 2B). Furthermore, there was no correlation between BMI and FMD ($r^2=0.01$; $P=0.512$; Figure 2C). The variance of FMD explained, when controlling for age and sex, by BMI was 1.1% and by MetS was 19.7%. There were negligible and non-statistically significant differences in baseline or peak arterial

198 diameter, shear rate or time to peak between groups (P>0.05). All vascular data are summarised in
199 Table 2.

200 **Cardio-respiratory fitness (CRF)**

201 $\dot{V}O_2$ peak was greatest in non-obese MetS-, similar in non-obese MetS+ and obese MetS-, and lowest
202 in obese MetS+ (Figure 1B). Obese MetS+ individuals had a significantly lower CRF than non-obese
203 MetS- by 13.9 $mL \cdot min^{-1} \cdot kg^{-1}$ (6.0, 21.7; P<0.0001). Differences between the MetS- groups just fell
204 short of conventional statistical significance (P=0.056). The between-group differences are also
205 consistent when $\dot{V}O_2$ peak is expressed relative to lean mass. Interestingly, when FMD was adjusted for
206 individual differences in CRF the difference in FMD between groups remained and was of similar
207 magnitude (P<0.05).

208 **MRS determination of liver fat**

209 Group differences in liver fat were non-significant (P=0.099), however the mean values for each group
210 suggest a trend toward greater levels of liver fat in the MetS+ groups (Figure 1C).

211 **Assessment of body composition (BIA and MRI)**

212 *BIA* Total body fat measured in percentage and mass was significantly lower in the non-obese groups
213 compared to the obese groups (P<0.05; Table 3), however there were no significant differences between
214 MetS- versus MetS+ within the BMI groups. Visceral fat rating was significantly lower in the non-
215 obese MetS- group (P<0.05) but there were no other significant differences. No significant differences
216 were observed in BIA derived fat free mass or muscle mass between any of the groups.

217 *MRI* Total subcutaneous adipose tissue (SAT) and whole-body fat were significantly lower in the non-
218 obese MetS- than both obese groups (P<0.05). Abdominal SAT was lower in both non-obese groups
219 (P<0.05). Visceral adipose tissue was significantly lower in non-obese MetS- when compared to obese
220 MetS-. Of note, there were no significant differences between MetS- versus MetS+ within the BMI
221 groups but the data was not available for all participants.

Discussion

The aim of study was to determine to what extent MetS or obesity are associated with endothelial function as a surrogate marker of cardiovascular health. The integration of measures of dietary intake and domains of physical activity, biochemical and anthropometric measures including characterisation of components of MetS (IDF consensus) and body composition using magnetic resonance imaging and spectroscopy enabled comprehensive phenotyping of individuals within age- and sex-matched groups. The major finding was that individuals with MetS (i.e. *metabolically unhealthy* individuals) exhibit endothelial dysfunction (lower FMD), irrespective of their obesity status. In contrast, individuals without MetS (i.e. *metabolically healthy* individuals), had relatively preserved endothelial function (higher FMD). Convincingly, MetS status is significantly associated with endothelial function whereas BMI is not. Alarmingly, the FMD differences between the metabolic phenotypes in this study (MetS+ *vs*. MetS-) was identified as ~4-6%, with indication towards an increased risk of incident CVD. Our data highlight the association of increased CVD risk in metabolically unhealthy individuals, irrespective of their obesity status, and suggest that preserved metabolic health may indeed confer a degree of cardiovascular protection and attenuate (but not necessarily eliminate) the risks associated with obesity.

Our findings support the existence of distinct phenotypes within different categories of BMI, where MetS+ individuals exhibit a cluster of metabolic abnormalities (e.g. insulin resistance, hypertension and dyslipidemia). The data suggests that endothelial dysfunction is not explained by the absolute fat mass, but rather is determined (in part) by associated cardio-metabolic dysfunction/risk factors alongside known and so far unidentified factors. Individuals with MetS (non-obese and obese) have an unfavourable cardiovascular profile with a lower FMD (an early marker of atherosclerotic disease), while those without MetS (non-obese and obese) have comparable endothelial function. This phenomenon whereby other measures of cardiovascular function differ between *metabolically healthy* versus *metabolically unhealthy* obese adults is observed not only for macrovascular complications, as here and in previous investigations (31) but also for microvascular function (32). Using identical phenotypic classification, we have previously shown similar trends for myocardial systolic and diastolic dysfunction (measured by tissue doppler imaging with transthoracic echocardiography). We observed

impaired myocardial performance related to poor metabolic health but not related to levels of fat mass nor to differing amounts of ectopic fat stores (visceral and liver) (33). Mechanisms such as inflammation, increased circulating free fatty acids and pro-inflammatory cytokines have been proposed as mediators of this impact on cardiovascular risk (34).

The increasing interest in the study of differing metabolic phenotypes has led many to investigate putative behavioural determinants (e.g. physical activity, diet), however findings remain equivocal (35). We found no difference between the groups for PA (even when domains of physical activity were analysed) nor in their total energy intake/macronutrient intake. We note the disparity between energy intake and expenditure, ostensibly showing the participants in a negative energy balance; however, we recognise that energy intake is largely under-reported, particularly in obese adults. Dietary assessment was not a primary outcome variable and was assessed using the best resources available. Cardiorespiratory fitness was highest in the healthy reference group (non-obese MetS-) and lowest in the obese MetS+ group perhaps as expected, although interestingly both groups of non-obese adults and obese MetS- had comparable fitness. A higher cardiorespiratory fitness is typically associated with a better metabolic profile and reduced CVD risk (36), and our data supports this. In the MetS- obese group, we observed FMD ~15%, this data is somewhat striking but not abnormal. While obesity has many comorbidities, the role of fitness is also recognised as an important prognostic marker that differs across phenotypes (37) and some researchers suggest that recommendations to reduce mortality risk should focus on increasing fitness rather than on weight loss (38). Although we interpret this data with caution it is reasonable to suggest that intrinsic biological mechanisms may contribute to the differences we observe in these phenotypes (such as subacute inflammation, levels of oxidative stress, levels of different regulatory microRNAs and adiponectin(39)).

Many authors suggest that cross-sectional observations of preserved metabolic health in obese adults likely represent a transient phenomenon and question their clinical utility and significance. Longitudinal studies are needed to address these important questions. One such study found that 50% of healthy obese progressed to an unhealthy metabolic status over a 10-year follow up period (40). Interpretation of such studies is hampered by the lack of an agreed definition of ‘metabolically healthy’ (41);

conclusions about the degree of protection against CV disease and T2D will clearly depend on the criteria of metabolic health. We opted for the IDF classification of MetS, as the most recent and internationally harmonised definition. Furthermore, FMD is often (as here) studied in the fasted state, yet humans spend a significant of their time in a post-prandial state. Examination of post-prandial endothelial function between the phenotypes described in this manuscript maybe warranted and highlight more profound differences. In particular, the post-prandial state following consumption of a high-fat meal, may be associated with oxidative stress and inflammation, which are potentially important mediators of impaired postprandial vascular function and may differ between these individuals.

We acknowledge limitations of the current study, including a relatively small sample size, its cross-sectional design. Participants were recruited via local advertisement, which limits external validity as this yielded only white Europeans; defining a causal relationship with validity at a global population level is therefore not possible. However, we believe the study has significant merit. The study was powered to detect meaningful differences in the primary outcome measure (FMD). It should be acknowledged that there are outliers (Figure 2C), but that removal of these data does not alter the outcome of statistical analyses, so the decision was made to include the data set in its entirety. It utilises objective monitoring of physical activity, a gold standard measurement of cardio-respiratory fitness combined with assessment of body composition including regional (VAT/SAT) and tissue specific (liver) fat and a novel prognostic marker for cardiovascular health, that of endothelial function. Liver fat was not our primary outcome and thus the study was not adequately powered for this outcome. Importantly, this measure was utilised to comprehensively phenotype the individuals. Based on previous work regarding fat deposition, we expected a greater propensity to deposit fat within the liver in the metabolically unhealthy (MetS+) phenotypes. This propensity was observed but did not reach statistical significance between groups. Whilst the present results demonstrate that endothelial function is impaired in those with MetS, larger studies are required with a follow-up design to determine measured cardiovascular function rather than predicted CVD. This has been undertaken to a limited extent in a multi-ethnic population study but did not include the classification of sub-phenotypes (42).

In conclusion, the current study provides evidence for impaired NO-mediated endothelial function in both non-obese and obese individuals who have multiple components of MetS (with comparable cardiovascular function in adults without MetS regardless of obesity status). Considering the definition of obesity as a disease (WHO), that recognises the impact of excessive fat accumulation on end-organ complications and the need to triage medical resources to those most in need, earlier detection and more focussed interventions in metabolically unhealthy individuals should be a priority rather than using a purely BMI-centric approach.

Declaration of interest

The authors have nothing to disclose.

Funding

This work was supported by Diabetes UK (grant number 13/0004719), with additional funding support from the MRC-Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing (CIMA), Institute of Ageing and Chronic Disease, University of Liverpool.

Acknowledgements

The authors would like to acknowledge Mr Andrew Irwin (Obesity and Endocrinology, Liverpool University Hospital NHS Foundation Trust, Liverpool, UK), for clinical assistance and Ms Val Adams, for radiographic expertise at LiMRIC. Further to Ms Olivia Pepper, Mr Daniel Watkin and Mr Rory O'Dolan, for their assistance during data collection.

References

1. Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis RJ, et al. Body-mass index and mortality among 1.46 million white adults. New England Journal of Medicine. 2010;363(23):2211-9.
2. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. Jama. 2013;309(1):71-82.
3. Troiano RP, Frongillo EA, Jr., Sobal J, Levitsky DA. The relationship between body weight and mortality: a quantitative analysis of combined information from existing studies. Int J Obes Relat Metab Disord. 1996;20(1):63-75.
4. Carnethon MR, Rasmussen-Torvik LJ, Palaniappan L. The obesity paradox in diabetes. Current cardiology reports. 2014;16(2):446.
5. Lavie CJ, Milani RV, Ventura HO. Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. J Am Coll Cardiol. 2009;53(21):1925-32.
6. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.
7. Caleyachetty R, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, Balachandran K, et al. Metabolically healthy obese and incident cardiovascular disease events among 3.5 million men and women. Journal of the American College of Cardiology. 2017;70(12):1429-37.
8. Phillips CM. Metabolically healthy obesity across the life course: epidemiology, determinants, and implications. Annals Of The New York Academy Of Sciences. 2017;1391(1):85-100.
9. Stefan N, Schick F, Häring H-U. Causes, Characteristics, and Consequences of Metabolically Unhealthy Normal Weight in Humans. Cell Metabolism. 2017;26(2):292-300.
10. Eckel N, Li Y, Kuxhaus O, Stefan N, Hu FB, Schulze MB. Transition from metabolic healthy to unhealthy phenotypes and association with cardiovascular disease risk across BMI categories in 90 257 women (the Nurses' Health Study): 30 year follow-up from a prospective cohort study. The Lancet Diabetes & Endocrinology. 2018;6(9):714-24.
11. Khan SS, Ning H, Wilkins JT, Allen N, Carnethon M, Berry JD, et al. Association of Body Mass Index With Lifetime Risk of Cardiovascular Disease and Compression of MorbidityAssociation of Body Mass Index With Cardiovascular Disease MorbidityAssociation of Body Mass Index With Cardiovascular Disease Morbidity. JAMA Cardiology. 2018;3(4):280-7.
12. Morkedal B, Vatten LJ, Romundstad PR, Laugsand LE, Janszky I. Risk of myocardial infarction and heart failure among metabolically healthy but obese individuals: HUNT (Nord-Trondelag Health Study), Norway. J Am Coll Cardiol. 2014;63(11):1071-8.
13. Hamer M, Stamatakis E. Metabolically healthy obesity and risk of all-cause and cardiovascular disease mortality. J Clin Endocrinol Metab. 2012;97(7):2482-8.

14. Mongraw-Chaffin M, Foster MC, Anderson CAM, Burke GL, Haq N, Kalyani RR, et al. Metabolically Healthy Obesity, Transition to Metabolic Syndrome, and Cardiovascular Risk. J Am Coll Cardiol. 2018;71(17):1857-65.
15. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980;288(5789):373-6.
16. Green DJ, Jones H, Thijssen D, Cable NT, Atkinson G. Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? Hypertension. 2011;57(3):363-9.
17. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, et al. The assessment of endothelial function: from research into clinical practice. Circulation. 2012;126(6):753-67.
18. Widlansky ME, Gokce N, Keaney JF, Jr., Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol. 2003;42(7):1149-60.
19. De Boer MP, Meijer RI, Wijnstok NJ, Jonk AM, Houben AJ, Stehouwer CD, et al. Microvascular dysfunction: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. Microcirculation. 2012;19(1):5-18.
20. Jonk AM, Houben AJ, de Jongh RT, Serne EH, Schaper NC, Stehouwer CD. Microvascular dysfunction in obesity: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. Physiology (Bethesda, Md). 2007;22:252-60.
21. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging. 2010;26(6):631-40.
22. Sprung VS, Jones H, Pugh CJ, Aziz NF, Daousi C, Kemp GJ, et al. Endothelial dysfunction in hyperandrogenic polycystic ovary syndrome is not explained by either obesity or ectopic fat deposition. Clin Sci (Lond). 2014;126(1):67-74.
23. Cuthbertson DJ, Shojaee-moradie F, Sprung VS, Jones H, Pugh CJA, Richardson P, et al. Dissociation between exercise-induced reduction in liver fat and changes in hepatic and peripheral glucose homeostasis in obese patients with Non-Alcoholic Fatty Liver Disease. Clin Sci (Lond). 2016;130(2):93-104.
24. Cuthbertson DJ, Irwin A, Gardner CJ, Daousi C, Purewal T, Furlong N, et al. Improved glycaemia correlates with liver fat reduction in obese, type 2 diabetes, patients given glucagon-like peptide-1 (GLP-1) receptor agonists. PLoS One. 2012;7(12):e50117.
25. Todd CS, Jones HE, Garber TC, Afnan-Holmes H, Woolgar H, Bekker LG, et al. Awareness and Interest in Intrauterine Contraceptive Device Use among HIV-Positive Women in Cape Town, South Africa. Infect Dis Obstet Gynecol. 2012;2012:956145.
26. Johannsen DL, Calabro MA, Stewart J, Franke W, Rood JC, Welk GJ. Accuracy of armband monitors for measuring daily energy expenditure in healthy adults. Med Sci Sports Exerc. 2010;42(11):2134-40.
27. Scheers T, Philippaerts R, Lefevre J. Variability in physical activity patterns as measured by the SenseWear Armband: how many days are needed? Eur J Appl Physiol. 2012;112(5):1653-62.
28. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behavior research methods. 2009;41(4):1149-60.
29. Atkinson G, Batterham AM, Thijssen DH, Green DJ. A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. J Hypertens. 2013;31(2):287-91.
30. Atkinson G, Batterham AM. Allometric scaling of diameter change in the original flow-mediated dilation protocol. Atherosclerosis. 2013;226(2):425-7.
31. Khan UI, Wang D, Thurston RC, Sowers M, Sutton-Tyrrell K, Matthews KA, et al. Burden of subclinical cardiovascular disease in "metabolically benign" and "at-risk"

overweight and obese women: the Study of Women's Health Across the Nation (SWAN). Atherosclerosis. 2011;217(1):179-86.
32. Brant LC, Wang N, Ojeda FM, LaValley M, Barreto SM, Benjamin EJ, et al. Relations of Metabolically Healthy and Unhealthy Obesity to Digital Vascular Function in Three Community-Based Cohorts: A Meta-Analysis. Journal of the American Heart Association. 2017;6(3).
33. Dobson R, Burgess MI, Sprung VS, Irwin A, Hamer M, Jones J, et al. Metabolically healthy and unhealthy obesity: differential effects on myocardial function according to metabolic syndrome, rather than obesity. International journal of obesity (2005). 2016;40(1):153-61.
34. Panagiotakos DB, Pitsavos C, Yannakoulia M, Chrysohoou C, Stefanadis C. The implication of obesity and central fat on markers of chronic inflammation: The ATTICA study. Atherosclerosis. 2005;183(2):308-15.
35. Phillips CM, Dillon C, Harrington JM, McCarthy VJ, Kearney PM, Fitzgerald AP, et al. Defining metabolically healthy obesity: role of dietary and lifestyle factors. PLoS One. 2013;8(10):e76188.
36. Myers J, McAuley P, Lavie CJ, Despres JP, Arena R, Kokkinos P. Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: their independent and interwoven importance to health status. Progress in cardiovascular diseases. 2015;57(4):306-14.
37. Ortega FB, Cadenas-Sanchez C, Sui X, Blair SN, Lavie CJ. Role of Fitness in the Metabolically Healthy but Obese Phenotype: A Review and Update. Progress in cardiovascular diseases. 2015;58(1):76-86.
38. Barry VW, Baruth M, Beets MW, Durstine JL, Liu J, Blair SN. Fitness vs. fatness on all-cause mortality: a meta-analysis. Progress in Cardiovascular Diseases. 2014;56:382-90.
39. Muñoz-Garach A, Cornejo-Pareja I, Tinahones FJ. Does metabolically healthy obesity exist? Nutrients. 2016;8(6):320.
40. Kouvari M, Panagiotakos DB, Yannakoulia M, Georgousopoulou E, Critselis E, Chrysohoou C, et al. Transition from metabolically benign to metabolically unhealthy obesity and 10-year cardiovascular disease incidence: the ATTICA cohort study. Metabolism. 2019.
41. Phillips CM. Metabolically healthy obesity: definitions, determinants and clinical implications. Reviews in endocrine & metabolic disorders. 2013;14(3):219-27.
42. Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. Circulation. 2009;120(6):502-9.

Figure legends

Figure 1. Individual participant plots for A) flow mediated dilation (FMD), B) cardio-respiratory fitness ($\dot{V}O_2$ peak) and, C) 'liver fat' intrahepatic cellular lipid (IHCL) percentage. Black circles, MetS-; grey circles, MetS+; non-obese are grouped left and obese are grouped right. Group mean ± SD data is presented as bar. *P<0.05, group difference.

Figure 2. Individual plots for all forty-four participants A) flow mediated dilation (FMD) categorised for number of metabolic syndrome (MetS) components, B) FMD categorised for (BMI) classifications and C) showing individual points for flow mediated dilation (FMD) and body mass index (BMI).