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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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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/m²) 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/m², 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% VO₂max. 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/height²
183 (kg/m²) 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, O₂ and CO₂ concentrations in expired
185 gases by using a computerized ergospirometer (Quark RMR, Cosmed, Italy). VO₂ and VCO₂
186 were measured and the respiratory quotient calculated (14).

187

188 **Hyperinsulinemic euglycemic clamp**

189 After an overnight fast, insulin (80 mU.m⁻².min⁻¹) 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 Δ 9, Δ 6 and Δ 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. Log₂-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^{-ΔCt} 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')₃ 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 α -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 ± 3 and
293 118 ± 2 cm in younger and older obese groups, respectively). Obesity duration based on self-
294 reporting assessment differed between the groups (10.1 ± 1.8 and 18.3 ± 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 / height² indicated that none of the
297 participants were sarcopenic (10.9 ± 0.2 and 10.7 ± 0.8 kg/m² 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 ± 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 ± 2.3 vs $10.2 \pm 1.9\%$ at baseline
359 vs 8 weeks for the younger obese group, and 16.1 ± 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 Δ 9-desaturase index related to
414 stearoyl desaturase activity and increased Δ 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/m²) 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 height² (F), prealbumin (G); retinol binding protein, RBP (H), sex hormone binding globulin,
534 SHBG (I); Δ -9 desaturase (D) activity (J), Δ 5D activity (K), and Δ 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 (Δ) 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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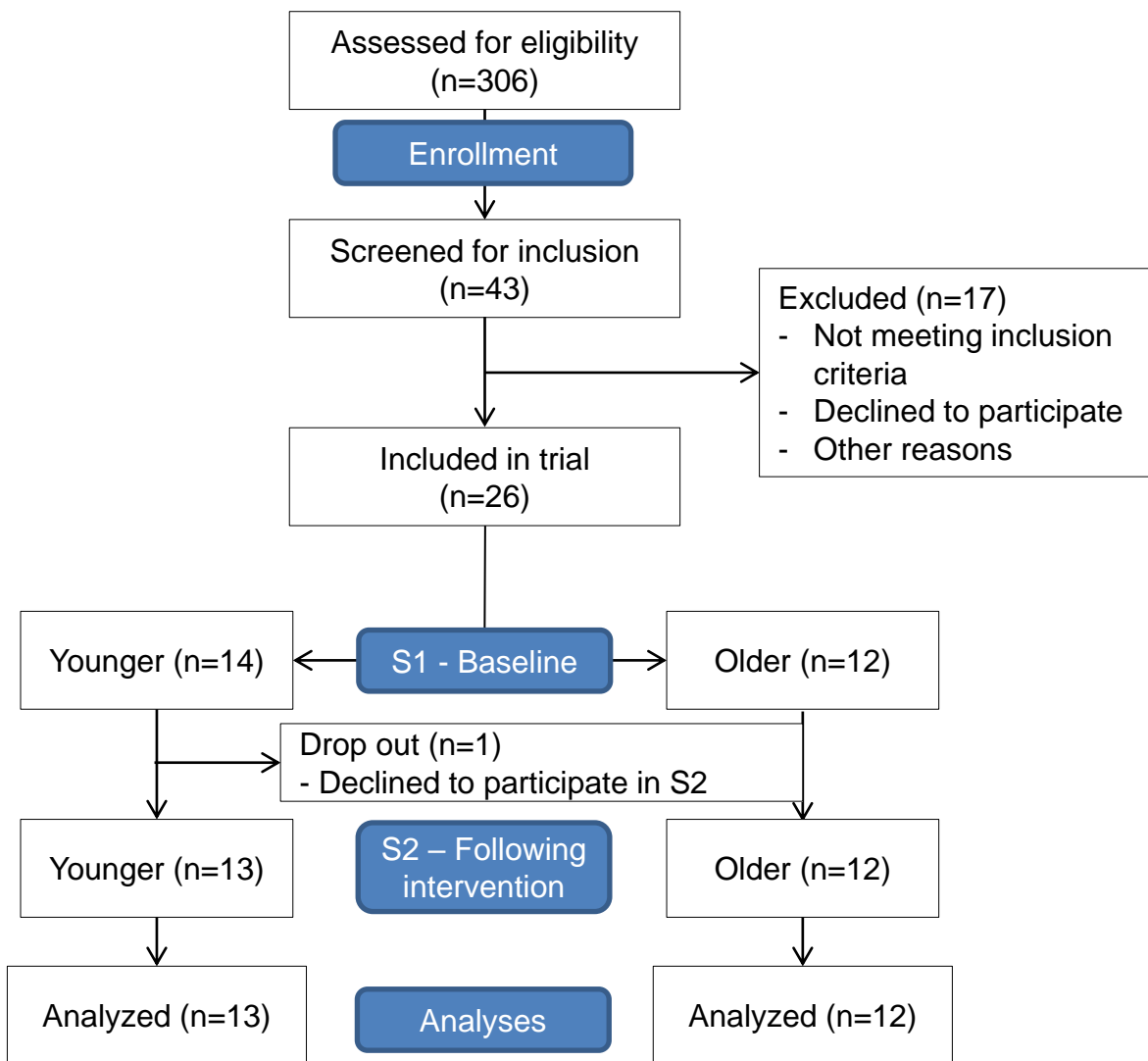
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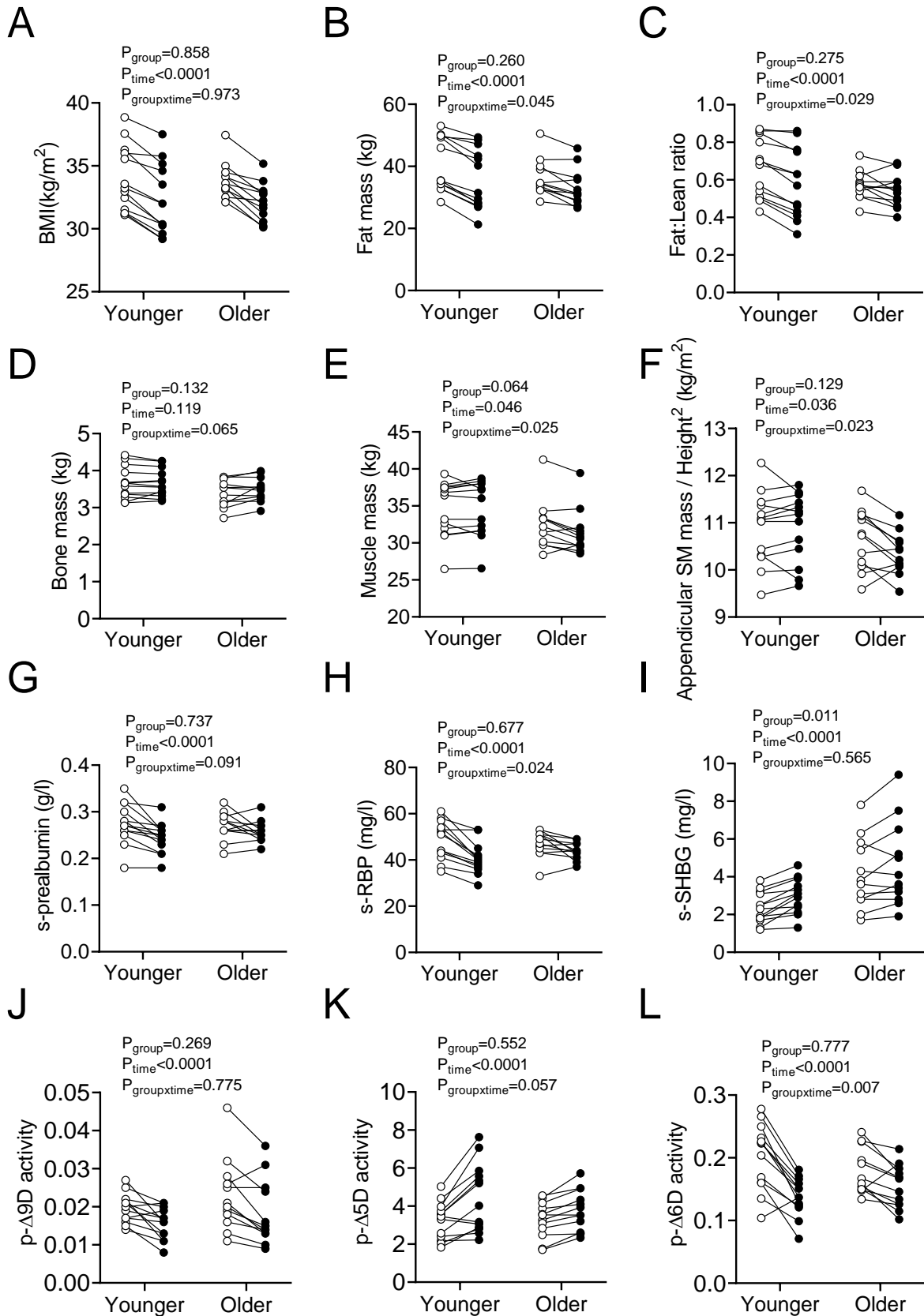
Table 1. *Body composition and whole-body energy metabolism variables at baseline and 8-weeks of lifestyle intervention in young and elderly obese participants*

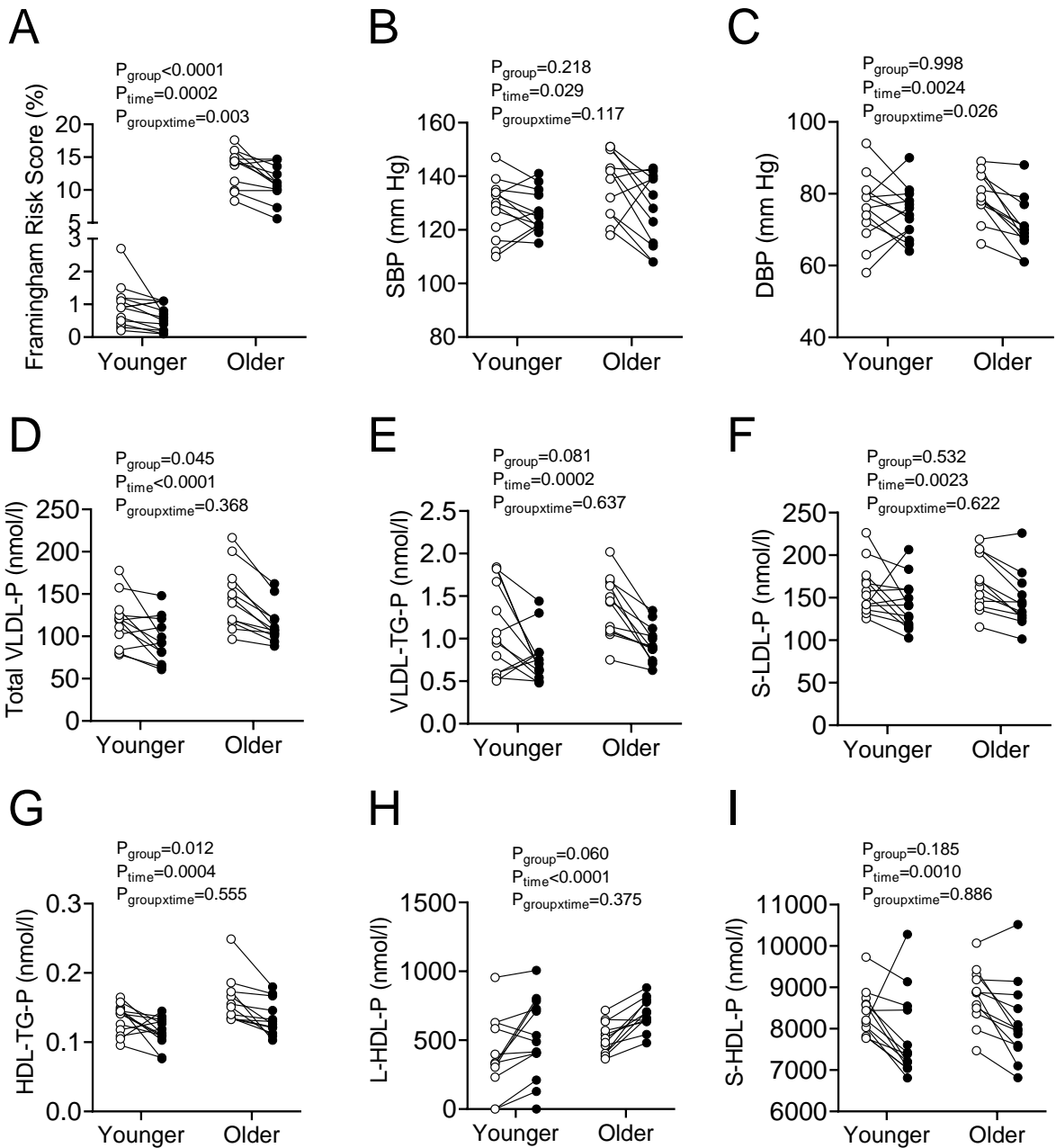
	Younger (n=13)		Older (n=12)		Group effect
	Baseline	8 weeks	Baseline	8 weeks	
Age (yr)	36.0±0.7		64.2±0.9		<0.001
Body composition					
Body weight (kg)	109±3	103±3***	103±3	97.6±2.8***	0.267
BMI (kg/m ²)	34.2±0.7	32.4±0.8***	33.6±2.7	32.1±0.4***	0.977
Fat mass (kg)	40.9±2.4	35.7±2.7***	36.3±3.3	33.1±3.1***	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
Energy metabolism					
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

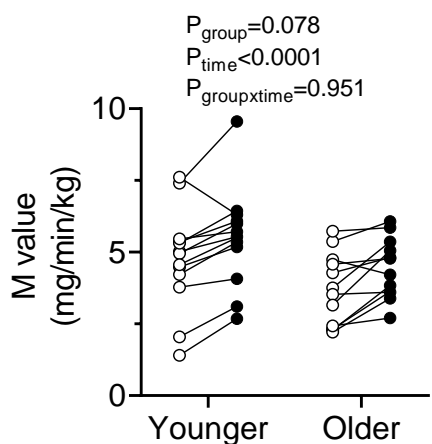
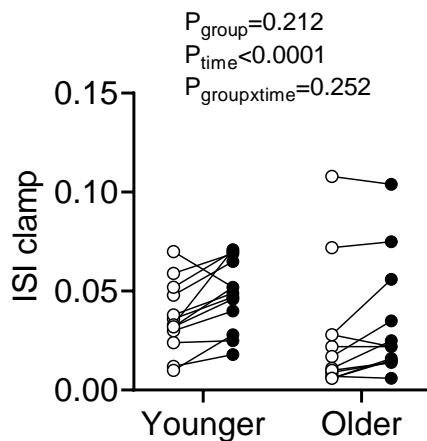
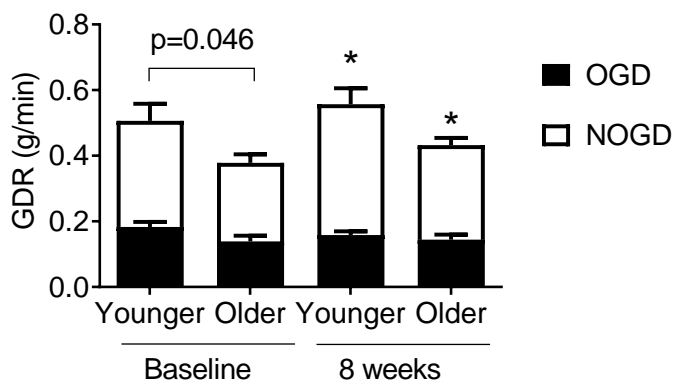
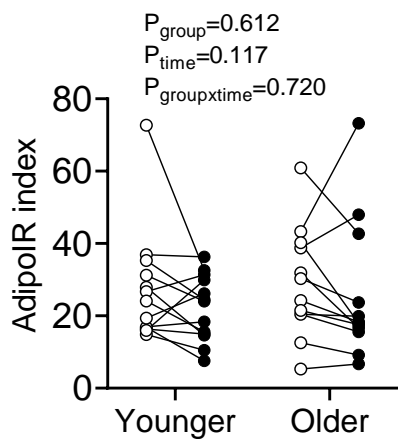
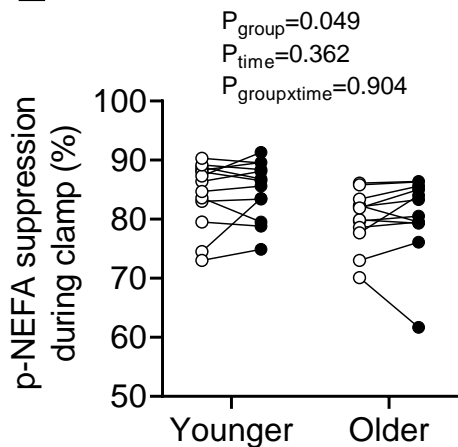
Abbreviations: BMI: body mass index; RMR: resting metabolic rate; TEE: total energy expenditure. Data are means±SEM. Age difference was assessed using Two-tailed unpaired Student t-test. Two-way repeated measures ANOVA was performed for other parameters.

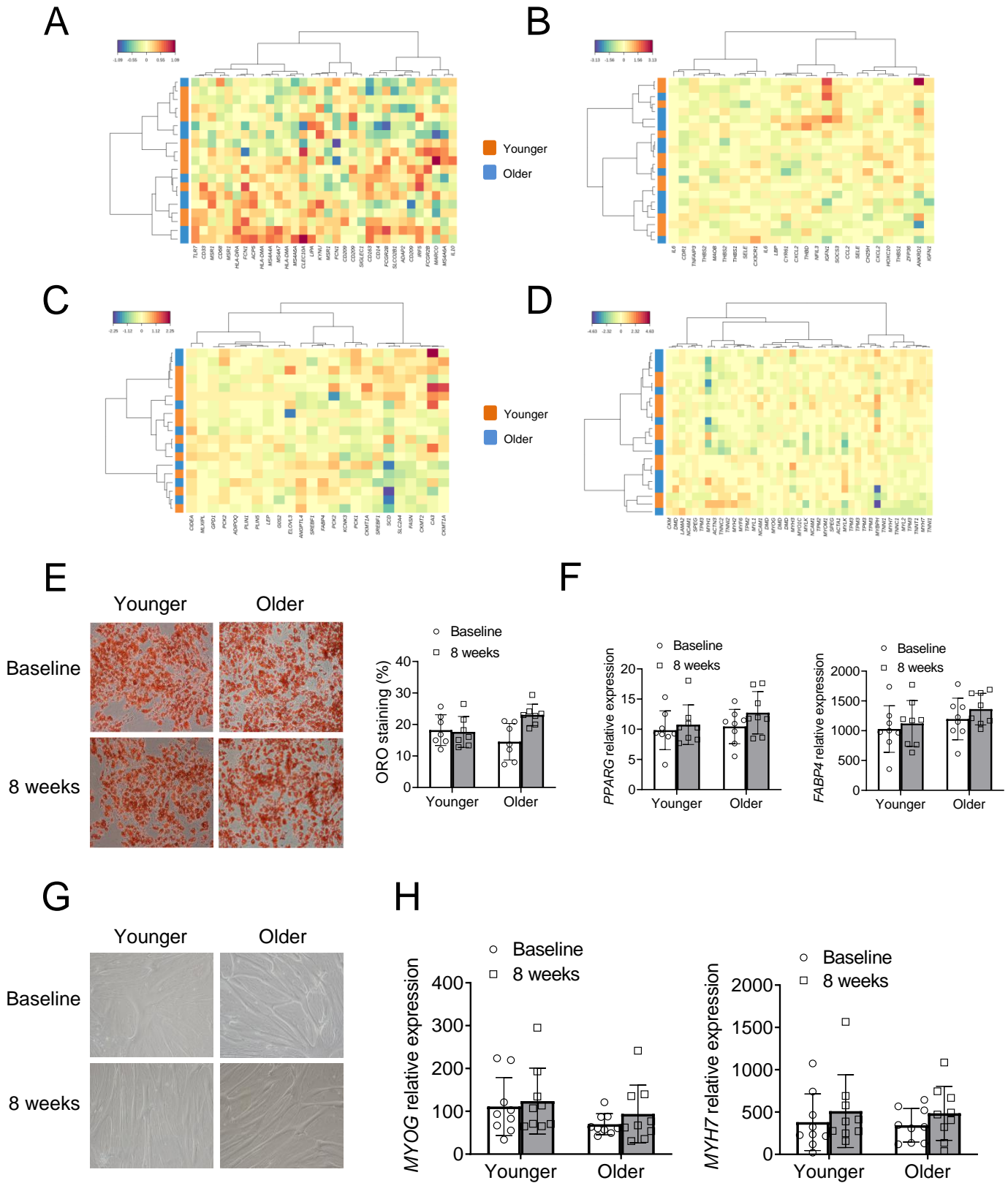
*p<0.05, **p<0.01, ***p<0.001 versus respective baseline.

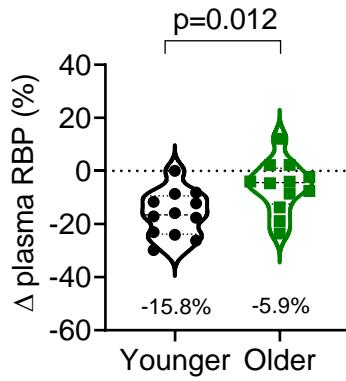
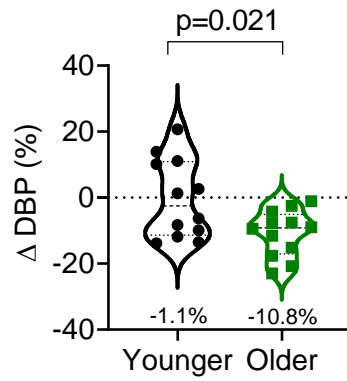
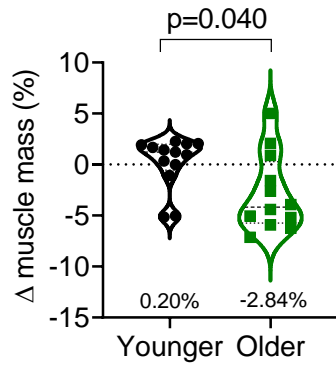
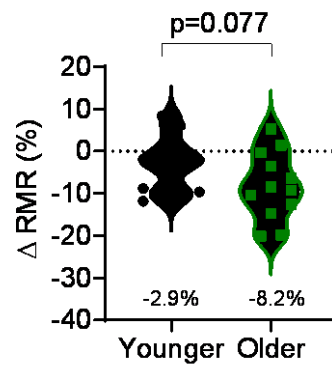
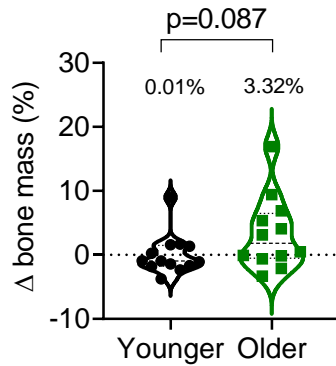
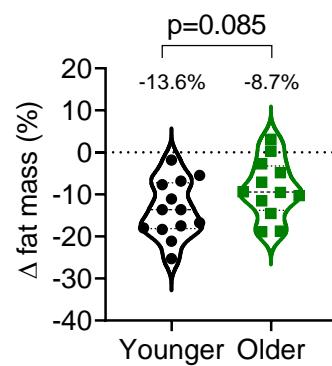






A**B****C****D****E**



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

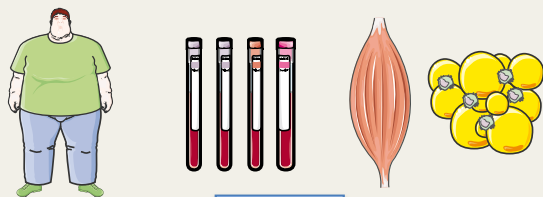
Cardiometabolic Adaptations in Younger and Older Obese Men

METHODS

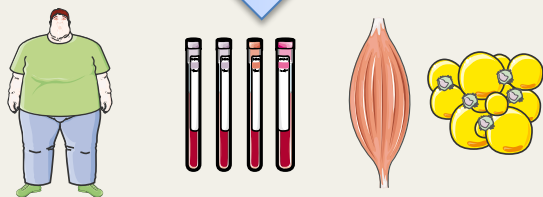
Obese men

30-40 yr old
n=13

60-70 yr old
n=12

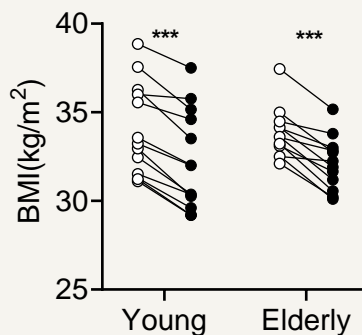


8 week lifestyle intervention

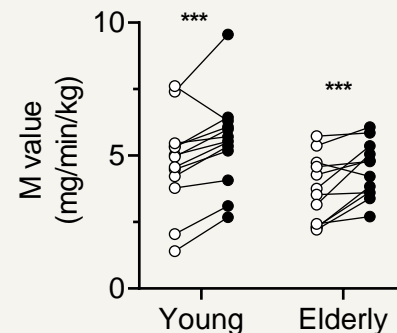
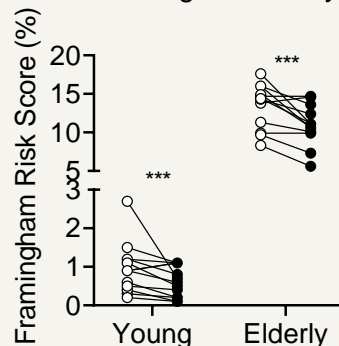


OUTCOME

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**.