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

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Abstract

Background: Data suggest that metabolic health status, incorporating components of metabolic syndrome (MetS), predicts cardiovascular disease (CVD) risk better than BMI. This study explored 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/m²) and according to the International Diabetes Federation criteria of MetS: ≤2 criteria MetS (MetS–) vs ≥3 criteria MetS (MetS+); (1.) non-obese MetS– vs (2.) non-obese MetS+ and (3.) obese MetS– vs (4.) obese MetS+. Flow-mediated dilation (FMD), body composition including liver fat (MRI and spectroscopy), dietary intake, intensities of habitual physical activity and cardio-respiratory fitness were determined. Variables were analysed using a one-factor between-groups 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.

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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). 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 with 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, 9, 10, 11).

When considering cardiovascular risk in these metabolically phenotyped groups, previous research has largely focused on the overall incidence of CVD (8, 9, 12, 13, 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 than 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).

Subjects 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 1 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 (^1H -MRS). Prior to each study visit, participants were required to fast overnight for >8 h, abstain from alcohol and caffeine for 24 h and from exercise for 48 h; up to 500 mL 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 770CE, 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 are 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 min rest, blood pressure was determined as an average of three 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 in the subsequent section.

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, 24, 25). Volumetric analysis of adipose tissue was used to quantify regional fat; proton magnetic resonance spectroscopy (1H -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 $\sim 98\%$) 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).

Dietary analysis

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

Individual phenotyping

Following physiological assessment, participants were phenotyped according to obesity status and presence or absence of MetS. Individuals were characterised into one of four groups based on BMI (non-obese <30 vs obese ≥ 30 kg/m 2) and the presence or absence of MetS according to IDF criteria (6); we refer to these groups as (1.) 'non-obese MetS–', (2.) 'non-obese MetS+', (3.) 'obese MetS–' and (4.) 'obese MetS+.

Sample size calculation

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

Statistical analysis

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

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- ($P \leq 0.028$) and light intensity PA was lower ($P \leq 0.001$). 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 $53 \pm 10\%$ carbohydrate, $26 \pm 9\%$ protein, and $21 \pm 4\%$ fat.

Flow mediated dilation

FMD was higher in the MetS- individuals in both the non-obese and obese groups (Fig. 1A). The non-obese MetS- individuals had a greater FMD than their MetS+

Table 1 Descriptive data, mean $\pm$ s.d. of clinical values, physical activity and dietary data of each group categorised for obesity and subsequently MetS.

	Non-obese		Obese	
	MetS- (n = 10)	MetS+ (n = 10)	MetS- (n = 12)	MetS+ (n = 12)
Gender				
Males (n)	9	8	7	6
Females (n)	1	2	5	6
Age (years)	43 $\pm$ 14	48 $\pm$ 9	43 $\pm$ 14	36 $\pm$ 11
BMI (kg/m ²)	24.6 $\pm$ 3.1	26.9 $\pm$ 2.0	33.7 $\pm$ 4.7	33.9 $\pm$ 2.6
Components of metabolic syndrome				
Waist circumference (cm)	89 $\pm$ 10	97 $\pm$ 6	105 $\pm$ 15	111 $\pm$ 9
Systolic BP (mmHg)	125 $\pm$ 13	143 $\pm$ 11	126 $\pm$ 14	149 $\pm$ 18
Diastolic BP (mmHg)	79 $\pm$ 13	95 $\pm$ 15	77 $\pm$ 5	92 $\pm$ 12
Fasting glucose (mmol/L)	5.0 $\pm$ 0.4	5.4 $\pm$ 0.3	4.9 $\pm$ 0.4	5.5 $\pm$ 1.1
Triglyceride (mmol/L)	1.1 $\pm$ 0.8	1.4 $\pm$ 0.5	1.2 $\pm$ 0.8	1.8 $\pm$ 1.0
HDL-cholesterol (mmol/L)	1.7 $\pm$ 0.4	1.7 $\pm$ 0.7	1.6 $\pm$ 0.5	1.3 $\pm$ 0.3
Physical activity				
Energy expenditure (kJ/day)	12 143 $\pm$ 3641	12 226 $\pm$ 1743	12 079 $\pm$ 3951	13 281 $\pm$ 3104
PA duration (>1.5 METS) (min/day)	482 $\pm$ 117	340 $\pm$ 137	304 $\pm$ 160	311 $\pm$ 179
Sedentary (<1.5 METS) (min/day)	909 $\pm$ 113*	1027 $\pm$ 91	1074 $\pm$ 166	1132 $\pm$ 125
Light (1.3–3 METS) (min/day)	321 $\pm$ 73*	253 $\pm$ 74	186 $\pm$ 96	176 $\pm$ 56
MVPA (>3 METS) (min/day)	165 $\pm$ 93	117 $\pm$ 52	121 $\pm$ 86	109 $\pm$ 104
Dietary analysis				
Energy intake (kJ/day)	9532 $\pm$ 2008	8272 $\pm$ 1441	9629 $\pm$ 2201	8019 $\pm$ 1217
Carbohydrate (g/day)	206 $\pm$ 79	209 $\pm$ 59	214 $\pm$ 76	236 $\pm$ 39
Protein (g/day)	95 $\pm$ 16	91 $\pm$ 13	130 $\pm$ 54	85 $\pm$ 14
Fat (g/day)	92 $\pm$ 24	73 $\pm$ 9	95 $\pm$ 26	65 $\pm$ 23

*significantly different to both obese groups.

BP, blood pressure; F, female; HDL, high-density lipoprotein; M, male; METS, metabolic equivalents; MetS, metabolic syndrome; MVPA, moderate-vigorous physical activity; PA, physical activity.

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 ($P=0.04$; Fig. 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/m²), none of the categories were statistically different for FMD ($P=0.27$; Fig. 2B). Furthermore, there was no correlation between BMI and FMD ($r^2=0.01$; $P=0.512$; Fig. 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 diameter, shear rate or time to peak between groups ($P>0.05$). All vascular data are summarised in Table 2.

Cardio-respiratory fitness (CRF)

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

MRS determination of liver fat

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

Assessment of body composition (BIA and MRI)

BIA

Total body fat measured in percentage and mass was significantly lower in the non-obese groups compared to

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 $\pm$ s.d. data are presented as bar. * $P<0.05$, group difference.

Figure 2

Individual plots for all 44 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).

the obese groups ($P < 0.05$; Table 3); however, there were no significant differences between MetS– vs MetS+ within the BMI groups. Visceral fat rating was significantly lower in the non-obese MetS– group ($P < 0.05$) but there were no other significant differences. No significant differences were observed in BIA derived fat free mass or muscle mass between any of the groups.

MRI

Total s.c. adipose tissue (SAT) and whole-body fat were significantly lower in the non-obese MetS– than both obese groups ($P < 0.05$). Abdominal SAT was lower in both non-obese groups ($P < 0.05$). Visceral adipose tissue was significantly lower in non-obese MetS– when compared to obese MetS–. Of note, there were no significant differences between MetS– vs MetS+ within the BMI groups but the data was not available for all participants.

Discussion

The aim of study was to determine to what extent MetS or obesity is 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 MRI 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. Alarming, 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

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Table 2 Differences in the brachial artery vascular function between groups categorised for obesity and subsequently MetS. Data are presented as mean $\pm$ s.d.

	Non-obese		Obese		P (ANOVA)
	MetS– (n = 10)	MetS+ (n = 10)	MetS– (n = 12)	MetS+ (n = 12)	
Males (n)	9	8	7	6	
Females (n)	1	2	5	6	
Flow-mediated dilation (%)	8.6 $\pm$ 1.2	4.7 $\pm$ 2.6	10.8 $\pm$ 3.6	4.6 $\pm$ 1.9	<0.001
Baseline diameter (mm)	0.42 $\pm$ 0.06	0.44 $\pm$ 0.06	0.40 $\pm$ 0.1	0.41 $\pm$ 0.09	0.75
Peak diameter (mm)	0.46 $\pm$ 0.06	0.45 $\pm$ 0.06	0.44 $\pm$ 0.1	0.43 $\pm$ 0.09	0.91
Shear rate _{AUC} (s ⁻¹ $\times$ 10 ³)	15 395 $\pm$ 8421	11 669 $\pm$ 7808	13 048 $\pm$ 23067	17 136 $\pm$ 11583	0.36
Time to peak (s)	44.4 $\pm$ 19.6	32.7 $\pm$ 19.2	71.9 $\pm$ 59.1	46.9 $\pm$ 21.4	0.32

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 vs 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 or 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) or 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

Table 3 Body composition data, mean $\pm$ s.d. derived from both bioelectrical impedance and MRI quantification presented for each group categorised for obesity and subsequently MetS.

	Non-obese		Obese	
	MetS–	MetS+	MetS–	MetS+
Bioelectrical impedance quantification				
n	10	10	12	12
Fat (%)	21.5 $\pm$ 5.6*	27.5 $\pm$ 5.1*	39.4 $\pm$ 7.0	39.4 $\pm$ 7.8
Fat mass (kg)	16.4 $\pm$ 5.5*	22.3 $\pm$ 3.4*	38.1 $\pm$ 10.9	39.1 $\pm$ 8.2
Fat free mass (kg)	58.5 $\pm$ 8.0	59.2 $\pm$ 8.0	58.4 $\pm$ 11.5	60.9 $\pm$ 12.9
Muscle mass (kg)	55.5 $\pm$ 7.7	56.2 $\pm$ 7.6	55.4 $\pm$ 7.7	57.9 $\pm$ 12.2
Visceral fat rating	8 $\pm$ 3*	10 $\pm$ 3	13 $\pm$ 5	14 $\pm$ 5
MRI quantification				
n	10	8	7	7
Total SAT (L)	15.3 $\pm$ 3.8*	17.9 $\pm$ 4.4	29.9 $\pm$ 11.9	28.6 $\pm$ 13.1
Abdominal SAT (L)	3.9 $\pm$ 1.8**	5.6 $\pm$ 1.1**	9.7 $\pm$ 4.2	12.9 $\pm$ 7.7
Visceral adipose tissue (L)	3.0 $\pm$ 1.9***	4.6 $\pm$ 1.7	6.0 $\pm$ 2.3	5.5 $\pm$ 2.1
Internal fat (L)	5.7 $\pm$ 2.6	8.1 $\pm$ 3.4	9.9 $\pm$ 3.6	9.3 $\pm$ 2.9
Whole-body fat (L)	21.0 $\pm$ 5.3*	26.0 $\pm$ 2.6*	39.7 $\pm$ 12.7	40.7 $\pm$ 10.4

*significantly lower than both obese groups; **significantly lower than obese mets+; ***significantly lower than obese MetS–. SAT, s.c. adipose tissue.

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 support 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 miRNA 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 and 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 (Fig. 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. While 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 declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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