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**Exercise-induced improvements in liver fat and endothelial function are not sustained 12 months following cessation of exercise supervision in non-alcoholic fatty liver disease (NAFLD).**

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### Article

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

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1 **Exercise-induced improvements in liver fat and endothelial function are not sustained**  
2 **12 months following cessation of exercise supervision in non-alcoholic fatty liver disease**  
3 **(NAFLD)**

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21 **Running title:** Liver fat, endothelial function and exercise in NAFLD

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30

31 Word Count (abstract): 218

32 Word count (excluding abstract, figures, tables and references): 1291

33 This study was funded by the European Federation for the Study of Diabetes (EFSD).

34 Clinicaltrials.gov identifier: NCT01834300

35 The authors have nothing to declare.

36 **Abstract**

37 **Aims** Supervised exercise reduces liver fat and improves endothelial function, a surrogate of  
38 cardiovascular disease risk, in non-alcoholic fatty liver disease (NAFLD). We hypothesised  
39 that after a 16-week supervised exercise program, patients would maintain longer-term  
40 improvements in cardiorespiratory fitness, liver fat and endothelial function.

41 **Materials and Methods.** Ten NAFLD patients [5/5 males/females, age  $51\pm 13$  years, BMI  
42  $31\pm 3\text{kg.m}^2$  (mean $\pm$ SD)] underwent a 16-week supervised moderate-intensity exercise  
43 intervention. Biochemical markers, cardiorespiratory fitness ( $\text{VO}_{2\text{peak}}$ ), subcutaneous, visceral  
44 and liver fat (measured by magnetic resonance imaging and spectroscopy respectively) and  
45 brachial artery flow-mediated dilation (FMD) were assessed at baseline, after 16 weeks  
46 supervised training and 12-months after ending supervision.

47 **Results** Despite no significant change in body weight, there were significant improvements in  
48  $\text{VO}_{2\text{peak}}$  [ $6.5\text{ml.kg}^{-1}.\text{min}^{-1}$  (95% CI 2.8, 10.1);  $P=0.003$ ], FMD [2.9% (1.5, 4.2);  $P=0.001$ ],  
49 liver transaminases ( $P<0.05$ ) and liver fat [-10.1% (-20.6, 0.5);  $P=0.048$ ] immediately after  
50 the 16-weeks supervised training. Nevertheless, 12-months after ending supervision,  $\text{VO}_{2\text{peak}}$   
51 [ $0.9\text{ml.kg}^{-1}.\text{min}^{-1}$  (-3.3 5.1);  $P=0.65$ ], FMD [-0.07% (-2.3, 2.2);  $P=0.95$ ], liver transaminases  
52 ( $P>0.05$ ) and liver fat [1.4% (-13.0, 15.9);  $P=0.83$ ] were not significantly different from  
53 baseline.

54 **Conclusions** Twelve months following cessation of supervision, exercise-mediated  
55 improvements in liver fat and other cardiometabolic variables had reversed with  
56 cardiorespiratory fitness at baseline levels. Maintenance of high cardiorespiratory fitness and  
57 stability of body weight are critical public health considerations for the treatment of NAFLD.

58

59

60

61 **Introduction**

62 Non-alcoholic fatty liver disease (NAFLD) increases liver-related morbidity and mortality<sup>1</sup>,  
63 yet cardiovascular disease (CVD) is the leading cause of its mortality<sup>2</sup>. We need effective  
64 sustainable interventions to reverse NAFLD and reduce cardiovascular risk. In the absence of  
65 approved pharmacological treatment, structured exercise and/or dietary modification are  
66 recommended first-line treatment in NAFLD<sup>3</sup>. The cardiometabolic benefits of supervised  
67 exercise, which include reduced liver fat, enhanced peripheral insulin sensitivity and  
68 microvascular and conduit-artery endothelial function<sup>4,5</sup>, do not require weight loss. Parallel  
69 improvements in liver fat and cardiac structure and function<sup>6</sup> emphasise the role of exercise  
70 as an intervention to reduce both hepatic and CVD risk.

71 We hypothesised that after a 16-week supervised exercise program, patients would maintain  
72 the longer-term improvements in cardiorespiratory fitness, liver fat and endothelial function.  
73 To test this we re-examined a subset of previously-reported patients<sup>4,5</sup> a year after ending  
74 exercise supervision.

75

76 **Methods**

77 At baseline, NAFLD was diagnosed by a hepatologist based on raised transaminases (after  
78 exclusion of secondary causes) with confirmation of elevated liver fat ( $\geq 5.5\%$ ) by magnetic  
79 resonance spectroscopy (<sup>1</sup>H MRS). All participants were physically inactive (<2 h/week low-  
80 intensity physical activity) Caucasians, with no history of excessive alcohol intake (males  
81 <21, females <14 units/week); normotensive, normoglycaemic non-smokers with no  
82 contraindications to exercise; females were post-menopausal.

83 Patients who completed a 16-week structured and supervised exercise intervention were  
84 offered the opportunity to repeat assessments 12-months later. From the original study cohort,  
85 10 patients who completed the exercise intervention<sup>4,5</sup> (5 males, 5 females; 51±13y; BMI

86 31±3kg.m<sup>-2</sup>) underwent repeat assessments 12-months later. All participants remained with  
87 similar alcohol intake and as normotensive, normoglycaemic non-smokers. Liverpool Central  
88 Research Ethics Committee approved the study, and all participants gave written informed  
89 consent.

90 Measurements were performed fasted at baseline, after 16-weeks supervised exercise training  
91 and 12-months after its end<sup>5</sup>. Anthropometric measurements were taken and blood samples  
92 collected for plasma glucose, lipid profiles and liver enzymes.

93 Magnetic resonance scanning at 1.5T was as previously described<sup>5</sup>. Abdominal visceral  
94 (VAT) and subcutaneous adipose tissue (SAT) were calculated from whole-body axial T1-  
95 weighted fast spin echo scans. Total abdominal adipose tissue (AT) = VAT + SAT. Liver fat  
96 was measured using <sup>1</sup>H MRS and expressed as % CH<sub>2</sub> lipid amplitude relative to water signal.

97 High-resolution ultrasound (Terason, t3000, Aloka, UK) was used to image the brachial  
98 artery after 30min supine rest. Endothelial-dependent function was assessed as flow-mediated  
99 dilation (FMD): brachial artery diameter, flow and shear stress were measured before and  
100 after 5min forearm cuff inflation, and FMD is peak artery diameter following hyperaemia,  
101 expressed as % increase using an allometric model. Endothelium-independent function was  
102 assessed by imaging 1min before and 10min after sublingual (400 µg) glyceryl trinitrate  
103 (GTN)<sup>7</sup>.

104 Cardiorespiratory fitness<sup>5</sup> was assessed on a treadmill ergometer, initially 2.7 km.h<sup>-1</sup> at 5°  
105 gradient, with step-wise increments every minute. VO<sub>2peak</sub> was calculated from expired gas  
106 (Oxycon Pro, Jaeger, Germany) as the highest consecutive 15s periods of oxygen uptake in  
107 the last minute before exhaustion. No self-reported or objective assessment of physical  
108 activity and/or exercise was made following the cessation the 16-week structured exercise  
109 intervention.

110 For the exercise training intervention, an exercise physiologist provided supervision and  
111 guidance. Based upon individual basal fitness, participants underwent 30min moderate  
112 intensity aerobic exercise 3 times/week at 30% heart rate reserve (HRR), progressing weekly  
113 based on HR responses in the initial 4-weeks. Intensity increased to 45% HRR for the  
114 following 4-weeks, until week 8, where HRR remained at 45% but each session increased to  
115 45min. From week 12, participants were exercising 5 times/week for 45min at 60% HRR.  
116 Upon completion of the supervised exercise patients had no contact from the research team  
117 for 12-months.

118 A general linear model with repeated measures was employed to evaluate differences  
119 between baseline, immediate and 12-months post-training data. Analyses were performed  
120 using SPSS 21.0 (SPSS, Chicago, Illinois). All data in the text, figure and table, including  
121 changes, are presented as mean (95% confidence intervals), except age and BMI (presented  
122 as mean and standard deviation). Intra-observer coefficients of variation for measurements of  
123 liver fat and FMD were 6.0<sup>8</sup> and 6.7 %<sup>9</sup>, respectively.

124

## 125 **Results**

126 Body weight did not change significantly from baseline over the training period [change = -  
127 1.9kg (-1.5, 5.2);  $P=0.29$ ], or 12-months following its completion [-0.2kg, (-3.6, 3.1);  
128  $P=0.90$ ; Figure 1].

129  $VO_{2peak}$  increased [6.5ml.kg<sup>-1</sup>.min<sup>-1</sup> (95% CI 2.8, 10.1);  $P=0.003$ ] and waist circumference  
130 decreased [-6cm (-9, -2);  $P=0.004$ ] following training, but had returned to baseline 12-months  
131 later [0.9ml.kg<sup>-1</sup>.min<sup>-1</sup> (-3.3, 5.1);  $P=0.67$ ; Figure 1 & -1cm (-7, 5);  $P=0.60$ ; Table 1  
132 respectively].

133 Liver fat [-10.1% (-20.6, 0.5);  $P=0.048$ ], ALT [-20u/L (-41, 1);  $P=0.05$ ] and AST [-11u/L (-  
134 21, -1);  $P=0.04$ ] decreased following training but had returned to baseline 12-months later  
135 [1.4% (-13.0, 15.9);  $P=0.83$ ]; Figure 1; 10u/L (-21, 41);  $P=0.48$  & 2u/l (-11, 16);  $P=0.70$ ;  
136 Table 1 respectively]. There were no significant changes in VAT, SAT or total AT ( $P>0.20$ ;  
137 Table 1).

138 FMD improved [2.9% (1.5, 4.2);  $P=0.001$ ] following training, but had returned to baseline 12  
139 months later [-0.07% (-2.3, 2.2);  $P=0.95$ ; Figure 1]. There were no significant differences in  
140 endothelium-independent (GTN-mediated) dilation ( $P=0.74$ ; Table 1).

141 Patients who lost the most weight during the 16-week intervention period had the smallest  
142 gain in liver fat between weeks 16 and 68 ( $P=0.03$ ); 1kg reduction in body weight at 16-  
143 weeks reduced the change in liver fat by ~4.5% in the following 52-week period.

144

## 145 **Conclusion**

146 Longitudinal data suggest that whilst vigorous physical activity can prevent liver fat  
147 accumulation, adherence to current national and international physical activity guidelines  
148 alone is not sufficient to prevent NAFLD <sup>10</sup>. A recent study demonstrated that 8-weeks  
149 aerobic exercise can reduce liver fat, irrespective of exercise volume and intensity <sup>11</sup>.  
150 Following 16-weeks of supervised exercise training in the present cohort, liver fat  
151 significantly decreased and FMD increased by 2.8%, extrapolated from meta-analysis data to  
152 confer a CVD risk reduction of ~17% <sup>12</sup>. Nevertheless, this improvement had disappeared 12-  
153 months after cessation of exercise supervision.

154 To the authors' knowledge, no study to date has undertaken longer-term follow-up of the  
155 exercise-induced improvements in liver and vascular health following cessation of



156 supervision. This study suggests that short-term exercise interventions have only short-term  
157 benefits.

158 By contrast, improvements in liver transaminases, liver fat and insulin resistance observed  
159 after a 6-month hypocaloric diet with dietary counselling, were maintained for 17-36 months  
160 after ending counselling, despite modest weight regain <sup>13</sup>; but this study did not examine the  
161 effects on CVD risk, the leading cause of mortality in NAFLD <sup>2, 14</sup>. In our study, changes in  
162 liver fat and FMD were strongly associated with changes in cardiorespiratory fitness,  
163 suggesting that maintenance of exercise-induced improvements in cardiometabolic  
164 parameters depends upon sustained cardiorespiratory fitness. It therefore appears that  
165 exercise and hypocaloric diet interventions modulate liver fat content across different time  
166 courses and perhaps via distinct mechanisms. Indeed, as little as 7 consecutive days of 60min  
167 treadmill walking improves liver fat and increases insulin sensitivity in obese individuals  
168 with NAFLD <sup>15</sup>. These data suggest that an increase in levels of physical activity with  
169 exercise training dynamically modulates liver fat, and that to achieve prolonged  
170 cardiometabolic benefits, higher levels of fitness must be maintained. Although the patients  
171 were counselled on the benefits of exercise and encouraged to maintain their exercise training  
172 without further guidance, physical fitness returned to pre-intervention level, suggesting that  
173 long-term supervision or alternative strategies of exercise provision are required.

174 Limitations of this exploratory pilot study include a relatively small patient cohort, and a lack  
175 of intermediate post-intervention assessments and measures of insulin resistance. Follow up  
176 assessments were based on patient choice and thus there is the possibility of cohort bias.

177 In summary, whilst 16-weeks of supervised exercise effectively improves liver fat and  
178 endothelial function in NAFLD, the cardiometabolic benefit of training is not sustained 1  
179 year after ending supervision. To overcome the NAFLD epidemic we need an effective  
180 mechanism to promote long-term maintenance of fitness.

181 **Acknowledgements** Thank you to all the patients for their participation in the study.

182 **Funding** We would like to thank the European Federation for the Study of Diabetes (EFSD)  
183 for funding the study.

184 **Duality of interest** Nil

185 **Contribution statement** G.J.K., P.R., A.M.U., D.J.G., N.T.C., H.J., and D.J.C. conception  
186 and design of research; C.J.P., V.S.S., F.S.-M., H.J., and D.J.C. performed experiments;  
187 C.J.P., V.S.S., G.J.K., F.S.-M., A.M.U., D.J.G., N.T.C., H.J., and D.J.C. analyzed data; C.J.P.,  
188 V.S.S., G.J.K., P.R., F.S.-M., A.M.U., D.J.G., N.T.C., H.J., and D.J.C. interpreted results of  
189 experiments; C.J.P., V.S.S., P.R., H.J., and D.J.C. prepared figures; C.J.P., H.J., and D.J.C.  
190 drafted manuscript; C.J.P., V.S.S., G.J.K., P.R., F.S.-M., A.M.U., D.J.G., N.T.C., H.J., and  
191 D.J.C. edited and revised manuscript; C.J.P., V.S.S., G.J.K., P.R., F.S.-M., A.M.U., D.J.G.,  
192 N.T.C., H.J., and D.J.C. approved final version of manuscript.

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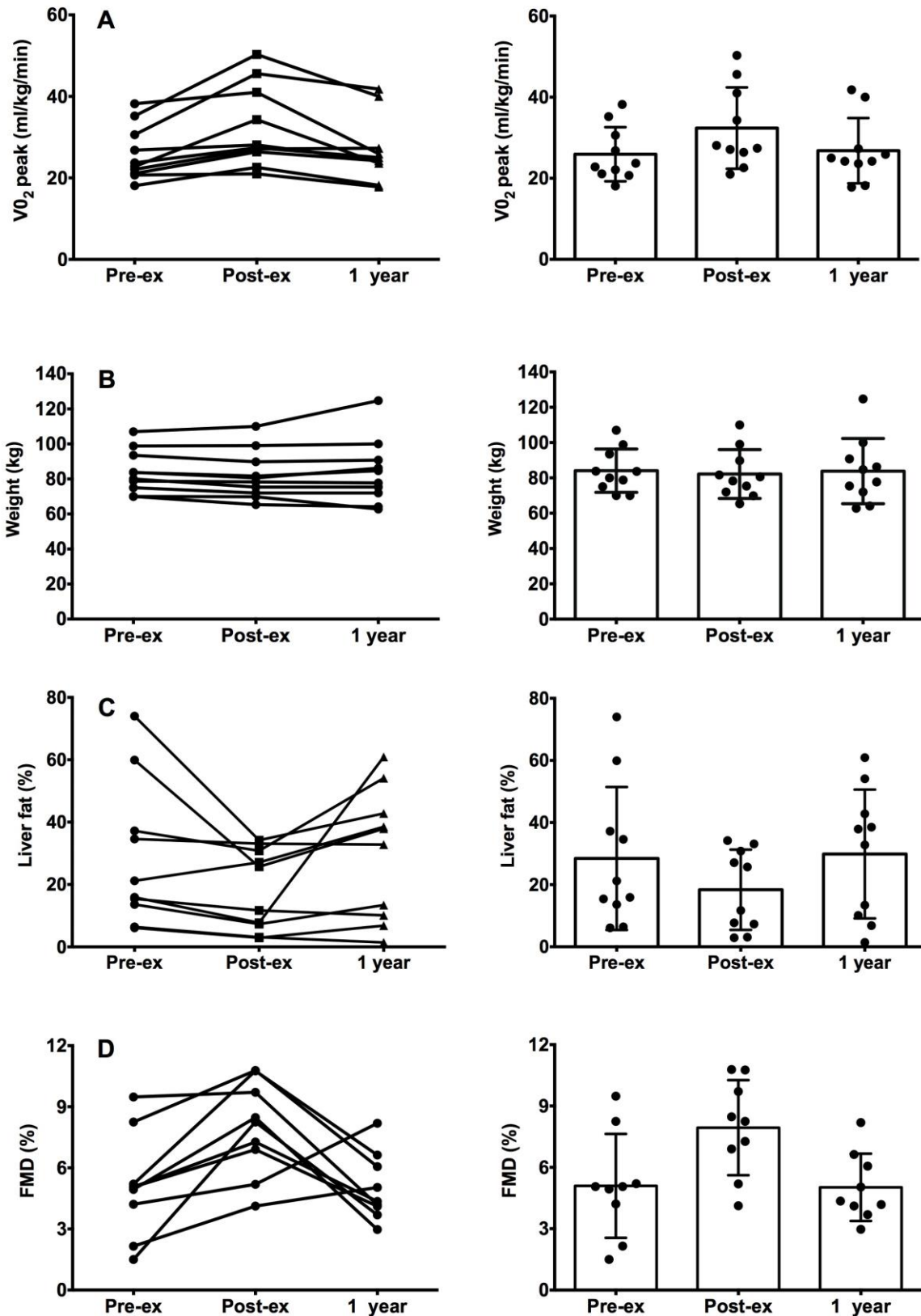
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203  
 204 **Figure 1** Changes in **A**) cardiorespiratory fitness (VO<sub>2peak</sub>), **B**) liver fat (%), **C**) flow  
 205 mediated dilatation (FMD) (%) and **D**) body weight at baseline ('Pre-ex'), following 16  
 206 weeks of supervised exercise training ('Post-ex') and 12-months following cessation of  
 207 exercise supervision ('1 year'). Data are presented as mean (95% CI) and as individual  
 208 patients' values.

209 **Table 1 Characteristics of NAFLD patients at baseline ('Pre-Ex'), immediately**  
 210 **following 16-weeks of supervised exercise training ('Post-ex') and 12 months following**  
 211 **('1 year') the cessation of supervised exercise.**

212

	<b>Pre-Ex</b>	<b>Post-Ex</b>	<b>1 year</b>	<b>P</b>
<b>Anthropometrics</b>				
Weight (kg)	84.4(75.6, 93.1)	82.1(72.7, 91.5)	83.8(70.6, 97.0)	0.40
BMI (kg.m <sup>-2</sup> )	30(28, 32)	29(27, 31)	30(27, 33)	0.37
Waist circumference (cm)	103(97, 108)	97(91, 104) <sup>†</sup>	101(97, 108) <sup>‡</sup>	0.03
Systolic BP (mmHg)	128(123, 134)	125(120, 130)	129(120,136)	0.23
Diastolic BP (mmHg)	79(74, 85)	76(74, 81)	78(71,85)	0.59
Fitness (L.min <sup>-1</sup> )	2.23 (1.61, 2.85)	2.73 (1.9,3.55) <sup>†</sup>	2.28 (1.63,2.93) <sup>‡</sup>	<0.01
<b>Liver Enzymes</b>				
ALT (u.l <sup>-1</sup> )	57(33, 81)	37(25, 48) <sup>†</sup>	67(40, 94) <sup>‡</sup>	0.05
AST (u.l <sup>-1</sup> )	39(26, 51)	28(24, 31) <sup>†</sup>	41(31, 51) <sup>‡</sup>	0.04
GGT (u.l <sup>-1</sup> )	85(18, 152)	60(18, 103)	68(38, 99)	0.26
<b>Glucose and Lipid Profile</b>				
Glucose (mmol.l <sup>-1</sup> )	5.0(4.6,5.4)	4.9(4.5, 5.3)	5.2(4.7, 5.6)	0.40
Cholesterol (mmol.l <sup>-1</sup> )	5.4(4.6, 6.1)	5.3(4.6, 5.9)	5.7(5.0, 6.5)	0.10
Triglyceride (mmol.l <sup>-1</sup> )	2.0(1.6,2.4)	1.9(1.6,2.2)	1.9(1.4, 2.4)	0.85
HDL (mmol.l <sup>-1</sup> )	1.4(1.2, 1.5)	1.4(1.3, 1.5)	1.5(1.3, 1.7)	0.16
LDL (mmol.l <sup>-1</sup> )	3.1(2.6, 3.6)	3.0(2.4, 3.6)	3.3(2.6, 4.0)	0.12
Chol:HDL ratio	3.8(3.3, 4.4)	3.8(3.1, 4.5)	3.9(3.2, 4.6)	0.89
<b>Adipose tissue deposition</b>				
VAT (l)	5.5(3.9, 7.1)	5.5(4.1, 6.8)	5.0(3.9, 6.0)	0.20
SAT (l)	8.2(6.0, 10.3)	7.7(5.6, 9.8)	7.9(5.0, 10.8)	0.27
Total abdominal AT (l)	13.7(11.3, 16.0)	13.1(11.2, 15.1)	12.8(9.1, 15.5)	0.23
<b>Brachial Artery Function</b>				
GTN-Mediated Dilation (%)	13.5(9.1, 17.8)	14.6(10.1, 19.0)	14.1(10.5, 18.7)	0.74

213

214 Data are presented as mean (95% CI). <sup>†</sup> Significantly different from baseline ( $P<0.05$ ).

215 <sup>‡</sup> Significantly different from immediately following 16 weeks of supervised exercise training  
 216 ( $P<0.05$ ).

217 **BMI** Body mass index, **BP** blood pressure, **ALT** Alanine aminotransferase, **AST** Aspartate  
 218 aminotransferase, **GGT** Gamma-glutamyltransferase, **HDL** High density lipoprotein, **LDL**  
 219 Low density lipoprotein, **VAT** Visceral adipose tissue **SAT** Subcutaneous adipose tissue **AT**  
 220 Adipose tissue **GTN** Glyceryl trinitrate

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