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## **Metabolic and cardiovascular adaptations to an 8-week lifestyle weight loss intervention in younger and older obese men**

Julie Vion, Veronika Sramkova, Emilie Montastier, Marie-Adeline Marquès, Sylvie Caspar-Bauguil, Thibaut Duparc, Laurent O Martinez, Virginie Bourlier, Isabelle Harant, Dominique Larrouy, et al.

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1 **RESEARCH ARTICLE**

2 **Metabolic and cardiovascular adaptations to an 8-week lifestyle weight loss**  
3 **intervention in younger and older obese men**

4

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10

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21

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23

24 Running head: Aging and Cardiometabolic Adaptations in Obesity

25

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29

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31

32 **Abstract**

33 The number of older obese adults is increasing worldwide. Whether obese adults show similar  
34 health benefits in response to lifestyle interventions at different ages is unknown. The study  
35 enrolled 25 obese men (BMI 31-39 kg/m<sup>2</sup>) in two arms according to age (30-40 and 60-70  
36 years old). Participants underwent an 8-week intervention with moderate calorie restriction  
37 (~20% below individual energy requirements) and supervised endurance training resulting in  
38 ~5% weight loss. Body composition was measured using dual energy X-Ray absorptiometry.  
39 Insulin sensitivity was assessed during a hypersinsulinemic euglycemic clamp.  
40 Cardiometabolic profile was derived from blood parameters. Subcutaneous fat and *vastus*  
41 *lateralis* muscle biopsies were used for *ex vivo* analyses. Two-way repeated-measure  
42 ANOVA and linear mixed models were used to evaluate the response to lifestyle intervention  
43 and comparison between the two groups. Fat mass was decreased and bone mass was  
44 preserved in the two groups after intervention. Muscle mass decreased significantly in older  
45 obese men. Cardiovascular risk (Framingham risk score, plasma triglyceride and cholesterol)  
46 and insulin sensitivity were greatly improved to a similar extent in the two age groups after  
47 intervention. Changes in adipose tissue and skeletal muscle transcriptomes were marginal.  
48 Analysis of the differential response to the lifestyle intervention showed tenuous differences  
49 between age groups. These data suggest that lifestyle intervention combining calorie  
50 restriction and exercise shows similar beneficial effects on cardiometabolic risk and insulin  
51 sensitivity in younger and older obese men. However, attention must be paid to potential loss  
52 of muscle mass in response to weight loss in older obese men.

53 **New & Noteworthy**

54 Rise in obesity and aging worldwide are major trends of critical importance in public health.  
55 This study addresses a current challenge in obesity management. Do older obese adults

56 respond differently to a lifestyle intervention composed of moderate calorie restriction and  
57 supervised physical activity than younger ones? The main conclusion of the study is that older  
58 and younger obese men similarly benefit from the intervention in terms of cardiometabolic  
59 risk.

60

61

## 62 INTRODUCTION

63 Life expectancy has increased over the past century. In parallel, obesity has reached  
64 epidemic proportion in many regions of the World. Adults over the age of 65 constitute 10%  
65 of the global population and are the fastest growing demographic age group. In the USA,  
66 more than a third of adults aged 60 and older are obese. The growing prevalence of obesity in  
67 the elderly is a major health concern as the condition favors an array of complications  
68 strongly associated with age and contributes to increased disability and mortality (1). The age-  
69 related decline in muscle mass and strength is exacerbated when obesity is present (2). Loss  
70 of muscle mass is a powerful predictor of late-life disability (3). A recent evaluation of  
71 cardiovascular morbidity revealed that overweight increases long-term risk for cardiovascular  
72 complications (4). Besides accumulation of co-morbidities and deterioration of the quality of  
73 life, excess body weight during midlife is associated with an increased risk of death (5).

74 Reducing the risk of cardiometabolic complications therefore constitutes a new  
75 important challenge in the elderly obese population. Obtaining clinically significant weight  
76 loss is possible in obese older men and women, notably as adherence to lifestyle interventions  
77 increases with age (6). However, the risk inherent to intensive lifestyle interventions in the  
78 aging population is real. The Look Ahead study revealed that intentional weight loss in  
79 overweight and obese older adults with diabetes results in an increase in frailty fractures later  
80 in life (7). Combined with a moderate calorie restriction to induce weight loss, obese older  
81 adults can safely perform moderate intensity physical activity. In the few randomized  
82 controlled trials designed to assess the effect of dietary interventions and physical exercise,  
83 the extent of weight loss was higher in groups with a calorie restriction component than in  
84 those with exercise alone (8). The combination of the two types of interventions preserved  
85 lean body mass and bone mineral density (9). A recent survey shows that there is congruent  
86 evidence for the benefit of intentional weight loss in advancing age obese patients (10).

87           The consequences of lifestyle interventions on metabolic and cardiovascular  
88 adaptations in elderly obese individuals are very imperfectly described. Whereas data from  
89 randomized control trials give indications on the type of lifestyle interventions that may be  
90 advised, information is scant on the differential response to such interventions between  
91 younger and older obese adults (11, 12). No comparisons between age groups has been  
92 performed to assess the impact on cardiometabolic risk of combined diet and physical  
93 exercise interventions. The aim of the present study was therefore to compare the  
94 cardiometabolic adaptations in two groups of obese adult men differing by age (30-40 vs. 60-  
95 70 year-old) who participated in an 8-week dietary intervention with moderate calorie  
96 restriction and supervised physical activity. Two day sessions of investigation performed  
97 before and at the end of the program included measurements of body composition, energy  
98 expenditure, insulin sensitivity, and an extensive characterization of cardiovascular and  
99 metabolic parameters along with biopsies of adipose tissue and skeletal muscle to assess the  
100 molecular adaptations in these tissues.

101

## 102 **METHODS**

103           The study complied to International Council for Good Clinical Practice guidelines  
104 from Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and was  
105 approved by Comité de Protection des Personnes and Agence Nationale de Sécurité du  
106 Médicament et des produits de santé (ANSM). Study was registered as ClinicalTrials.gov  
107 Identifier NCT02161926.

108

### 109 **Study criteria, case report form and adherence**

#### 110 ***Inclusion criteria***

- 111 • Men
- 112 • Age between 30 and 40 years for the younger group and 60 and 70 years for the older
- 113 group
- 114 • Body mass index between 30 and 40 kg/m<sup>2</sup>, both inclusive
- 115 • Obesity duration > 5 years
- 116 • Stable weight for 3 months immediately prior the study (<5% change)

117 ***Exclusion criteria***

- 118 • Women
- 119 • Untreated dyslipidemia
- 120 • Untreated hypertension
- 121 • Type 1 or type 2 diabetes with insulin therapy
- 122 • HbA1c > 7%
- 123 • History of cardiovascular disease
- 124 • History of bariatric surgery
- 125 • Dietary restrictions (*e.g.* vegetarianism and veganism)
- 126 • Smoker (> 10 cigarettes/day)
- 127 • Alcohol abuse (> 66g/day)
- 128 • Known malignancy
- 129 • Unable to perform physical exercise

130 Among older obese participants, three were taking metformin, two were under  
131 cholesterol-lowering drugs and two were treated for hypertension. After obtaining signed  
132 informed consent from participants, recruiting clinicians completed case report forms for each  
133 participant to record socio-demographic information, anthropometric measurements, vital  
134 signs, information about medical history, concomitant medication, physical examination and  
135 dietary habits. Calorie restriction adherence was checked during dietitian follow up and



136 scheduled medical examinations. The caloric deficit ranged between 167 and 704 kcal/day.  
137 Compliance with training was checked by a training diary including day-to-day activities. The  
138 percentage of completed sessions was greater than 90%.

139

## 140 **Study design**

141 Twenty-five obese male adults were enrolled in two groups according to age ranges in  
142 the MONA (Metabolism, Obesity, Nutrition and Age) clinical trial (Table 1, Fig. 1).  
143 Participants followed an 8-week lifestyle intervention combining moderate calorie restriction  
144 and regular physical activity. Each participant performed two sessions at the clinical  
145 investigation center at baseline (S1) and after lifestyle intervention (S2) after a 10h overnight  
146 fast. Measurements started after a 1h rest period. On one experimental day, blood parameters  
147 were assessed at rest and during a 45 min acute exercise bout performed at 50% VO<sub>2</sub>max. On  
148 another experimental day one week apart, resting metabolic rate was measured prior to  
149 subcutaneous abdominal adipose tissue and *vastus lateralis* muscle biopsies. One hour after  
150 the last biopsy, a hyperinsulinemic euglycemic clamp was performed. All these parameters  
151 were investigated at baseline and at the end of the lifestyle intervention 8 weeks later.  
152 According to validated procedures at Toulouse University Hospitals and manufacturers  
153 recommendations, blood was collected in dry tubes with or without separating gel and in  
154 EDTA tubes, and immediately processed for plasma or serum storage at -80°C.

155

## 156 **Lifestyle intervention**

157 The calorie restriction program (minus 20% caloric intake below individual energy  
158 requirements) was prescribed to the study participants in order to induce moderate weight  
159 loss. Energy requirements/expenditure were calculated based on resting metabolic rate

160 measured by indirect calorimetry at baseline  $\times 1.3$  for sedentary individuals. Energy deficit  
161 was calculated as follows: [(Total energy intake at S2 (kcal) – Total energy expenditure at S2  
162 (kcal)) / Total energy intake at S2 (kcal)]  $\times 100$ . Total energy intake was estimated based on  
163 48h dietary records. Participants were instructed to eat meals containing  $\sim 50\%$  carbohydrates,  
164  $\sim 30\%$  fat and  $\sim 20\%$  proteins. Older adults were advised to consume 1.2 g/kg/day of protein.  
165 Participants were instructed to fill in three-day (Tuesday, Thursday and Sunday) food diaries  
166 at weeks 2, 4, 6 and 8. A dietitian called each participant every week to ensure proper follow  
167 up which was also checked during medical monitoring week 4 at the clinical investigation  
168 center.

169 Physical exercise sessions aimed at maintaining the decline of lean body mass  
170 typically associated with calorie restriction. Participants exercised 2 times per week under  
171 direct supervision and 3 times per week on their own following detailed instructions. All daily  
172 sessions consisted of 10 min warm-up at 40%  $VO_{2max}$  on bicycle ergometer followed by  
173 endurance training with progressive increase in exercise intensity (at 60%  $VO_{2max}$ ) and  
174 duration (5-15 min), intense 1 min exercise bouts at 80-90%  $VO_{2max}$  followed by 4 min at  
175 40%  $VO_{2max}$  (the number of which progressively increased during the intervention) and,  
176 muscle strengthening of the lower and upper limbs. Muscle stretching completed each  
177 session.

178

### 179 **Body composition and indirect calorimetry**

180 After completing the screening visit, fat mass was measured on a Dual Energy X-Ray  
181 Absorptiometer (Lunar Prodigy Advance DPX). The fat-lean ratio was calculated by dividing  
182 absolute fat mass (kg) to absolute lean body mass (kg). The appendicular muscle mass/height<sup>2</sup>  
183 (kg/m<sup>2</sup>) was used as cut-off for sarcopenia (13). Breath-by-breath measurements were taken at

184 rest and throughout exercise to assess air flow and, O<sub>2</sub> and CO<sub>2</sub> concentrations in expired  
185 gases by using a computerized ergospirometer (Quark RMR, Cosmed, Italy). VO<sub>2</sub> and VCO<sub>2</sub>  
186 were measured and the respiratory quotient calculated (14).

187

### 188 **Hyperinsulinemic euglycemic clamp**

189 After an overnight fast, insulin (80 mU.m<sup>-2</sup>.min<sup>-1</sup>) and 20% glucose (to maintain  
190 plasma glucose at 90 mg/dL) were administered for 2 h (15). Glucose and insulin were  
191 measured in 3 independent blood plasma samples 10 min apart at baseline and again at  
192 steady-state during the last 30 min of the clamp. The glucose disposal rate (GDR) was  
193 normalized to body weight (M value) and the Insulin Sensitivity Index (ISI clamp) was  
194 calculated as follow: [M value/(Mean blood glucose at clamp steady-state (mM)×0.18)]/Mean  
195 blood insulin at clamp steady-state (μU/ml). Plasma NEFA suppression during the clamp was  
196 calculated as follows: 100-[(Plasma NEFA at clamp steady-state/Plasma NEFA at  
197 baseline)×100]. Oxidative glucose disposal was calculated as the total carbohydrate oxidation  
198 at clamp steady-state (g/min). The non-oxidative glucose disposal was determined by the  
199 difference between GDR and oxidative glucose disposal.

200

### 201 **Blood analyses**

202 Routine cardiometabolic, nutritional and inflammation-related parameters were  
203 determined using standard methods at Toulouse University Hospitals COFRAC-accredited  
204 laboratories. Other blood analyses used commercial assays described in Supplementary Table  
205 1. The adipo-IR index was calculated by the product of [plasma insulin at baseline (pmol/l) ×  
206 (plasma NEFA at baseline (μmol/l)/1000)] as previously described (16). The Framingham risk

207 score is a composite estimate of the 10 year coronary heart disease risk (%) including the  
208 following variables: age, sex, smoking status, p-total cholesterol, p-HDL-C, and systolic  
209 blood pressure(17).

210

### 211 **Fatty acid profiles in red blood cells and adipose tissue**

212 FA composition was determined by capillary gas chromatography. Red blood cells  
213 were separated from plasma and washed three times with physiological saline. The lipid layer  
214 remaining after RNA extraction was collected from subcutaneous adipose tissue from  
215 abdominal region (18). Adipose tissue and red blood cell lipids were extracted according to  
216 the Folch method. Phospholipid (for red blood cells) and triacylglycerol (for adipose tissue)  
217 fraction were isolated by thin layer chromatography on silica glass plates (Merck, Darmstadt,  
218 Germany) using petrol ether/diethylether/acetic acid (80V/20V/1V) as the mobile phase.  
219 Separation and quantitation of FA methyl esters generated by transmethylation was achieved  
220 on a GC 2010 (Shimadzu, Champs sur Marne, France) with flame ionization detection as  
221 previously described (19). Fatty acid profiles were reported as molar %. C16  $\Delta$ 9,  $\Delta$ 6 and  $\Delta$ 5  
222 desaturase indexes were calculated from the following ratios: C16:1n-7/C16:0, C20:3 n-  
223 6/C18:3 n-6 and C20:4 n-6/C20:3 n-6.

224

### 225 **Adipose tissue and skeletal muscle analyses**

226 A needle biopsy (~1.5 g) of subcutaneous abdominal adipose tissue was performed  
227 under local anesthesia at a distance of 10 - 15 cm to the left of the umbilicus (20). Samples of  
228 muscle *vastus lateralis* weighing 60–100 mg were obtained by needle biopsy using the  
229 Bergström technique, blotted and cleaned (21). Samples were snap-frozen in liquid nitrogen.  
230 DNA microarray experiments were performed according to manufacturer's recommendations

231 (Agilent Technologies, Supplementary Table 1). Sample datasets were processed together  
232 following standard methods (22). Arrays were scanned using an InnoScan®710 scanner  
233 (Innopsys, Carbonne, France). Images were quantified using MAPIX® v7.2.0 software  
234 (Innopsys). Microarray processing used R/limma for background correction, loess  
235 normalization and within-array replicate probes average (23). Transcript levels were  
236 expressed as log ratios with regard to baseline. Log<sub>2</sub>-transformed data were analyzed by  
237 principal components analysis using centered and scaled variables. Heat maps were ordered  
238 by dendrograms derived from hierarchical clustering using Euclidian distance as the similarity  
239 measure and Ward methodology (24). Gene expression data from DNA microarray analyses  
240 reported in this study have been deposited at Gene Expression Omnibus (GSE159809 for  
241 adipose tissue; GSE158850 for skeletal muscle). Reverse transcription and quantitative PCR  
242 data were normalized to reference gene PPIA (adipocyte) and TBP (myotubes) and expressed  
243 as 2<sup>-ΔCt</sup> values (Supplementary Table 1). Deparaffinated sections of subcutaneous abdominal  
244 adipose tissue were processed for immunohistochemical detection of CD206 macrophage  
245 marker according using CD206 primary antibody (MAB25341 R&D Systems) and anti-  
246 mouse immunoglobulin/biotinylated rabbit F(ab')<sub>3</sub> secondary antibody (EO413, Dako).  
247 Sections were processed with hematoxylin-eosin staining. Images were generated using  
248 Nanozoomer (Hamamatsu). CD206-labelled macrophages were located and marked. Then,  
249 surrounding adipocytes were counted at 10x magnification. Fields representing 500  
250 adipocytes were analysed. Data are presented as number of macrophages for 100 adipocytes.

251

## 252 **Adipocyte and myotube cultures**

253 Subcutaneous abdominal adipose tissue was digested with collagenase (Sigma  
254 Aldrich). Following filtration and centrifugation, pellet containing cells from the stromal

255 vascular fraction was incubated in erythrocyte lysis buffers. Cells were then filtrated,  
256 centrifuged, resuspended in PM4 medium with 132 nmol/L insulin and plated into 35 mm  
257 Petri dish (25). PM4 was replaced every second day. Cells were sub-cultured at 70%  
258 confluence. Experiments were performed at passages 3 or 4. Differentiation of 2-day post-  
259 confluent cells and Oil-Red O staining was performed as described (26, 27). Satellite cells  
260 were isolated and grown from *vastus lateralis* muscle biopsies (28). Briefly, cells from stroma  
261 vascular fraction were grown in DMEM supplemented with 10% FBS and various factors  
262 (human epidermal growth factor, BSA, dexamethasone, gentamycin, fungizone, fetuin).  
263 Quiescent and activated satellite cells (*i.e.* myoblasts) were immunopurified using PE-Vio770  
264 mouse anti-human CD56 and fluorescent-activated cell sorting analysis. Differentiation of  
265 myoblasts into myotubes was initiated at ~ 80–90% confluence, by switching to  $\alpha$ -Minimum  
266 Essential Medium with 2% penicillin-streptomycin, 2% FBS, and fetuin (0.5 mg/ml). The  
267 medium was changed every 2 days and cells were grown up to 4 days.

268

## 269 **Statistical analysis**

270 Except for analyses of DNA microarray data described above, statistical analyses were  
271 performed using GraphPad Prism 8.4 for Windows (GraphPad Software Inc., San Diego,  
272 CA). Normal distribution and homogeneity of variance of the data were tested using Shapiro-  
273 Wilk and F tests, respectively. Group matching at baseline was assessed using two-tailed  
274 unpaired Student's t-tests (Table 1). Two-way repeated-measure ANOVA and linear mixed  
275 models were used for determination of both group and time (intervention) effects. Partial  
276 least-squares discriminant analysis was applied to classify 53 variables of metabolic and  
277 cardiovascular risk from the most to the less relevant in discriminating age groups

278 (Supplementary Table 2, Supplementary Fig. 1). All values in figures and tables are presented  
279 as means  $\pm$  SEM. Statistical significance was set at  $p \leq 0.05$ .

280

## 281 **RESULTS**

### 282 **Participant characteristics**

283 Following assessment for eligibility and inclusion criteria, younger and older obese  
284 men were included in two study groups (Fig. 1). Age ranges were selected on two grounds.  
285 First, we wished to investigate the effect of aging without occurrence of severe pathological  
286 and frailty conditions often associated with advancing age. Second, participants below 70  
287 year-old were enrolled on the basis of known benefits of lifestyle interventions with good  
288 compliance in this age range (12). As comparison of two age groups with thorough  
289 cardiometabolic phenotyping had not been performed before, this initial study enrolled men to  
290 avoid the confounding effect of menopausal status. The participants showed similar body  
291 weight, body mass index and fat mass indicating proper matching of the groups in terms of  
292 body composition (Table 1). No difference was seen for waist circumference ( $117 \pm 3$  and  
293  $118 \pm 2$  cm in younger and older obese groups, respectively). Obesity duration based on self-  
294 reporting assessment differed between the groups ( $10.1 \pm 1.8$  and  $18.3 \pm 2.1$  years in younger  
295 and older obese groups, respectively,  $p < 0.01$ ). Based on current European guidelines,  
296 assessment of appendicular skeletal muscle mass / height<sup>2</sup> indicated that none of the  
297 participants were sarcopenic ( $10.9 \pm 0.2$  and  $10.7 \pm 0.8$  kg/m<sup>2</sup> in younger and older obese  
298 groups, respectively) (13).

299

### 300 **Body composition, energy balance and nutritional parameters**

301 The mixed intervention combining moderate calorie restriction and physical activity  
302 induced a decrease in body weight ( $-4.6\pm 0.7$  and  $-5.0\pm 0.7\%$  from baseline in younger and  
303 older obese participants, respectively). BMI and fat mass were decreased in the two groups  
304 (Fig. 2A and 2B, Table 1). Accordingly, fat-to-lean ratio (Fig. 2C) and plasma leptin level  
305 (Supplementary Table 3) decreased. Fat mass loss in response to lifestyle intervention was  
306 lower in older obese participants ( $P_{\text{Group}\times\text{Time}}=0.045$ ). Bone mass showed a trend towards an  
307 increase in older obese adults after lifestyle intervention ( $P_{\text{Group}\times\text{Time}}=0.065$ ) (Fig. 2D). In the  
308 context of the potential adverse effects of calorie restriction in elderly individuals, muscle  
309 mass was unchanged in younger obese but decreased significantly in older obese men after  
310 lifestyle intervention ( $P_{\text{Group}\times\text{Time}}=0.025$ ) (Fig. 2E and 2F). In agreement with the lower energy  
311 intake, calorie restriction was associated with a drop in total energy expenditure and resting  
312 metabolic rate (Table 1) (29, 30). Resting metabolic rate showed a trend towards a higher  
313 decrease in older obese adults after lifestyle intervention ( $P_{\text{Group}\times\text{Time}}=0.058$ ) that may be due  
314 to the reduced muscle mass in this age group.

315 In accordance with the duration of the calorie restriction, we observed a decrease in  
316 serum prealbumin (Fig. 2G) while albumin levels remained unchanged (data not shown). The  
317 respective decrease and increase in serum retinol binding protein (Fig. 2H) and sex hormone  
318 binding globulin (Fig. 2I) levels were also coherent with a state of energy deficit (31, 32). The  
319 decrease in serum retinol binding protein after lifestyle intervention was lower in older obese  
320 individuals ( $P_{\text{Group}\times\text{Time}}=0.024$ ). Fatty acid composition was, respectively, assessed in plasma  
321 phospholipids and adipose tissue triacylglycerols (Supplementary Tables 4 and 5). Following  
322 weight loss, plasma C16  $\Delta 9$ -desaturase index was significantly decreased in the two groups  
323 (Fig. 2J). Plasma  $\Delta 5$ - (Fig. 2K) and  $\Delta 6$ -desaturase (Fig. 2L) indexes were, respectively, up-  
324 and down-regulated in younger and older obese adults. In line with the slower turnover rate in



325 adipose tissue compared to plasma fatty acids, variation in the fatty acid profiles of adipose  
326 tissue was minimal during the weight loss program (Supplementary Table 5).

327

### 328 **Cardiovascular risk, insulin sensitivity and inflammation**

329 The main variations in parameters of cardiovascular risk are shown in Fig. 3 and  
330 Supplementary Table 3. Parameters derived from deep analysis of the profiles of lipoprotein  
331 classes by nuclear magnetic resonance are shown in Supplementary Table 6 (33). A  
332 composite index, the Framingham Risk Score (Fig. 3A), which measures the risk in percent to  
333 develop a cardiovascular disease in the next 10 years, was markedly decreased in the two  
334 groups (17). The weight loss program induced a decrease in systolic (Fig. 3B) and diastolic  
335 (Fig. 3C) arterial blood pressures. The decrease in diastolic blood pressure after lifestyle  
336 intervention was significantly greater in older obese individuals ( $P_{\text{Group} \times \text{Time}} = 0.026$ ). Plasma  
337 triacylglycerol and cholesterol levels were reduced in the two groups (Supplementary Table  
338 3). Accordingly, the plasma concentration in total VLDL particles (Fig. 3D), the  
339 triacylglycerol and cholesterol contents of VLDL (Fig. 3E and Supplementary Table 6) and,  
340 plasma apoB level were decreased (Supplementary Table 3). LDL-cholesterol level was  
341 significantly lowered in the two groups (Supplementary Table 3). This was associated with a  
342 decrease in the proatherogenic small fraction of LDL particles (Fig. 3F). Whereas HDL-  
343 cholesterol levels and the concentration of HDL particles were not modified by the  
344 intervention, there was a decrease in the triacylglycerol content of HDL that was associated  
345 with an increase of total HDL size and a reciprocal change in the concentrations of large and  
346 small HDL particles (Fig. 3G, 3H and 3I; Supplementary Tables 3 and 6).

347 At baseline, older obese participants had slightly deteriorated glycaemic control  
348 (Supplementary Table 7). The weight loss program lowered HbA1c (Supplementary Table 3)

349 and fasting plasma glucose levels (Supplementary Table 7) in the two groups. Various  
350 components of insulin sensitivity were derived from euglycemic hyperinsulinemic clamp  
351 (Fig. 3, Supplementary Table 7). M-value (Fig. 4A) and insulin sensitivity index (Fig. 4B)  
352 were increased in both groups. It was also the case for the glucose disposal rate, notably the  
353 non-oxidative glucose disposal component (Fig. 4C).

354 Inflammatory status was investigated at plasma and tissue levels. There were little  
355 changes in plasma parameters except a moderate down regulation of interleukin 8  
356 (Supplementary Table 3). In subcutaneous adipose tissue, CD206 immunostaining revealed  
357 little infiltration by macrophages and no significant change induced by the lifestyle  
358 intervention (number of macrophages for 100 adipocytes:  $15.1 \pm 2.3$  vs  $10.2 \pm 1.9\%$  at baseline  
359 vs 8 weeks for the younger obese group, and  $16.1 \pm 2.5$  vs  $14.0 \pm 2.4\%$  at baseline vs 8 weeks  
360 for the older obese group). Accordingly, DNA microarray analyses of subcutaneous adipose  
361 tissue and *vastus lateralis* skeletal muscle showed no evidence for major group differences in  
362 variations of adipose tissue macrophage-specific and skeletal muscle inflammation-related  
363 genes (Fig. 5A and 5B) (34, 35).

364

### 365 ***In vitro* and *in vivo* characteristics of adipocytes and myocytes**

366 Gene expression profiling using DNA microarrays revealed no changes during the  
367 lifestyle intervention in human adipocyte and myocyte specific genes (Fig. 5C and 5D) (20,  
368 36). To gain further insight on cell-autonomous regulation, precursors from the stroma-  
369 vascular fractions of the respective tissues were differentiated *in vitro* into adipocytes and  
370 myotubes. Preadipocytes at day 12 of differentiation showed similar triacylglycerol  
371 accumulation at baseline in the two groups (Fig. 5E). The weight loss program did not  
372 influence the triacylglycerol content. Accordingly, expression of genes prototypical of white

373 adipocytes such as *PPARG* and *FABP4* was not modified (Fig. 5F). The *ex vivo* and *in vitro*  
374 data are in agreement with adipose tissue-related parameters derived from the euglycemic  
375 hyperinsulinemic clamp (Fig. 4D and 4E). Adipose tissue insulin sensitivity reflected by both  
376 the Adipo-IR index and the suppression of plasma non-esterified fatty acid (NEFA) levels  
377 during the clamp did not change significantly during the intervention. Differentiation of  
378 myoblasts and expression of two prototypical myogenic genes *MYOG* and *MYH7* were similar  
379 in the two groups at baseline and in response to the 8-week lifestyle intervention (Fig. 5G and  
380 5H). Therefore, analyses of whole tissue transcriptome and *in vitro* differentiated progenitor  
381 cells yield similar conclusions in the two tissues.

382

### 383 **Differences between younger and older obese men**

384 At baseline, older obese men had significantly lower energy intake, resting metabolic  
385 rate, non-oxidative glucose disposal and plasma NEFA suppression measured during a  
386 hyperinsulinemic euglycemic clamp, compared to younger obese men (Fig. 4C and 4E, Table  
387 1). In contrast, older obese participants had higher plasma HDL-C, HDL-TG, Apo A1, total  
388 VLDL, sex hormone binding globulin, FABP3, interleukin 6, glucose and Framingham risk  
389 score compared to younger ones (Fig. 2I, 3A, 3D and 3G, Supplementary Tables 3 and 6). To  
390 assess the differential response to the weight loss program between the two groups, we  
391 selected 53 non redundant parameters associated with metabolic and cardiovascular risk  
392 (Supplementary Table 2). Partial least square-discriminant analysis using the percentage of  
393 variation during the lifestyle intervention showed discrimination between younger and older  
394 participants (Supplementary Fig. 1). The rank order of the main contributing variables was:  
395 plasma retinol binding protein (Fig. 6A), diastolic blood pressure (Fig. 6B), muscle mass (Fig.  
396 6C), resting metabolic rate (Fig. 6D), bone mass (Fig. 6E) and fat mass (Fig. 6F).

397

## 398 **DISCUSSION**

399 Life expectancy has increased over the past century. In parallel, obesity has reached  
400 epidemic proportion in many regions of the World. As a result, more than a third of adults  
401 aged 60 and older are obese in the USA. The growing prevalence of obesity in the elderly is a  
402 major health concern as the condition favors an array of complications strongly associated  
403 with age and contributes to increased disability and mortality (1). Whereas lifestyle  
404 interventions suggest a beneficial effect of weight loss in older obese adults, the  
405 cardiovascular and metabolic response to such interventions has not been compared between  
406 age groups (8, 9, 37). The present study aimed at determining whether older obese men  
407 respond differently than younger ones to a lifestyle intervention program targeted for ~5%  
408 weight loss and whether some of the responses may be considered as detrimental during  
409 advancing age.

410 The two age groups responded to the lifestyle intervention and showed beneficial  
411 outcomes. Evolution of nutritional parameters showed proper adherence to the calorie  
412 restriction protocol in the two groups. In agreement with a recent randomized control trial,  
413 moderate calorie restriction resulted in decreased plasma C16  $\Delta$ 9-desaturase index related to  
414 stearoyl desaturase activity and increased  $\Delta$ 5-desaturase index reflecting fatty acid desaturase  
415 1 activity (38). Of note, changes in fatty acid desaturase activity have previously been  
416 associated with improvements in glucose tolerance and insulin sensitivity (see below) during  
417 lifestyle intervention (39). The five percent weight loss reached at the end of the intervention  
418 is considered clinically significant to observe long lasting benefits (40). The decrease in fat  
419 mass was not accompanied with a diminution in bone mass suggesting that, using this type of  
420 lifestyle intervention combining moderate calorie restriction and physical training, significant

421 weight loss can be achieved without marked increase in the risks of bone fractures in older  
422 obese men (2, 8). Despite good compliance in the context of a supervised training program,  
423 older obese men showed significant loss in muscle mass. This is a point of attention which  
424 requires further investigation as we could not ascribe muscle loss to specific changes in  
425 plasma parameters or skeletal muscle transcriptomic profile. Whether the duration of obesity  
426 may contribute needs further investigation. Moreover, as the weight loss-induced decrease in  
427 resting metabolic rate is a determinant of weight regain, further studies are required to  
428 determine whether obese men in their sixties are more at risk of body weight relapse than  
429 younger ones (41).

430       Cardiovascular and metabolic risk was decreased with improvement at the levels of  
431 blood pressure, plasma lipid profile and insulin sensitivity. Lipoprotein parameters notably  
432 the decrease in those related to LDL particles shows the mitigating effect of the intervention  
433 on atherogenic risk. Evolution of the HDL profile may result from an induction of lipoprotein  
434 lipase in skeletal muscle upon physical activity leading to the release of surface components  
435 resulting from lipolysis of VLDL to HDL particles (42). This results in the formation of large  
436 size HDL (43). Altogether, data reveal that the cardiovascular risk is diminished in the two  
437 age ranges. As age is the most important risk factor for cardiovascular diseases, lifestyle  
438 interventions such as the one studied here appears of high relevance in the elderly obese  
439 population (44). Whole-body insulin sensitivity measured by euglycemic hyperinsulinemic  
440 clamp increased to a similar extent in younger and older obese individuals, although older  
441 obese exhibited lower insulin sensitivity at baseline. This was particularly obvious for the  
442 non-oxidative glucose disposal component representing mostly glycogen storage in skeletal  
443 muscle (45). Defect in non-oxidative glucose disposal contributes to the development of  
444 insulin resistance, which is independently increased by obesity and age (46). Altogether, our  
445 data are in agreement with previous studies showing a beneficial effect of lifestyle

446 interventions combining calorie restriction and physical exercise on insulin sensitivity (47,  
447 48). They reveal that whole-body insulin sensitivity measured by euglycemic  
448 hyperinsulinemic clamp increased to a similar extent in the two groups. Importantly, this  
449 suggests that, older obese men without marked complication and frailty profiles respond as  
450 well as younger ones to the amelioration of insulin action during a weight loss intervention.  
451 Of note, the improvement in insulin sensitivity observed here with a lifestyle intervention  
452 inducing ~5% weight loss occurs without prominent changes in inflammatory status and  
453 immune response at plasma and tissue levels. Irrespective of the age range, a modulation in  
454 inflammation therefore does not contribute to the amelioration in systemic insulin action.

455         The lifestyle intervention had little impact on adipose tissue and skeletal muscle. *Ex*  
456 *vivo* and *in vitro* analyses revealed no effect on metabolism and immune response in the two  
457 tissues. Whereas protocols with more severe calorie deficit result in marked changes of gene  
458 expression profiles, moderate calorie restriction such as used here does not (49, 50). Lack of  
459 changes in adipose tissue gene expression were supported by immunohistochemistry and lipid  
460 species analyses as well as characterization of differentiated preadipocytes. Similar  
461 congruence between whole tissue transcriptomic analyses and differentiation of progenitor  
462 cells was observed in skeletal muscle. Altogether, these data show that the systemic  
463 improvement in cardiometabolic parameters may occur without major changes in adipose  
464 tissue and skeletal muscle.

465         This study shows several limitations. It was a pilot non-randomized two arms study  
466 with parallel assignment of small sample size. High homogeneity of the study groups was  
467 achieved through recruitment limited to restricted age (10-year window) and BMI (30-40  
468 kg/m<sup>2</sup>) ranges and to a single sex. Design providing paired samples increased statistical  
469 power, especially when adjustment for multiple comparisons has to be applied such as in  
470 ‘omics data analyses (51). Establishing whether the results can be extrapolated to overweight

471 or morbidly obese individuals and to obese women requires further investigation. Other types  
472 of lifestyle interventions may lead to different outcomes. As discussed above, a more severe  
473 calorie restriction is likely to lead to other responses, notably at tissue levels and for  
474 inflammation-related parameters. Different types of physical exercise programs combined  
475 with calorie restriction show different efficiency in improving functional status in obese older  
476 adults (37). Moreover, it will be useful to ascertain that lifestyle interventions of longer  
477 duration do result in similar response in obese adults of various age ranges and do not  
478 provoke adverse effects on skeletal muscle and bone mass.

479

## 480 **CONCLUSIONS**

481 Whereas lifestyle interventions suggest a beneficial effect of weight loss in older obese  
482 adults, the cardiovascular and metabolic response to such interventions had not been  
483 compared between age groups (8, 9, 37). We conclude from the present study that an 8-week  
484 lifestyle intervention combining moderate calorie restriction and physical activity results in  
485 clinically significant weight loss with improvement of cardiometabolic parameters in obese  
486 men of different age ranges. This improvement occurs without changes in inflammatory  
487 markers and molecular adaptations at adipose tissue and skeletal muscle levels. Another  
488 salient feature is the limited differences in response between age groups. Analyses of the  
489 percentage of variation revealed discriminating parameters between younger and older obese  
490 men but scant differences were observed. A higher volume of physical activity may be needed  
491 to limit muscle mass loss in older obese individuals. When combined to calorie restriction,  
492 progressive resistance training proves more efficient in preserving fat-free mass than aerobic  
493 endurance training (11). Adherence to the program was good in the elderly obese group in  
494 accordance with age being a prominent predictor of adherence to lifestyle interventions (6).

495 Altogether, the study shows the interest in implementing lifestyle interventions in the rising  
496 population of older obese adults.

497

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#### 514 **DISCLOSURES**

515 No authors report conflicting interest.

#### 516 **AUTHOR CONTRIBUTIONS**



517 C.T. and D.Lan. designed the clinical protocol with other co-authors. J.V., V.S., M.-A.M.,  
518 T.D. and S.B. analyzed samples and contributed to data analyses. E.M., I.H., D.Lar. and C.T.  
519 contributed to clinical phenotyping. E.M., S.C.-B., L.O.M., V.B., C.V., C.D., P.V., J.-S.S.-B,  
520 J.P.S., C.T., C.M. and D.L. performed data analyses. N.V. supervised transcriptomic analyses  
521 with contribution from N.M.. C.M. and D.Lan. wrote the manuscript, with all coauthors  
522 contributing to revisions.

523 **Figure legends**

524

525 **Figure 1.** CONSORT diagram of the MONA clinical trial. Overall, 25 obese (13 younger and  
526 12 older) male participants were enrolled in an 8-week lifestyle intervention combining  
527 moderate calorie restriction and physical exercise. Two sessions (S1 and S2) of two days of  
528 investigation were performed before and at the end of the program.

529

530 **Figure 2.** Body composition, serum (s-) and plasma (p-) nutritional parameters during a  
531 lifestyle intervention in younger and older obese men. Changes in BMI (A), fat mass (B), fat-  
532 to-lean ratio (C), bone mass (D), muscle mass (E), appendicular skeletal muscle mass /  
533 height<sup>2</sup> (F), prealbumin (G); retinol binding protein, RBP (H), sex hormone binding globulin,  
534 SHBG (I);  $\Delta$ -9 desaturase (D) activity (J),  $\Delta$ 5D activity (K), and  $\Delta$ 6D activity (L). White  
535 circles indicate baseline and black circles 8 weeks after the lifestyle intervention. Data are  
536 means $\pm$ SEM; n=13 younger, n=12 older. Two-way repeated measures ANOVA was  
537 performed to indicate group and time effects.

538

539 **Figure 3.** Cardiovascular risk parameters during a lifestyle intervention in younger and older  
540 obese men. Changes in Framingham risk score (A); systolic blood pressure, SBP (B); and  
541 diastolic blood pressure, DBP (C); total VLDL particles (-p) (D), VLDL-triglyceride, VLDL-  
542 TG (E); small LDL particles, S-LDL-P (F); HDL-TG (G), large HDL particles, L-HDL-p (H),  
543 and S-HDL-p (I). White circles indicate baseline and black circles 8 weeks after the lifestyle  
544 intervention. Data are means $\pm$ SEM; n=13 younger, n=12 older. Two-way repeated measures  
545 ANOVA was performed to indicate group and time effects.

546

547 **Figure 4.** Whole-body and adipose tissue insulin sensitivity during a lifestyle intervention in  
548 younger and older obese men. Changes in M-value (A); insulin sensitivity index, ISI clamp  
549 (B); main components of the glucose disposal rate, GDR, including oxidative GDR, OGD,  
550 and non-oxidative GDR, NOGD (C); the adipose tissue insulin resistance index, adipo-IR  
551 index (D); and plasma non esterified fatty acid, p-NEFA, suppression during the clamp (E).  
552 White circles indicate baseline and black circles 8 weeks after the lifestyle intervention  
553 (panels A, B, D and E). Data are means±SEM; n=13 younger, n=12 older. Two-way repeated  
554 measures ANOVA was performed to indicate group and time effects, \* p<0.05 vs. baseline.

555

556 **Figure 5.** *In vivo* and *in vitro* adipose tissue and skeletal muscle characteristics during a  
557 lifestyle intervention in younger and older obese men. Hierarchical clustering of DNA  
558 microarray data for human adipose tissue macrophage-specific genes (A), skeletal muscle  
559 inflammation-related genes (B), adipocyte metabolism-specific genes (C) and myocyte  
560 marker genes (D). Agglomerative hierarchical clustering was derived using the Euclidean  
561 distance as the similarity measure and Ward methodology. The resulting heatmaps display  
562 participants in rows and genes in columns with red indicating up regulation, violet down  
563 regulation and yellow no change. Gene symbols are shown at the bottom of the heatmaps. On  
564 the left hand side of the heat maps, younger and older individuals are, respectively, colored in  
565 orange and blue. n=13 younger, n=12 older. *In vitro* differentiation of preadipocyte (E, F) and  
566 myoblasts (G, H) at baseline and after 8-weeks of lifestyle intervention in younger and older  
567 obese participants. (E) Oil Red O staining and, (F) *PPARG* and *FABP4* mRNA levels in  
568 adipocytes at 12 days of differentiation. (G) Representative picture of myotubes and, (H)  
569 *MYOG* and *MYH7* mRNA levels at 4 days of differentiation. Data are means±SEM. n=8-10

570 participants per group for *in vitro* data. Two-way repeated measures ANOVA was performed  
571 to investigate group and time effects.

572

573 **Figure 6.** Differential responses in body composition, nutritional and cardiovascular  
574 parameters at baseline and 8-weeks of lifestyle intervention in younger and older obese  
575 participants. Variations ( $\Delta$ ) in plasma retinol binding protein, RBP (A); diastolic blood  
576 pressure, DBP (B); muscle mass (C); resting metabolic rate, RMR (D); bone mass (E); and fat  
577 mass (F) was calculated in younger and older obese participants as the percent change from  
578 baseline. Data are presented as violin box plots; n=13 younger, n=12 older. The mean percent  
579 changes and p values from two-tailed unpaired Student t-test are shown.

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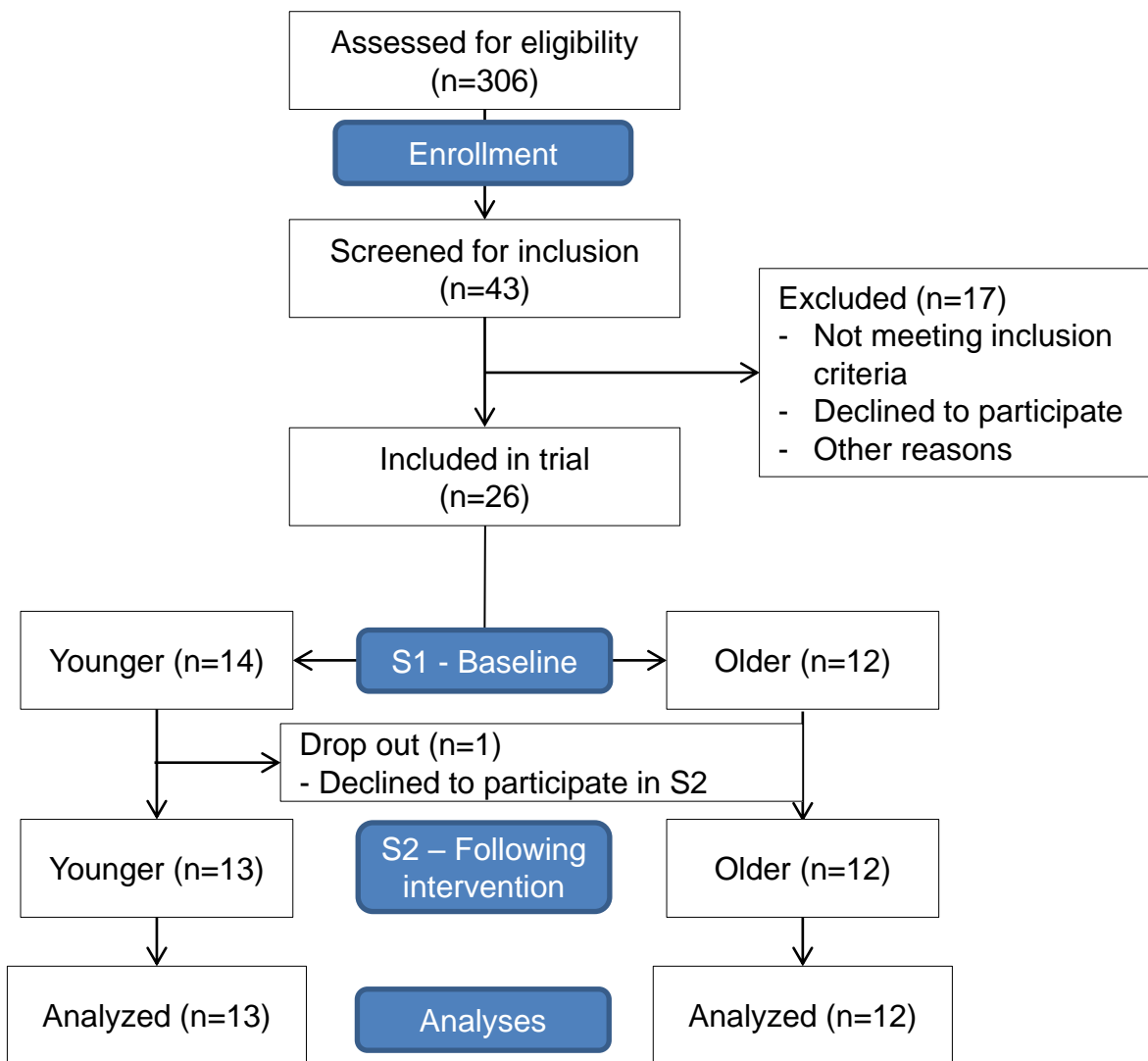


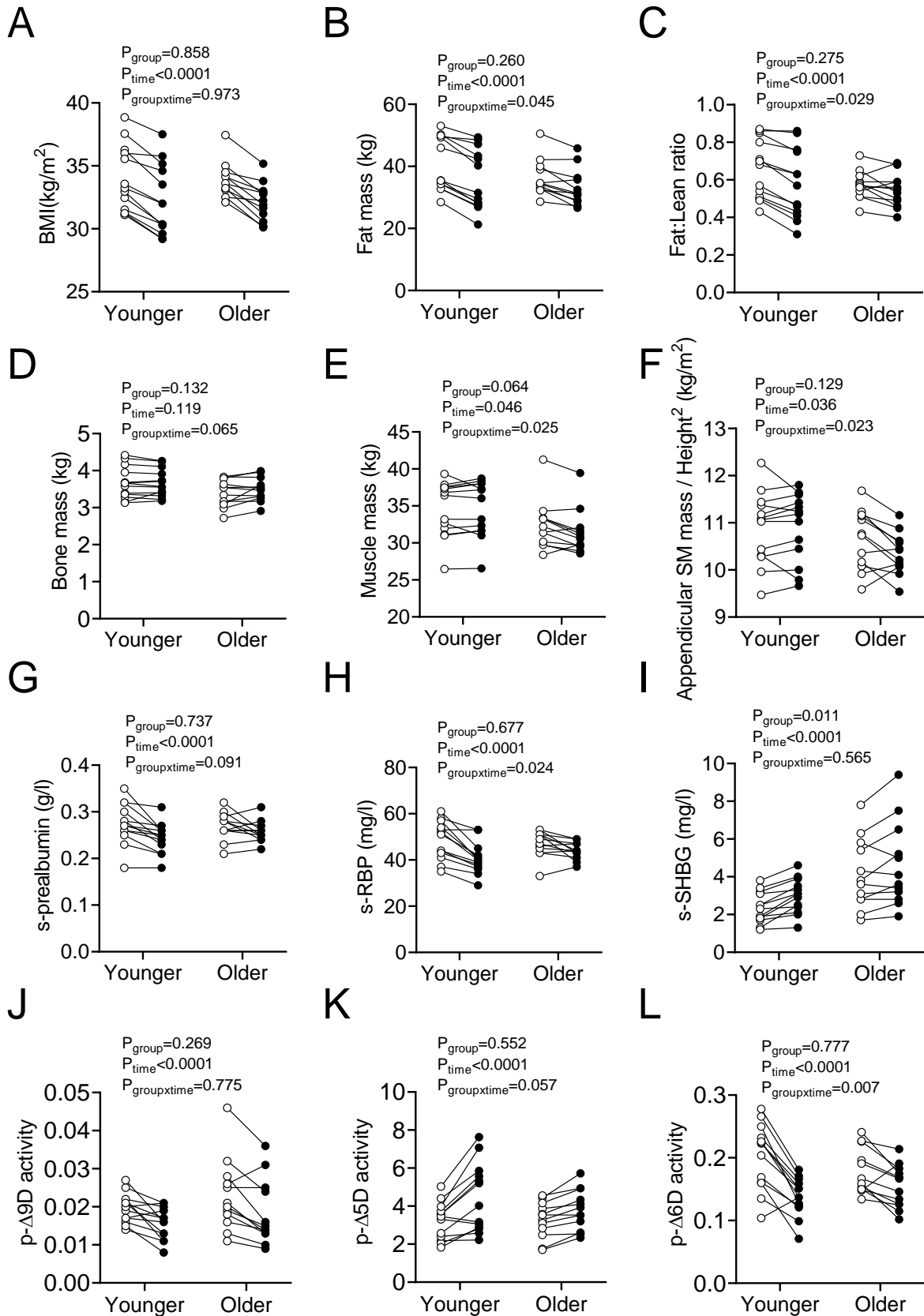
**Table 1.** *Body composition and whole-body energy metabolism variables at baseline and 8-weeks of lifestyle intervention in young and elderly obese participants*

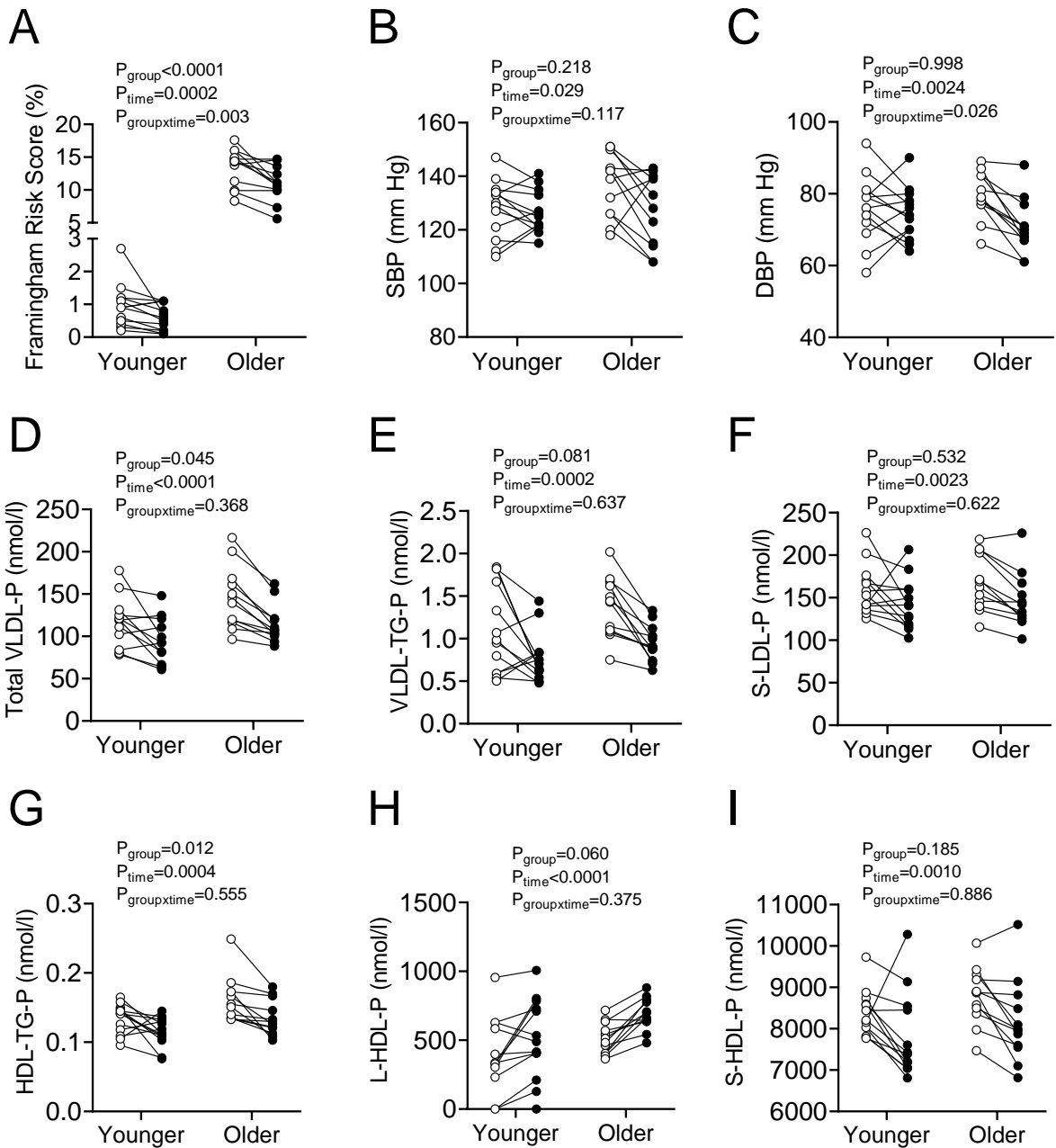
	Younger (n=13)		Older (n=12)		Group effect
	Baseline	8 weeks	Baseline	8 weeks	
Age (yr)	36.0±0.7		64.2±0.9		<0.001
<b>Body composition</b>					
Body weight (kg)	109±3	103±3***	103±3	97.6±2.8***	0.267
BMI (kg/m <sup>2</sup> )	34.2±0.7	32.4±0.8***	33.6±2.7	32.1±0.4***	0.977
Fat mass (kg)	40.9±2.4	35.7±2.7***	36.3±3.3	33.1±3.1***	0.086
Lean mass (kg)	63.1±1.7	62.9±1.8	63.5±5.4	61.5±5.2**	0.854
Bone mass (kg)	3.67±0.12	3.67±0.10	3.39±0.29	3.49±0.29	0.358
Muscle mass (kg)	34.5±1.0	34.6±1.1	32.4±2.8	31.4±2.7	0.035
<b>Energy metabolism</b>					
Energy intake (kcal/d)	3026±50	2156±52***	2678±83	2105±70***	0.022
TEE (kcal/d)	2695±65	2616±71***	2632±88	2404±73***	0.179
RMR (kcal/d)	2073±50	2012±55***	2024±68	1850±57***	0.045

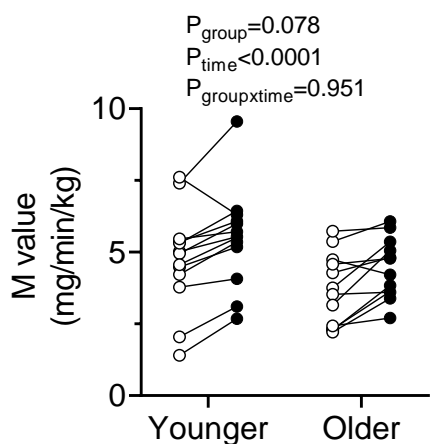
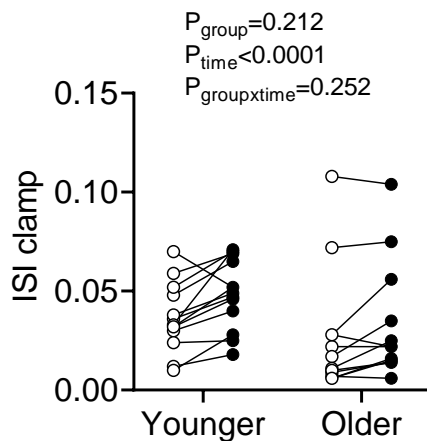
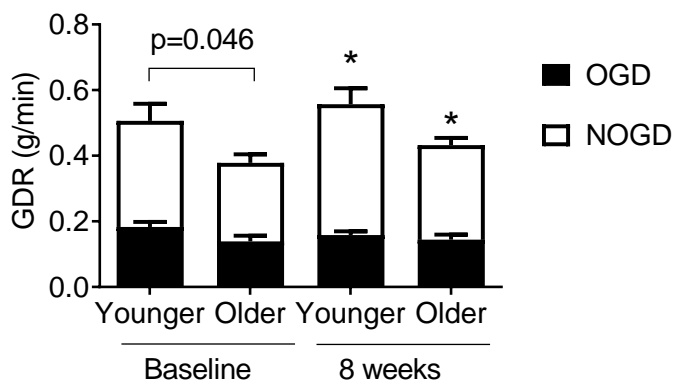
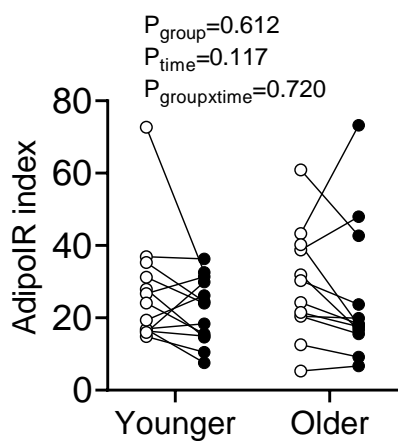
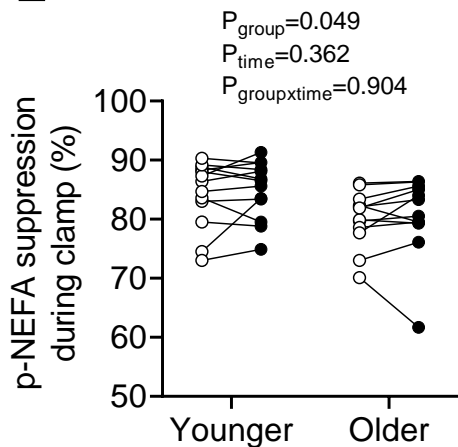
Abbreviations: BMI: body mass index; RMR: resting metabolic rate; TEE: total energy expenditure. Data are means±SEM. Age difference was assessed using Two-tailed unpaired Student t-test. Two-way repeated measures ANOVA was performed for other parameters.

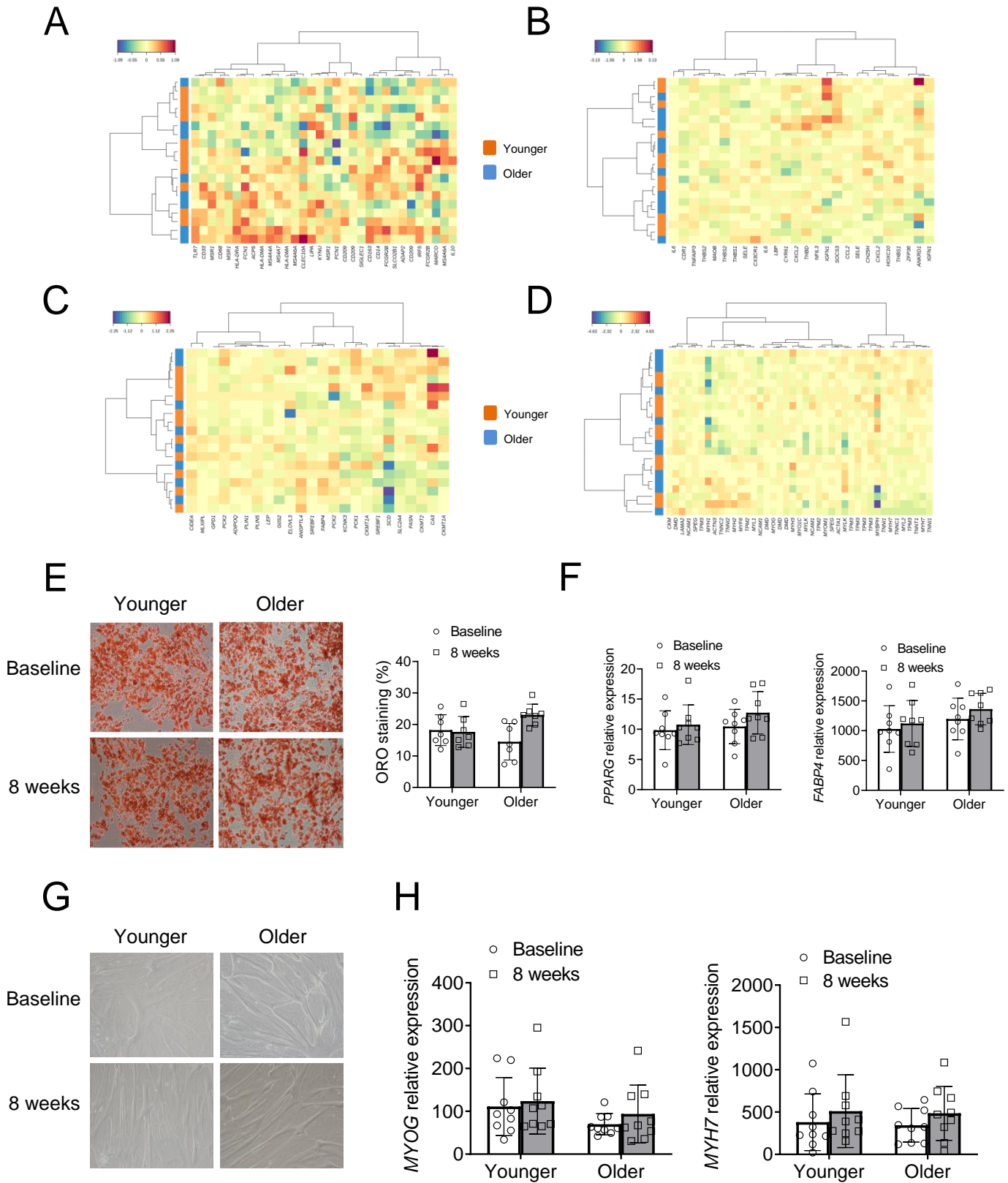
\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 versus respective baseline.

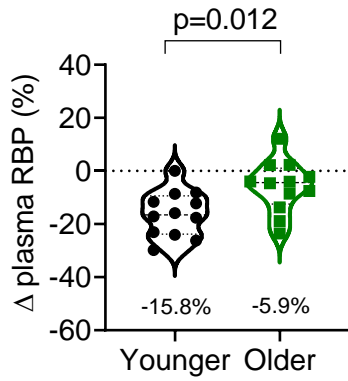
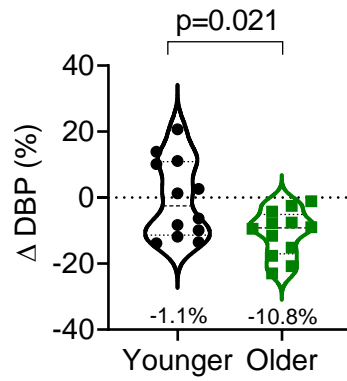
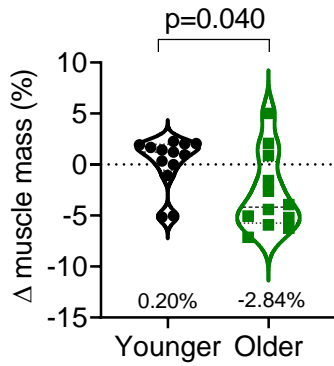
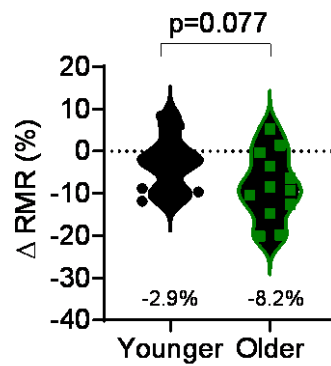
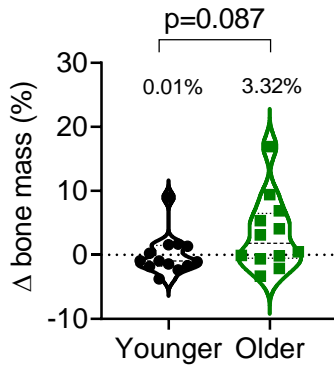
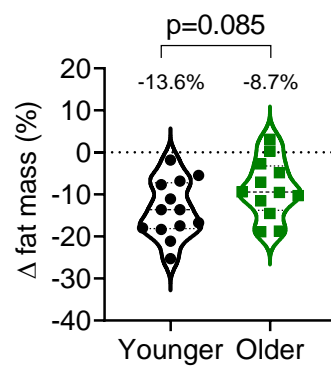






**A****B****C****D****E**



**A****B****C****D****E****F**

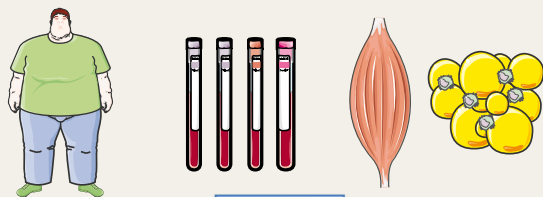
# Cardiometabolic Adaptations in Younger and Older Obese Men

## METHODS

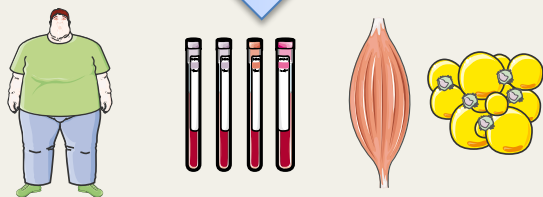
Obese men

30-40 yr old  
n=13

60-70 yr old  
n=12

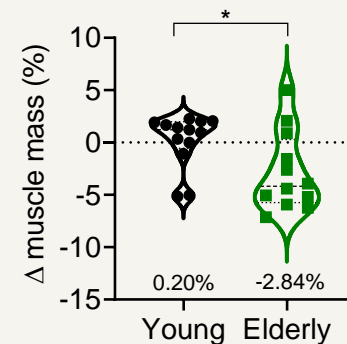
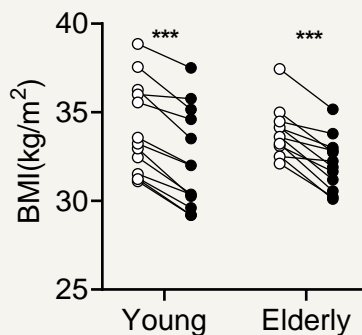


8 week lifestyle intervention

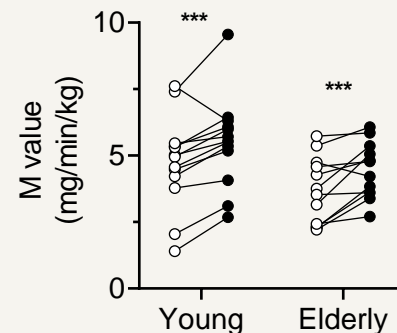
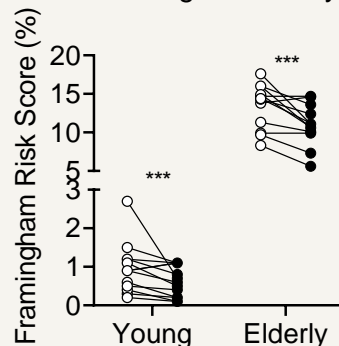


## OUTCOME

Body composition



Cardiometabolic parameters



**CONCLUSION** Calorie restriction combined with physical exercise shows **similar beneficial effects on cardiometabolic risk in younger and older obese men**. Attention must be paid to **loss of muscle mass in response to weight loss in older obese men**.