

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

3 *Short title: Metabolic health, obesity and FMD*

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26 **Abstract**

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

31 **Methods:** Forty-four participants were phenotyped according to BMI as non-obese *vs.* obese
32 (<30 or >30 kg/m²) and according to the International Diabetes Federation criteria of MetS: ≤ 2
33 criteria MetS (MetS-) *vs.* ≥ 3 criteria MetS (MetS+); **i)** *non-obese MetS-* *vs.* **ii)** *non-obese MetS+*
34 **and iii)** *obese MetS-* *vs.* **iv)** *obese MetS+*. Flow-mediated dilation (FMD), body composition
35 including liver fat (magnetic resonance imaging and spectroscopy), dietary intake, intensities
36 of habitual physical activity and cardio-respiratory fitness, were determined. Variables were
37 analysed using a one-factor between-groups analysis of variance (ANOVA) and linear
38 regression; mean (95% CI) are presented.

39 **Results:** Individuals with MetS+ displayed lower FMD than those with MetS-. For non-obese
40 individuals mean difference between MetS+ and MetS- was 4.1% [(1.0, 7.3); $P=0.004$] and
41 obese individuals had a mean difference between MetS+ and MetS- of 6.2% [(3.1, 9.2);
42 $P<0.001$]. Although there was no association between BMI and FMD ($P=0.27$), an increased
43 number of MetS components was associated with a lower FMD ($P=0.005$), and after
44 adjustment for age and sex, 19.7% of the variance of FMD was explained by MetS whereas
45 only 1.1% was explained by BMI.

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

49 **Introduction**

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

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

73 When considering cardiovascular risk in these metabolically phenotyped groups, previous
74 research has largely focused on the overall incidence of CVD (8, 9, 12-14). While this is
75 important, endothelial function, an early, prognostic and reversible marker of CVD, is much
76 less explored. The endothelium plays a pivotal role in vascular homeostasis (15), and brachial
77 artery flow-mediated dilation (FMD) is predictive of future CVD risk (16). Endothelial
78 dysfunction, characterised by decreased nitric oxide (NO) bioavailability, resulting in vascular
79 inflammation, vasoconstriction, and thrombosis (17, 18), has been mechanistically related to
80 the greater risk of cardiovascular events in people with obesity (19, 20). To put this
81 measurement into a pathophysiological perspective, a meta-analysis reports that a 1% increase
82 in FMD is associated with a pooled relative risk reduction in CVD of 0.87 (95% CI, 0.83- 0.91)
83 (21). Furthermore, there is evidence that FMD has independent prognostic value to predict
84 cardiovascular events that may better that of traditional risk factors (16). Evidence is lacking
85 on how MetS alone, or in combination with obesity, affects FMD.

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

93 **Materials and Methods**

94 **Participants**

95 Forty-four individuals (30 male, 14 female) with a mean age of 46 ± 11 years were recruited via
96 local advertisement across hospital departments and university campuses. Exclusions included

97 cardiovascular, respiratory, kidney, liver and/or endocrine complications, smoking and >14
98 units/week of alcohol consumption; all participants were medication free. The study conformed
99 to the *Declaration of Helsinki* and was approved by the North West Research Ethics Committee
100 (14/NW/1145; 14/NW/1147; 15/NW/0550). All participants were informed of the protocol
101 verbally and in writing before providing written informed consent prior to any assessments.

102 **Study design**

103 All participants completed habitual monitoring of physical activity (PA) and dietary
104 consumption over a period of 4 days (including one weekend day), followed by two assessment
105 visits. The first assessment visit, at Aintree University Hospital, comprised anthropometry,
106 fasting biochemistry, and cardio-respiratory fitness ($\dot{V}O_2$ peak). The second assessment at the
107 University of Liverpool MRI Centre (LiMRiC) comprised flow mediated dilation (FMD) and
108 proton magnetic resonance spectroscopy ($^1\text{H-MRS}$). Prior to each study visit, participants were
109 required to fast overnight for >8 hours, abstain from alcohol and caffeine for 24 hours and from
110 exercise for 48 hours; up to 500ml of water was permitted in the morning of a visit.

111 **Brachial artery flow mediated dilation (FMD)**

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

120 **Cardio-respiratory fitness**

121 $\dot{V}O_2$ peak was determined using the modified Bruce protocol on a treadmill (Model 770CE,
122 RAM Medisoft Group, Manchester, UK) with breath-by-breath monitoring and analysis of
123 expiratory gases and ventilation (Love Medical Cardiopulmonary Diagnostics, Manchester,
124 UK). The $\dot{V}O_2$ peak was determined by any of the following: respiratory exchange ratio >1.15,
125 heart rate >90% predicted maximum, plateau in $\dot{V}O_2$, or exhaustion, data is presented relative
126 to total body mass and lean mass determined by BIA.

127 **Biochemical measures**

128 Blood samples were collected and analysed using the Olympus AU2700 analyser (Beckman
129 Coulter, High Wycombe, UK) with standard proprietary reagents as follows: glucose with
130 hexokinase, total cholesterol and HDL-cholesterol with cholesterol esterase/oxidase and
131 triglyceride with glycerol kinase. LDL-cholesterol was calculated according to the Friedewald
132 formula.

133 **Anthropometric measures**

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

142 **MR determination of adipose tissue and liver fat**

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

148 **Habitual physical activity monitoring and dietary analysis**

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

157 *Dietary analysis* Total energy consumption, carbohydrate, protein and fat content were
158 determined from dietary records by a registered nutritionist (KLM) using Nutritics (Nutrition
159 Analysis Software for Professionals; <https://www.nutritics.com/p/home>; accessed
160 17/07/2017).

161 **Individual phenotyping**

162 Following physiological assessment, participants were phenotyped according to obesity status
163 and presence or absence of MetS. Individuals were characterised into one of four groups based
164 on BMI (non-obese <30 vs obese ≥30 kg/m²) and the presence or absence of MetS according

165 to IDF criteria (6); we refer to these groups as i) 'non-obese MetS-', ii) 'non-obese MetS+', iii)
166 'obese MetS-' and iv) 'obese MetS+'.

167 **Sample size calculation**

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

172 **Statistical analysis**

173 All data were explored for normality by visual inspection. Comparisons of group demographics
174 were explored using one factor between-groups analysis of variance (ANOVA) for continuous
175 variables and chi-squared for categorical outcomes. The main outcome variables (e.g. FMD,
176 cardio-respiratory fitness, and liver fat) were analysed using a one factor between-groups
177 ANOVA, with Bonferroni correction for multiple comparisons. All FMD data were analysed,
178 and are presented, as covariate-controlled for baseline artery diameter measured prior to the
179 introduction of hyperaemia in each test; this approach is more accurate for scaling changes in
180 artery diameter than simple percentage change (29, 30). Regression models, adjusted for age
181 and sex, were fitted to categories of BMI and number of MetS components to explore the
182 association with FMD. Finally, we estimated the amount of variance explained in FMD by
183 BMI and number of MetS components using an incremental sums of squares approach.
184 Distribution data are presented as mean \pm SD and outcomes of ANOVA as mean (95% CI). The
185 alpha level of statistical significance was set at $P<0.05$. Statistical analysis was performed using
186 SPSS for Windows (Version 24.0, SPSS, Chicago, IL, USA).

187 **Results**

188 **Participant characteristics**

189 Gender, age and BMI for each of the groups are summarised in Table 1. The differences
190 between the mean BMI and components of MetS were in line with WHO and IDF
191 classifications, respectively. Age and gender were not significantly different between groups
192 ($P>0.05$). Overall, habitual physical activity did not differ between BMI categories of MetS;
193 however, sedentary behaviour was greater in both of the obese groups compared to non-obese
194 MetS- ($P\leq 0.028$) and light intensity PA was lower ($P\leq 0.001$). Total energy consumption,
195 carbohydrate, protein and fat did not differ significantly between groups ($P>0.05$) (Table 1).
196 Macronutrient percentages of all groups combined were $53\pm 10\%$ carbohydrate, $26\pm 9\%$ protein,
197 and $21\pm 4\%$ fat.

198 **Flow mediated dilation**

199 FMD was higher in the MetS- individuals in both the non-obese and obese groups (Figure 1A).
200 The non-obese MetS- individuals had a greater FMD than their MetS+ counterparts [4.1% (1.0,
201 7.3; $P=0.004$)] and obese MetS+ [4.3% (1.3, 7.3; $P=0.002$)], with no difference compared to
202 obese MetS-. The mean difference between the obese MetS- and obese MetS+ was 6.2% (3.1,
203 9.2; $P<0.0001$), and non-obese MetS+ was 6.0% (2.8, 9.2; $P<0.0001$). There was no significant
204 difference between the MetS+ groups. An increased number of MetS components was
205 associated with a lower FMD ($P=0.04$; Figure 2A), differences were observed from the healthy
206 reference group (0 components) for those with 3 ($P=0.005$) or ≥ 4 ($P=0.023$) components of
207 MetS. In contrast, when using a healthy BMI as a reference group ($18.5\text{-}24.9\text{ kg/m}^2$), none of
208 the categories were statistically different for FMD ($P=0.27$; Figure 2B). Furthermore, there
209 was no correlation between BMI and FMD ($r^2=0.01$; $P=0.512$; Figure 2C). The variance of
210 FMD explained, when controlling for age and sex, by BMI was 1.1% and by MetS was 19.7%.
211 There were negligible and non-statistically significant differences in baseline or peak arterial

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

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

215 $\dot{V}O_2$ peak was greatest in non-obese MetS-, similar in non-obese MetS+ and obese MetS-, and
216 lowest in obese MetS+ (Figure 1B). Obese MetS+ individuals had a significantly lower CRF
217 than non-obese MetS- by $13.9 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ (6.0, 21.7; $P<0.0001$). Differences between the
218 MetS- groups just fell short of conventional statistical significance ($P=0.056$). The between-
219 group differences are also consistent when $\dot{V}O_2$ peak is expressed relative to lean mass.
220 Interestingly, when FMD was adjusted for individual differences in CRF the difference in FMD
221 between groups remained and was of similar magnitude ($P<0.05$).

222 **MRS determination of liver fat**

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

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

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

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

237 **Discussion**

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

254 Our findings support the existence of distinct phenotypes within different categories of BMI,
255 where MetS+ individuals exhibit a cluster of metabolic abnormalities (e.g. insulin resistance,
256 hypertension and dyslipidemia). The data suggests that endothelial dysfunction is not explained
257 by the absolute fat mass, but rather is determined (in part) by associated cardio-metabolic
258 dysfunction/risk factors alongside known and so far unidentified factors. Individuals with MetS
259 (non-obese and obese) have an unfavourable cardiovascular profile with a lower FMD (an early
260 marker of atherosclerotic disease), while those without MetS (non-obese and obese) have
261 comparable endothelial function. This phenomenon whereby other measures of cardiovascular

262 function differ between *metabolically healthy* versus *metabolically unhealthy* obese adults is
263 observed not only for macrovascular complications, as here and in previous investigations (31)
264 but also for microvascular function (32). Using identical phenotypic classification, we have
265 previously shown similar trends for myocardial systolic and diastolic dysfunction (measured
266 by tissue doppler imaging with transthoracic echocardiography). We observed impaired
267 myocardial performance related to poor metabolic health but not related to levels of fat mass
268 nor to differing amounts of ectopic fat stores (visceral and liver) (33). Mechanisms such as
269 inflammation, increased circulating free fatty acids and pro-inflammatory cytokines have been
270 proposed as mediators of this impact on cardiovascular risk (34).

271 The increasing interest in the study of differing metabolic phenotypes has led many to
272 investigate putative behavioural determinants (e.g. physical activity, diet), however findings
273 remain equivocal (35). We found no difference between the groups for PA (even when domains
274 of physical activity were analysed) nor in their total energy intake/macronutrient intake. We
275 note the disparity between energy intake and expenditure, ostensibly showing the participants
276 in a negative energy balance; however, we recognise that energy intake is largely under-
277 reported, particularly in obese adults. Dietary assessment was not a primary outcome variable
278 and was assessed using the best resources available. Cardiorespiratory fitness was highest in
279 the healthy reference group (non-obese MetS-) and lowest in the obese MetS+ group perhaps
280 as expected, although interestingly both groups of non-obese adults and obese MetS- had
281 comparable fitness. A higher cardiorespiratory fitness is typically associated with a better
282 metabolic profile and reduced CVD risk (36), and our data supports this. In the MetS- obese
283 group, we observed FMD ~15%, this data is somewhat striking but not abnormal. While obesity
284 has many comorbidities, the role of fitness is also recognised as an important prognostic marker
285 that differs across phenotypes (37) and some researchers suggest that recommendations to
286 reduce mortality risk should focus on increasing fitness rather than on weight loss (38).

287 Although we interpret this data with caution it is reasonable to suggest that intrinsic biological
288 mechanisms may contribute to the differences we observe in these phenotypes (such as
289 subacute inflammation, levels of oxidative stress, levels of different regulatory microRNAs
290 and adiponectin(39)).

291 Many authors suggest that cross-sectional observations of preserved metabolic health in obese
292 adults likely represent a transient phenomenon and question their clinical utility and
293 significance. Longitudinal studies are needed to address these important questions. One such
294 study found that 50% of healthy obese progressed to an unhealthy metabolic status over a 10-
295 year follow up period (40). Interpretation of such studies is hampered by the lack of an agreed
296 definition of ‘metabolically healthy’ (41); conclusions about the degree of protection against
297 CV disease and T2D will clearly depend on the criteria of metabolic health. We opted for the
298 IDF classification of MetS, as the most recent and internationally harmonised definition.
299 Furthermore, FMD is often (as here) studied in the fasted state, yet humans spend a significant
300 of their time in a post-prandial state. Examination of post-prandial endothelial function
301 between the phenotypes described in this manuscript maybe warranted and highlight more
302 profound differences. In particular, the post-prandial state following consumption of a high-fat
303 meal, may be associated with oxidative stress and inflammation, which are potentially
304 important mediators of impaired postprandial vascular function and may differ between these
305 individuals.

306 We acknowledge limitations of the current study, including a relatively small sample size, its
307 cross-sectional design. Participants were recruited via local advertisement, which limits
308 external validity as this yielded only white Europeans; defining a causal relationship with
309 validity at a global population level is therefore not possible. However, we believe the study
310 has significant merit. The study was powered to detect meaningful differences in the primary
311 outcome measure (FMD). It should be acknowledged that there are outliers (Figure 2C), but

312 that removal of these data does not alter the outcome of statistical analyses, so the decision was
313 made to include the data set in its entirety. It utilises objective monitoring of physical activity,
314 a gold standard measurement of cardio-respiratory fitness combined with assessment of body
315 composition including regional (VAT/SAT) and tissue specific (liver) fat and a novel
316 prognostic marker for cardiovascular health, that of endothelial function. Liver fat was not our
317 primary outcome and thus the study was not adequately powered for this outcome. Importantly,
318 this measure was utilised to comprehensively phenotype the individuals. Based on previous
319 work regarding fat deposition, we expected a greater propensity to deposit fat within the liver
320 in the metabolically unhealthy (MetS+) phenotypes. This propensity was observed but did not
321 reach statistical significance between groups. Whilst the present results demonstrate that
322 endothelial function is impaired in those with MetS, larger studies are required with a follow-
323 up design to determine measured cardiovascular function rather than predicted CVD. This has
324 been undertaken to a limited extent in a multi-ethnic population study but did not include the
325 classification of sub-phenotypes (42).

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

334 **Declaration of interest**

335 The authors have nothing to disclose.

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346 **References**

- 347 1. Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis
348 RJ, Moore SC, Tobias GS, Anton-Culver H, Freeman LB, et al. Body-mass index and
349 mortality among 1.46 million white adults. *New England Journal of Medicine*.
350 2010;363(23):2211-9.
- 351 2. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with
352 overweight and obesity using standard body mass index categories: a systematic review and
353 meta-analysis. *Jama*. 2013;309(1):71-82.
- 354 3. Troiano RP, Frongillo EA, Jr., Sobal J, Levitsky DA. The relationship between body
355 weight and mortality: a quantitative analysis of combined information from existing studies.
356 *Int J Obes Relat Metab Disord*. 1996;20(1):63-75.
- 357 4. Carnethon MR, Rasmussen-Torvik LJ, Palaniappan L. The obesity paradox in diabetes.
358 *Current cardiology reports*. 2014;16(2):446.
- 359 5. Lavie CJ, Milani RV, Ventura HO. Obesity and cardiovascular disease: risk factor,
360 paradox, and impact of weight loss. *J Am Coll Cardiol*. 2009;53(21):1925-32.
- 361 6. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC,
362 James WP, Loria CM, Smith SC Jr. Harmonizing the metabolic syndrome: a joint interim
363 statement of the International Diabetes Federation Task Force on Epidemiology and
364 Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World
365 Heart Federation; International Atherosclerosis Society; and International Association for the
366 Study of Obesity. *Circulation*. 2009;120(16):1640-5.
- 367 7. Caleyachetty R, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, Balachandran
368 K, et al. Metabolically healthy obese and incident cardiovascular disease events among
369 3.5 million men and women. *Journal of the American College of Cardiology*.
370 2017;70(12):1429-37.

- 371 8. Phillips CM. Metabolically healthy obesity across the life course: epidemiology,
372 determinants, and implications. *Annals Of The New York Academy Of Sciences*.
373 2017;1391(1):85-100.
- 374 9. Stefan N, Schick F, Häring H-U. Causes, Characteristics, and Consequences of
375 Metabolically Unhealthy Normal Weight in Humans. *Cell Metabolism*. 2017;26(2):292-300.
- 376 10. Eckel N, Li Y, Kuxhaus O, Stefan N, Hu FB, Schulze MB. Transition from metabolic
377 healthy to unhealthy phenotypes and association with cardiovascular disease risk across BMI
378 categories in 90 257 women (the Nurses' Health Study): 30 year follow-up from a prospective
379 cohort study. *The Lancet Diabetes & Endocrinology*. 2018;6(9):714-24.
- 380 11. Khan SS, Ning H, Wilkins JT, Allen N, Carnethon M, Berry JD, Sweis RN, Lloyd-
381 Jones DM. Association of Body Mass Index With Lifetime Risk of Cardiovascular Disease and
382 Compression of Morbidity Association of Body Mass Index With Cardiovascular Disease
383 Morbidity Association of Body Mass Index With Cardiovascular Disease Morbidity. *JAMA*
384 *Cardiology*. 2018;3(4):280-7.
- 385 12. Morkedal B, Vatten LJ, Romundstad PR, Laugsand LE, Janszky I. Risk of myocardial
386 infarction and heart failure among metabolically healthy but obese individuals: HUNT (Nord-
387 Trondelag Health Study), Norway. *J Am Coll Cardiol*. 2014;63(11):1071-8.
- 388 13. Hamer M, Stamatakis E. Metabolically healthy obesity and risk of all-cause and
389 cardiovascular disease mortality. *J Clin Endocrinol Metab*. 2012;97(7):2482-8.
- 390 14. Mongraw-Chaffin M, Foster MC, Anderson CAM, Burke GL, Haq N, Kalyani RR, et
391 al. Metabolically Healthy Obesity, Transition to Metabolic Syndrome, and Cardiovascular
392 Risk. *J Am Coll Cardiol*. 2018;71(17):1857-65.
- 393 15. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation
394 of arterial smooth muscle by acetylcholine. *Nature*. 1980;288(5789):373-6.
- 395 16. Green DJ, Jones H, Thijssen D, Cable NT, Atkinson G. Flow-mediated dilation and
396 cardiovascular event prediction: does nitric oxide matter? *Hypertension*. 2011;57(3):363-9.
- 397 17. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg
398 NM, Lüscher TF, Shechter M, Taddei S, Vita JA, Lerman A. The assessment of endothelial
399 function: from research into clinical practice. *Circulation*. 2012;126(6):753-67.
- 400 18. Widlansky ME, Gokce N, Keaney JF, Jr., Vita JA. The clinical implications of
401 endothelial dysfunction. *J Am Coll Cardiol*. 2003;42(7):1149-60.
- 402 19. De Boer MP, Meijer RI, Wijnstok NJ, Jonk AM, Houben AJ, Stehouwer CD, Smulders
403 YM, Eringa EC, Serné EH. Microvascular dysfunction: a potential mechanism in the
404 pathogenesis of obesity-associated insulin resistance and hypertension. *Microcirculation*.
405 2012;19(1):5-18.
- 406 20. Jonk AM, Houben AJ, de Jongh RT, Serne EH, Schaper NC, Stehouwer CD.
407 Microvascular dysfunction in obesity: a potential mechanism in the pathogenesis of obesity-
408 associated insulin resistance and hypertension. *Physiology (Bethesda, Md)*. 2007;22:252-60.
- 409 21. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by
410 flow-mediated vasodilatation of brachial artery: a meta-analysis. *Int J Cardiovasc Imaging*.
411 2010;26(6):631-40.
- 412 22. Sprung VS, Jones H, Pugh CJ, Aziz NF, Daousi C, Kemp GJ, Green DJ, Cable NT,
413 Cuthbertson DJ. Endothelial dysfunction in hyperandrogenic polycystic ovary syndrome is not
414 explained by either obesity or ectopic fat deposition. *Clin Sci (Lond)*. 2014;126(1):67-74.
- 415 23. Cuthbertson DJ, Shojaee-Moradie F, Sprung VS, Jones H, Pugh CJ, Richardson P,
416 Kemp GJ, Barrett M, Jackson NC, Thomas EL, et al. Dissociation between exercise-induced
417 reduction in liver fat and changes in hepatic and peripheral glucose homeostasis in obese
418 patients with Non-Alcoholic Fatty Liver Disease. *Clin Sci (Lond)*. 2016;130(2):93-104.
- 419 24. Cuthbertson DJ, Irwin A, Gardner CJ, Daousi C, Purewal T, Furlong N, Goenka N,
420 Thomas EL, Adams VL, Pushpakom SP, et al. Improved glycaemia correlates with liver fat

421 reduction in obese, type 2 diabetes, patients given glucagon-like peptide-1 (GLP-1) receptor
422 agonists. *PLoS One*. 2012;7(12):e50117.

423 25. Todd CS, Jones HE, Garber TC, Afnan-Holmes H, Woolgar H, Bekker LG, Myer L.
424 Awareness and Interest in Intrauterine Contraceptive Device Use among HIV-Positive Women
425 in Cape Town, South Africa. *Infect Dis Obstet Gynecol*. 2012;2012:956145.

426 26. Johannsen DL, Calabro MA, Stewart J, Franke W, Rood JC, Welk GJ. Accuracy of
427 armband monitors for measuring daily energy expenditure in healthy adults. *Med Sci Sports
428 Exerc*. 2010;42(11):2134-40.

429 27. Scheers T, Philippaerts R, Lefevre J. Variability in physical activity patterns as
430 measured by the SenseWear Armband: how many days are needed? *Eur J Appl Physiol*.
431 2012;112(5):1653-62.

432 28. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power
433 3.1: tests for correlation and regression analyses. *Behavior research methods*. 2009;41(4):1149-
434 60.

435 29. Atkinson G, Batterham AM, Thijssen DH, Green DJ. A new approach to improve the
436 specificity of flow-mediated dilation for indicating endothelial function in cardiovascular
437 research. *J Hypertens*. 2013;31(2):287-91.

438 30. Atkinson G, Batterham AM. Allometric scaling of diameter change in the original flow-
439 mediated dilation protocol. *Atherosclerosis*. 2013;226(2):425-7.

440 31. Khan UI, Wang D, Thurston RC, Sowers M, Sutton-Tyrrell K, Matthews KA, Barinas-
441 Mitchell E, Wildman RP. Burden of subclinical cardiovascular disease in "metabolically
442 benign" and "at-risk" overweight and obese women: the Study of Women's Health Across the
443 Nation (SWAN). *Atherosclerosis*. 2011;217(1):179-86.

444 32. Brant LC, Wang N, Ojeda FM, LaValley M, Barreto SM, Benjamin EJ, Mitchell GF,
445 Vasan RS, Palmisano JN, Münzel T, et al. Relations of Metabolically Healthy and Unhealthy
446 Obesity to Digital Vascular Function in Three Community-Based Cohorts: A Meta-Analysis.
447 *Journal of the American Heart Association*. 2017;6(3).

448 33. Dobson R, Burgess MI, Sprung VS, Irwin A, Hamer M, Jones J, Daousi C, Adams V,
449 Kemp GJ, Shojaee-Moradie F, et al. Metabolically healthy and unhealthy obesity: differential
450 effects on myocardial function according to metabolic syndrome, rather than obesity.
451 *International journal of obesity (2005)*. 2016;40(1):153-61.

452 34. Panagiotakos DB, Pitsavos C, Yannakoulia M, Chrysohoou C, Stefanadis C. The
453 implication of obesity and central fat on markers of chronic inflammation: The ATTICA study.
454 *Atherosclerosis*. 2005;183(2):308-15.

455 35. Phillips CM, Dillon C, Harrington JM, McCarthy VJ, Kearney PM, Fitzgerald AP,
456 Perry IJ. Defining metabolically healthy obesity: role of dietary and lifestyle factors. *PLoS
457 One*. 2013;8(10):e76188.

458 36. Myers J, McAuley P, Lavie CJ, Despres JP, Arena R, Kokkinos P. Physical activity and
459 cardiorespiratory fitness as major markers of cardiovascular risk: their independent and
460 interwoven importance to health status. *Progress in cardiovascular diseases*. 2015;57(4):306-
461 14.

462 37. Ortega FB, Cadenas-Sanchez C, Sui X, Blair SN, Lavie CJ. Role of Fitness in the
463 Metabolically Healthy but Obese Phenotype: A Review and Update. *Progress in cardiovascular
464 diseases*. 2015;58(1):76-86.

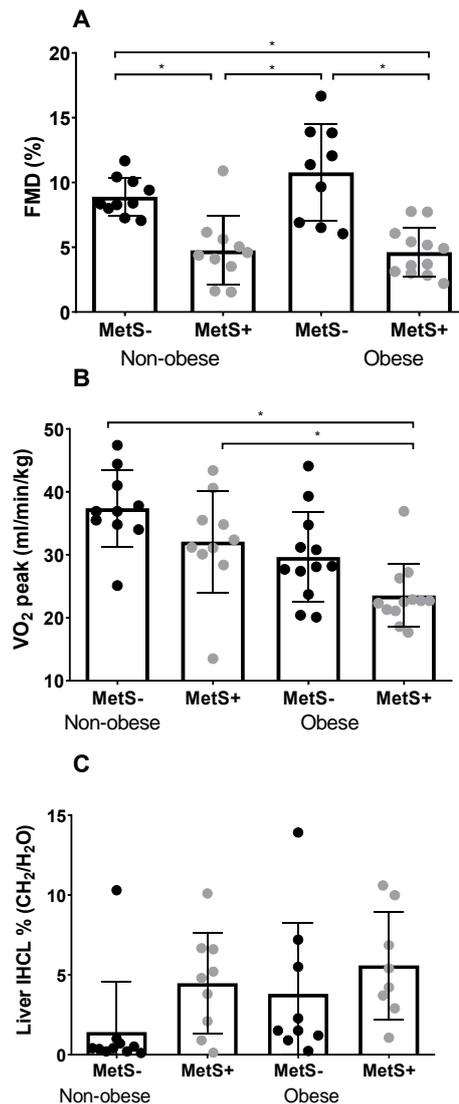
465 38. Barry VW, Baruth M, Beets MW, Durstine JL, Liu J, Blair SN. Fitness vs. fatness on
466 all-cause mortality: a meta-analysis. *Progress in Cardiovascular Diseases*. 2014;56:382-90.

467 39. Muñoz-Garach A, Cornejo-Pareja I, Tinahones FJ. Does metabolically healthy obesity
468 exist? *Nutrients*. 2016;8(6):320.

469 40. Kouvari M, Panagiotakos DB, Yannakoulia M, Georgousopoulou E, Critselis E,
470 Chrysohoou C, Tousoulis D, Pitsavos C; ATTICA Study Investigators. Transition from

471 metabolically benign to metabolically unhealthy obesity and 10-year cardiovascular disease
 472 incidence: the ATTICA cohort study. *Metabolism*. 2019.
 473 41. Phillips CM. Metabolically healthy obesity: definitions, determinants and clinical
 474 implications. *Reviews in endocrine & metabolic disorders*. 2013;14(3):219-27.
 475 42. Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, Lima JA, Crouse JR,
 476 Herrington DM. Predictive value of brachial flow-mediated dilation for incident cardiovascular
 477 events in a population-based study: the multi-ethnic study of atherosclerosis. *Circulation*.
 478 2009;120(6):502-9.

479 **Figures**



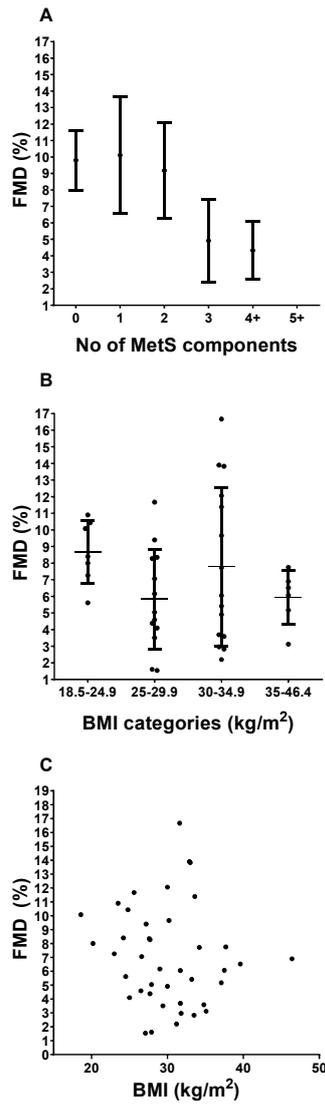
480

481 **Figure 1.** Individual participant plots for A) flow mediated dilation (FMD), B) cardio-

482 respiratory fitness ($\dot{V}O_2$ peak) and, C) 'liver fat' intrahepatic cellular lipid (IHCL)

483 percentage. Black circles, MetS-; grey circles, MetS+; non-obese are grouped left and obese

484 are grouped right. Group mean \pm SD data is presented as bar. * $P < 0.05$, group difference.



485

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

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

	Non-obese		Obese	
	MetS- (<i>n</i> =10)	MetS+ (<i>n</i> =10)	MetS- (<i>n</i> =12)	MetS+ (<i>n</i> =12)
Gender	M <i>n</i> =9; F <i>n</i> =1	M <i>n</i> =8; F <i>n</i> =2	M <i>n</i> =7; F <i>n</i> =5	M <i>n</i> =6; F <i>n</i> =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)	12143 \pm 3641	12226 \pm 1743	12079 \pm 3951	13281 \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

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

*sig different to both obese groups

Table 2. Differences in the brachial artery vascular function between groups categorised for obesity and subsequently MetS, mean \pm SD.

	Non-obese		Obese		<i>P</i> (ANOVA)
	MetS- (<i>n</i> =10)	MetS+ (<i>n</i> =10)	MetS- (<i>n</i> =12)	MetS+ (<i>n</i> =12)	
	M <i>n</i> =9; F <i>n</i> =1	M <i>n</i> =8; F <i>n</i> =2	M <i>n</i> =7; F <i>n</i> =5	M <i>n</i> =6; F <i>n</i> =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 x 10 ³)	15395 \pm 8421	11669 \pm 7808	13048 \pm 23067	17136 \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

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

	Non-obese		Obese	
	MetS- (<i>n</i> =10)	MetS+ (<i>n</i> =10)	MetS- (<i>n</i> =12)	MetS+ (<i>n</i> =12)
Bioelectrical impedance quantification of:				
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 of:				
	MetS- (<i>n</i>=10)	MetS+ (<i>n</i>=8)	MetS- (<i>n</i>=7)	MetS+ (<i>n</i>=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

SAT, subcutaneous adipose tissue

*sig lower than both obese groups

**sig lower than obese MetS+

***sig lower than obese MetS-