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Weight loss induces changes in adaptive thermogenesis in female and male physique athletes

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Keywords: energy restriction, metabolic rate, competition preparation, bodybuilding, sex differences, fitness

38 **ABSTRACT**

39

40 Physique athletes lose substantial weight preparing for competitions, potentially altering systemic
41 metabolism. We investigated sex differences in body composition, resting energy expenditure (REE),
42 and appetite-regulating and thyroid hormone changes during a competition preparation among drug-
43 free physique athletes. The participants were female (10 competing (COMP) and 10 non-dieting
44 controls (CTRL)) and male (13 COMP) and 10 CTRL)) physique athletes. COMP were tested before
45 they started their diet 23 weeks before competing (PRE), during their diet one week before competing
46 (MID), and 23 weeks after competing (POST) whereas CTRL were tested at similar intervals but did
47 not diet. Measurements included body composition by DXA, muscle size, and subcutaneous fat
48 thickness (SFA) by ultrasound, REE by indirect calorimetry, circulating ghrelin, leptin T3, and T4
49 hormone analysis. Fat mass (FM) and SFA decreased in both sexes ($p < 0.001$), while males ($p < 0.001$)
50 lost more lean mass (LM) than females ($p < 0.05$). Weight loss, decreased energy intake, and increased
51 aerobic exercise ($p < 0.05$) led to decreased LM and FM-adjusted REE ($p < 0.05$), reflecting metabolic
52 adaptation. Absolute leptin levels decreased in both sexes ($p < 0.001$) but more among females
53 ($p < 0.001$) due to higher baseline leptin levels. These changes occurred with similar decreases in T3
54 ($p < 0.001$) and resting heart rate ($p < 0.01$) in both sexes. CTRL, who were former or upcoming
55 physique athletes, showed no systematic changes in any measured variables. In conclusion, while
56 dieting, female and male physique athletes experience REE and hormonal changes leading to adaptive
57 thermogenesis. However, responses seemed temporary as they returned toward baseline after the
58 recovery phase. ClinicalTrials.gov (NCT04392752).

59 **INTRODUCTION**

60

61 Weight loss is a standard practice in several sport disciplines, including physique sport. When using
62 more extended weight loss periods (>10 weeks), athletes aim to alter body composition by reducing
63 fat mass (FM) while retaining lean mass (LM). This process may lead to several metabolic adaptations
64 to weight loss, such as reduced resting energy expenditure and associated hormonal alternations
65 (Trexler, Smith-Ryan and Norton, 2014). In physique sport, these weight-loss periods are typically
66 the longest and result in the lowest FM levels compared to other sports disciplines. Specifically,
67 females have been reported to undergo 18–24-week (Alwan *et al.*, 2019) and males 12–26-week
68 (Mitchell *et al.*, 2018) competition preparation periods.

69 Competition preparation involves prolonged energy restriction, high-volume resistance
70 training, and concurrent increases in aerobic exercise to reduce FM while maintaining LM to enhance
71 muscular appearance (Helms *et al.*, 2014; Hulmi *et al.*, 2017; Mitchell *et al.*, 2018). At the culmination
72 of competition preparation, females have achieved body fat percentages (BF%) of 9 – 16 % (Alwan
73 *et al.*, 2019), while case studies of male physique athletes sometimes report BF%s lower than 5 %
74 (Rossow *et al.*, 2013; Pardue, Trexler and Sprod, 2017). While competitors of both sexes reduce FM
75 substantially, female and male physique athletes may face differing unfavorable physiological
76 adaptations in the course of reaching their division-specific body composition requirements.

77 One such unfavorable physiological adaptation is adaptive thermogenesis, which refers
78 to the decrease in resting energy expenditure due to weight loss beyond what would be predicted from
79 the loss of body weight (BW) and corresponding changes in fat or lean mass alone (Rosenbaum *et al.*
80 *et al.*, 2008). In general, a decrease in resting energy expenditure has been observed during weight loss
81 in obese and non-obese people (Magkos, 2022). Previously, based on previous case studies, adaptive
82 thermogenesis has also been reported in male physique athletes (Rossow *et al.*, 2013) but not in
83 female athletes during competition preparation (Rohrig *et al.*, 2017).

84 Several characteristics differ between females and males, potentially contributing to or hindering
85 successful body composition change in physique competition preparation. This could be observed as
86 metabolic and other physiological adaptations. Females have higher levels of essential FM and, thus,
87 at the same BW, have a lower LM and higher FM than males (Ivey *et al.*, 2000). This contributes to
88 lower resting and total energy expenditure among females at the same BW as males, which is only
89 similar when adjusted to LM (Jagim *et al.*, 2018). Further, there are slight differences between sexes
90 in skeletal muscle mass distribution, as males carry proportionately more LM than females,
91 specifically in the upper body (Janssen *et al.*, 2000). However, the sexes also share similarities, as
92 both females and males can increase muscle size and strength in response to resistance training to
93 similar relative degrees (Hubal *et al.*, 2005; Roberts, Nuckols and Krieger, 2020). One aspect of sex
94 differences among athletes, which has not been thoroughly investigated, is the physiological response
95 to prolonged energy restriction when FM losses approach essential levels. Further, the unique
96 demands of competition preparation, and their effects on resting energy expenditure, body
97 composition, and hormonal balance, specifically between male and female physique athletes, are yet
98 unexplored.

99 Therefore, the primary aim of this study was to evaluate changes in body composition,
100 appetite-regulating and thyroid hormone concentrations, and resting energy expenditure during
101 competition preparation among drug-free female and male physique athletes. Based on our previous
102 study (Hulmi *et al.*, 2017) and the current body of literature on physique athletes during the
103 competition preparation (Alwan *et al.*, 2019), we hypothesized that both female and male athletes
104 would experience substantial FM reductions, but only males might experience significant LM and
105 skeletal muscle size losses. Further, we hypothesized that changes in FM and decreased energy intake
106 would be accompanied by reductions in leptin and thyroid hormone levels and resting energy
107 expenditure without major systematic differences between sexes.

108

109 **MATERIALS AND METHODS**

110 **PARTICIPANTS**

111 A total of 48 males and 46 females volunteered to participate in this study via the web pages and
112 associated social media of the University and the governing sports body for physique sport. An online
113 screening questionnaire was sent to the competition (COMP), and control (CTRL) group volunteers
114 who claimed to meet the inclusion criteria. Participants who were diagnosed with chronic diseases,
115 reported using prescribed medications (excluding birth control pills), or any substances or methods
116 prohibited by WADA, or those competing in the junior (below 19 years of age) or master (over 40
117 years of age) categories or in a non-drug tested competition were excluded from the study.
118 Participants who had competed within 6 months before the first measurement or aimed to compete
119 within 6 months after the last measurement were also excluded.

120 All COMP group participants were amateur athletes who aimed to lose fat mass and
121 maintain muscle mass for competition. In contrast, CTRL participants maintained their normal off-
122 season nutrition and training plans. The participants in the COMP groups were required to prepare
123 for and compete in the Finnish Fitness Sports Association's 2019 national championships during the
124 investigation and be registered under the national doping control and testing organization under the
125 World Antidoping Agency (WADA) to participate.

126 The CTRL participants were matched with the COMP group participants based on their
127 age, height, BW, and training experience, as reported on the online pre-study questionnaire. The
128 participants selected for the study completed a health questionnaire subsequently reviewed by the
129 study physician. As a result, ten female and 13 male COMP, 12 female and ten male CTRL were
130 analyzed and measured at PRE-MID measurement. Figure 1 summarizes the participant study flow.
131 Table 1 displays the participants' baseline pre-test (PRE) values.

132 **INSERT TABLE 1 HERE**

133 Participants were given a full explanation of the study design, protocols and potential risks. All
134 participants gave informed consent. The study was conducted from 2019 to 2020. The researchers
135 followed the guidelines of the Declaration of Helsinki, and the study was reviewed by the Ethics
136 Committee of the Central Finland Health Care District (19U/2018), Finland. The study was registered
137 at ClinicalTrials.gov ID: NCT04392752.

138

139 **Study design**

140 We provided no intervention; thus, all groups followed their preferred diet and exercise regimen. All
141 participants were asked to meet the researchers for three laboratory testing sessions over 46 weeks of
142 competition preparation (23 weeks) and recovery (23 weeks) (Figure 1). PRE measurements were
143 obtained before the dieting phase for the competition, then one week before the competition (MID),
144 and finally after the recovery period (POST), during which the participants were advised to continue
145 their regular training and diet regimen. The CTRL groups were instructed to maintain their usual
146 physical activity and nutrition throughout the study. All measurements were conducted at the same
147 time of day (within \pm 1 hour). Participants arrived at the laboratory between 07.00 and 09.00 am. All
148 laboratory measurements were performed in the same visit, in the same order. The CTRL groups were
149 measured at the same relative time points as the COMP groups.

150 **INSERT FIGURE 1 HERE**

151 If participants traveled over 50 km to the laboratory, they were provided a hotel room for the night
152 before the measurement day. Participants were advised to avoid physical activity such as walking,
153 jogging and cycling on the morning of the assessment day. The participants from the hotel were
154 transported to the laboratory by car. The participants arrived for testing after an 8-hour food and fluid
155 fast and after instruction to sleep for 8 h and abstain from alcohol and caffeine for 12 h, and exercise
156 for 24 h prior to the first measurements (i.e., blood sampling, Dual-energy X-ray absorptiometry, and
157 resting energy expenditure measurements, see details below). After that, participants were provided

158 a standardized breakfast, including a protein drink and bar (Celcius Finland Ltd) and a medium-sized
159 banana, containing a total of 36 g protein, 53 g carbohydrates, and 11 g fat. After breakfast, ultrasound
160 and skinfold measurements were conducted (see details below).

161

162 **Resistance and aerobic training**

163 The participants followed their planned training programs and were asked to provide their training
164 diaries throughout the study period. When participants made changes to their training program, they
165 were asked to report these adjustments to the investigators. Resistance training volumes were
166 calculated from the training diaries. Volume was determined as total sets per week per muscle group.
167 Aerobic training duration and type were also determined from diaries. Aerobic training volume was
168 calculated as total minutes per week. Complete resistance training programs were available for seven
169 female and 12 male COMP group participants, and 12 female and seven male CTRL group
170 participants from PRE to MID. Further, complete resistance training programs from MID to POST
171 were available from five female and nine male COMP group participants and nine female and seven
172 male CTRL group participants. Complete aerobic training volumes were available from 9 female and
173 12 male COMP group participants and 12 female and nine male CTRL group participants from PRE
174 to MID, and six female and ten male COMP group participants, and eight female and eight male
175 CTRL group participants from MID to POST.

176

177 **Nutrition intake and supplementation**

178 Daily energy and macronutrient intake and dietary supplementation guidance were provided to the
179 athletes by their coaches throughout the study. To estimate energy intake, the COMP groups provided
180 nutrition logs throughout the study period, where they recorded the nutrition information of their
181 diets. When athletes changed their nutrition, they reported these adjustments to the investigators. To
182 obtain nutrition information from the CTRL groups, they completed 4-day food records for three

183 weekdays and one weekend day, which were analyzed at PRE, MID, and POST-equivalent
184 measurement time-points using nutrient analysis software (Aivodiet, Flow-team Oy, Oulu, Finland).
185 Nutrients in food supplements were included in the analysis. Daily energy, carbohydrate, protein, and
186 fat intake were adjusted to BW. Complete dietary information was available from 9 female and ten
187 male COMP group participants, and ten female and eight male CTRL group participants from PRE
188 to MID, and five female and five male COMP group participants, and five female and eight male
189 CTRL group participants from MID to POST period.

190

191 **Body composition**

192 Body composition was estimated by Dual-energy X-ray absorptiometry (DXA, Lunar Prodigy
193 Advance EnCore version 14.10.022, GE Medical Systems—Lunar, Madison WI USA) using the half-
194 body symmetry method on the right side of the body. Participants were measured with their arms at
195 their sides with minimal clothing (i.e., underwear). Non-elastic straps secured their legs at the ankles.
196 All metal objects were removed from the participant before scanning. The analysis provided BW,
197 bone-free LM, FM, and BF%. The typical error of measurement of DXA for active people when using
198 repeated measurements has been reported as 0.4% and 1.9% for LM and FM, respectively (Nana,
199 Gary J. Slater, *et al.*, 2012), and half-body scans produced no significant differences in body
200 composition compared to whole-body scans (Nana, Gary J Slater, *et al.*, 2012).

201

202 **Resting metabolism and heart rate**

203 Resting energy expenditure (REE) was assessed with a Vmax Encore 29 metabolic cart
204 (Sensormedics, Yorba Linda, CA, USA) using the canopy method. The manufacturer's
205 recommendations for gas and flow calibrations were followed. The measurements were performed
206 for 20 min in a dimmed, thermoneutral and quiet laboratory. If necessary, the measurement time was
207 extended to ensure that each participant reached a steady state. Resting heart rate was measured

208 simultaneously with a heart rate monitor (Polar V800, Kempele, Finland). The first 5 min of gas
209 exchange data were discharged. Then a 5 min steady-state period with a coefficient of variation for
210 VO₂ and VCO₂ <10% with a respiratory exchange ratio (RER) between 0.7–0.91 was located
211 (Fullmer *et al.*, 2015). REE was calculated with the modified Weir equation (Weir, 1949). To
212 investigate the presence of adaptive thermogenesis, Nunes *et al.* (2022) recommend using a regression
213 equation created based on the sample baseline information. (Nunes *et al.*, 2022) Therefore, a multiple
214 linear regression model with FM and LM as covariates were first used to produce a prediction
215 equation in the PRE measurement data: REE (kcal/day) = 449.5 + 9.3 FM (kg) + 19.0 LM (kg). Sex
216 was not included as a covariate because it did not improve model fit, and it does not change in time.
217 The model explained measured REE (mREE) at the PRE measurement well (R² = 0.83, adjusted R²
218 = 0.82, p<0.001). The homogeneity of the sample explains the high coefficient of determination of
219 the REE prediction model. Remaining unexplained variance likely results from intrinsic differences
220 between individuals in REE and measurement error regarding body composition assessment and
221 indirect calorimetry. (Müller *et al.*, 2018.) The figure of adjusted REE with regression line and 95%
222 confidence intervals is in Supplementary Information (Figure S1). Predicted REE (pREE) was
223 estimated for each participant at every measurement they participated in using the equation. REE
224 residuals, termed as adjusted REE from herein, were calculated as mREE – pREE. Last, the adaptive
225 thermogenesis at MID and POST was calculated by subtracting the adjusted REE at PRE from the
226 respective estimation. REE calculations were performed with R version 4.0.5 (R Core Team, 2021).

227

228 **Ultrasound for muscle cross-sectional area and subcutaneous fat thickness**

229 Vastus lateralis (VL) muscle cross-sectional area (CSA) and subcutaneous fat thickness (SFA) were
230 examined as earlier (Hulmi *et al.*, 2017) at the mid-thigh using a B-mode axial plane ultrasound
231 (model SSD- α 10, Aloka, Tokyo, Japan) with a 10 MHz linear-array probe (60 mm width) in
232 extended-field-of-view mode (23 Hz sampling frequency). Triceps brachii muscle and subcutaneous

233 fat thickness were measured by the same device. The scanning head was overlay with water-soluble
234 transmission gel to provide acoustic contact. The reliability and validity of ultrasound to detect a
235 change in resistance training-induced muscle CSA is reported as very high when compared with
236 magnetic resonance imaging, e.g., ICC>0.9 (Ahtiainen *et al.*, 2010). Also, for muscle thickness, the
237 reliability of ultrasound is high, with ICC> 0.87 (Nijholt *et al.*, 2020). Likewise, SFA standard error
238 estimate and reliability for this method are also reported as high, 0.55 mm and ICC>0.998 (Müller *et*
239 *al.*, 2016). The CSA of VL was measured from two points; the first measurement was 40 % from the
240 superior point of the patella to the spina iliaca anterior superior, and the second measurement was 2
241 cm distally from the first. The thickness of subcutaneous fat in the thigh was measured from the 40
242 % line mentioned above but at the medial-lateral axis (Nijholt *et al.*, 2020). Three images were
243 scanned at each measurement point. VL CSA and fat and muscle thickness were analyzed using
244 ImageJ software (version 1.53a; National Institutes of Health, Bethesda, MD). The average values
245 were used for statistical analyses. The same researcher performed all ultrasound measurements and
246 analyses.

247

248 **Blood parameters**

249 Venous blood samples were collected from the antecubital vein into serum tubes (Venosafe; Terumo
250 Medical Co., Leuven, Hanau, Belgium) using standard laboratory procedures. Samples were stored
251 at room temperature for 30 minutes after being centrifuged at 3500 rpm for 10 min (Megadure 1.0 R
252 Heraeus; DJB Lab Care Germany). Free thyroxine (T4), insulin, free triiodothyronine (T3), and
253 thyroid-stimulating hormone (TSH) were analyzed from serum with the Immunolite 2000 XPi,
254 immunoassay system (Seimen Healtineers, Erlangen Germany) using Immulite® 2000 Free T3
255 (L2KF32), Immulite® 2000 Free T4 (L2KFT42), and Immulite® 2000 Third Generation TSH
256 (L2KTS2) commercial kits. Serum ghrelin and leptin were analyzed with the Dynex Ds 2 ELISA
257 processing System (DYNEX Technologies, Chantilly, VA, USA) using a commercial kit (Human

258 Leptin ELISA, Clinical Range, REF RD191001100, Human Unacylated ghrelin express ELISA, REF
259 RA194063400R). These hormones are routinely analyzed in our laboratory, and day-to-day reliability
260 (CV%) for these hormones in our laboratory is <8%. The detection limit of leptin was 0.2 ng/ml.

261

262 **Statistical analysis**

263 Means and standard deviations (SD) were calculated for all test parameters. Statistical analyses were
264 conducted using IBM SPSS statistical analysis software (SPSS version 27; Chicago, IL). The
265 normality of the data was analysed using the Shapiro-Wilk test. Due to the SARS-CoV-2 pandemic,
266 we failed to retain all participants for the POST measurements. Therefore, we used an analysis of
267 variance (ANOVA) for normally distributed or Mann Whitney tests for non-normally distributed data
268 to assess the differences between groups at baseline (PRE). Then we analyzed the absolute changes
269 from PRE to MID and MID to POST within groups by paired t-test (normally distributed data) or
270 Wilcoxon signed-rank test (non-normally distributed data). Next, we used an ANOVA to assess the
271 absolute differences between COMP groups and CTRL groups from PRE to MID and MID to POST.
272 After that, we analyzed the differences in responses between female and male COMP groups from
273 PRE to MID and MID to POST by ANOVA. Also, differences in percentage changes from PRE to
274 MID were analyzed by ANOVA for body composition, hormone, resting energy expenditure, and
275 ultrasound variables between sexes. Pearson's (normally distributed data) or Spearman's (non-
276 normally distributed data) correlation coefficients were used to report correlations between the
277 changes in the variables of interest. Statistical significance was set to < 0.05. Data generated or
278 analyzed during this study are provided in full within the published article.

279

280 **RESULTS**

281 **Nutrition intake**

282 Daily energy intake before the weight-loss period was 2609.7 ± 475.9 kcal / day and 3254.1 ± 429.0
283 kcal / day in the female and male COMP groups, respectively (Table 2). Energy intake decreased in
284 both the female -889.2 ± 322.7 kcal / day ($p < 0.01$) and male -1248.8 ± 462.6 kcal / day ($p < 0.001$)
285 COMP groups, and these changes were greater than CTRL ($p < 0.01-0.001$). The reduction in energy
286 intake was explained by reductions in carbohydrate and fat intake in the female COMP group
287 ($p < 0.001$ and $p < 0.01$), and the changes were statistically different than CTRL ($p < 0.05$ and $p < 0.001$,
288 respectively). Also, the male COMP group reduced their carbohydrate and fat intake ($p < 0.01$ and
289 $p < 0.001$), but only carbohydrate intake was lower than CTRL ($p < 0.01$).

290 From MID to POST, energy, carbohydrate, and fat intake increased in the male COMP
291 group compared with CTRL ($p < 0.01$), while in the female COMP group only carbohydrate intake
292 increased compared with CTRL ($p < 0.01$).

293 **INSERT TABLE 2 HERE**

294

295 **Exercise training**

296 From PRE to MID, aerobic training volume increased in both female and male COMP groups
297 ($p < 0.01$) and the changes were greater in female and male COMP groups than CTRL ($p < 0.01$, Table
298 2). In addition, the volume of resistance training remained unaltered in all groups throughout the
299 study period.

300 From MID to POST, aerobic training volume decreased within the female and male
301 COMP groups ($p < 0.01$, $p < 0.05$), and changes were greater in both female and male COMP groups
302 than in the CTRL ($p < 0.001$ and $p < 0.05$, respectively), where the aerobic training volume remained
303 unaltered.

304

305 **Body composition**

306 From PRE to MID, BW and FM decreased in female and male COMP groups ($p < 0.001$), while FM
307 increased in both female and male CTRL groups ($p < 0.05$). BW and FM changes were statistically
308 different in female and male COMP groups compared to CTRL ($p < 0.001$, Figure 2). The BF%
309 differed between the sexes ($p < 0.05$). BF% decreased from $26.1 \pm 5.4\%$ to $12.6 \pm 6.0\%$ in the female
310 COMP group and from $15.7 \pm 4.7\%$ to $5.6 \pm 0.2\%$ in the male COMP group ($p < 0.001$), with greater
311 changes than CTRL ($p < 0.001$). LM decreased in the male COMP group ($p < 0.05$), while an increase
312 was observed in the female COMP group ($p < 0.05$). LM changes in the COMP groups did not differ
313 from CTRL. For LM, a statistically significant difference was observed from PRE to MID between
314 female and male COMP groups ($p < 0.001$). Changes in LM were positively associated with the
315 changes in aerobic training volume in the male COMP group ($r = 0.66$, $p < 0.05$).

316 From MID to POST, BW increased in the female and male COMP groups ($p < 0.001$ and
317 $p < 0.01$, respectively), and changes were greater in the female and male COMP groups than CTRL
318 ($p < 0.001$). FM increased more in the male COMP group than CTRL ($p < 0.001$), while no significant
319 differences were observed in the female COMP group compared to CTRL. No significant changes in
320 LM were observed in any group.

321 **INSERT FIGURE 2 HERE**

322

323 **Muscle CSA, muscle thickness and fat thickness**

324 From PRE to MID, VL-muscle CSA and fat thickness of the VL and triceps decreased in the female
325 and male COMP groups ($p < 0.001$), and changes were statistically different compared to CTRL
326 (Figure 3). In addition, VL muscle CSA tended to decrease more in the male COMP group than in
327 the female COMP group ($p = 0.06$).

328 From MID to POST, triceps muscle thickness increased in the male COMP group
329 ($p < 0.01$). VL fat thickness increased in the female and male COMP groups compared to CTRL

330 (p<0.001 and p<0.05, respectively). Triceps fat thickness also increased in the female COMP group
331 compared to CTRL (p<0.05).

332 **INSERT FIGURE 3 HERE**

333

334 **Resting metabolism and heart rate**

335 From PRE to MID, absolute REE decreased in the female COMP and in the male COMP group
336 (p<0.001), with greater changes than CTRL (p<0.01 and p<0.001, Figure 4). FM and LM-adjusted
337 REE decreased in the female COMP and male COMP groups (p<0.01) and changes were greater than
338 CTRL (p<0.05 and p<0.01, respectively). In addition, resting heart rate decreased in the female
339 COMP group and in the male COMP group (p<0.01), and changes were greater than CTRL (p<0.05
340 and p<0.001, respectively). Changes in heart rate were positively associated with changes in FM
341 (r=0.80, p<0.01) and negatively associated with changes in LM (r=-0.64, p<0.05), carbohydrate
342 intake (r=-0.75, p<0.05), and the change in resistance training volume (r=-0.85, p<0.05) in the female
343 COMP group. In turn, changes in heart rate were positively associated with changes in adjusted REE
344 (i.e. adaptive thermogenesis, r=0.77, p<0.01) and relative REE (r=0.73, p<0.05), and VL-muscle CSA
345 (r=0.59, p<0.05) in the male COMP group. From MID to POST, absolute REE increased in the male
346 COMP group compared to CTRL (p<0.05).

347 **INSERT FIGURE 4 HERE**

348

349 **Appetite-regulating and thyroid hormones**

350 From PRE to MID, serum leptin decreased in both female and male COMP groups (p<0.01 and
351 p<0.05), and the changes were greater than CTRL (p<0.05 and p<0.001, Figure 5). A statistically
352 significant difference (p<0.001) was observed between the sexes in the absolute change in serum
353 leptin (Figure 5I), but not in the relative changes (71.3 ± 74.2 % in females vs. 77.9 ± 84.5 % in
354 males, p=0.473). Serum ghrelin concentrations increased in both female and male COMP groups

355 (p<0.05 and p<0.01, respectively), with a greater change compared to CTRL (p<0.05). T3 decreased
356 in both female and male COMP groups (p<0.001), with a greater decrease in both sexes compared to
357 CTRL (p<0.001). Changes in T3 were positively associated with the changes in energy intake (r=0.78,
358 p<0.05) and carbohydrate intake (r=0.92, p<0.001) in the female COMP group. Changes in T3 were
359 positively associated with changes in LM (r=0.68, p<0.01) in the male COMP group. Changes in
360 ghrelin were positively associated with the change in RER (r=0.67, p<0.05), and changes in leptin
361 were positively associated with the change in FM (r=0.57, p<0.05) and protein intake (r=0.71, p<0.05)
362 in the male COMP group.

363 From MID to POST, serum leptin levels increased in the female COMP group (p<0.01)
364 compared to CTRL. Serum ghrelin levels decreased in the male COMP group compared to CTRL
365 (p<0.01). T3 increased in both female and male COMP groups (p<0.001).

366 **INSERT FIGURE 5 HERE**

367

368 **DISCUSSION**

369 The main finding from this study was that female and male physique athletes experienced similar
370 changes in REE (both in absolute and body composition adjusted), suggesting adaptive
371 thermogenesis. Both sexes lost BW, FM, and muscle size similarly, but only males lost LM during
372 the 20-week competition preparation. Sex differences were observed for serum leptin, but this was
373 expected given the sex differences in BF%, as it is known that there is a strong relationship between
374 leptin and BF % (Considine *et al.*, 1996).

375 In this study, female physique athletes maintained their LM during competition
376 preparation, while it decreased in male athletes. The loss of LM may have been greater in male
377 physique athletes because they also substantially reduced BF% during the weight-loss period,
378 finishing the dieting period with under 3 kg of FM (i.e., 4–6 % of body fat), which represents the
379 lower limit for healthy males (Friedl *et al.*, 1994). However, in males, a very low body fat level may

380 be a prerequisite for achieving an optimal physique for a competition (Rossow *et al.*, 2013).
381 Therefore, greater LM loss in male athletes may be due to lower energy availability. At the end of the
382 weight-loss period, the energy intake of the male COMP group was below the suggested levels (below
383 25 kcal/kg LM) necessary to maintain LM in male physique athletes (Fagerberg, 2018). Unlike the
384 LM results, there were no significant differences between sexes in VL-muscle CSA or triceps muscle
385 thickness changes. In contrast, VL-muscle CSA decreased in female and male athletes during
386 competition preparation. These results suggest that male physique athletes may experience greater
387 LM loss than females, but the difference between sexes in LM change may not be entirely due to
388 skeletal muscle. Greater LM losses in male physique athletes may be partly explained by reaching
389 lower body fat and energy availability levels by the end of the competition preparation period.

390 Absolute REE decreased in both sexes during competition preparation following weight
391 loss which aligns with previous studies on female and male physique athletes (Pardue *et al.* 2017;
392 Tinsley *et al.* 2018). However, REE relative to FM and LM also decreased, indicating adaptive
393 thermogenesis, which refers to a more significant decline in energy expenditure than predicted based
394 on changes in body tissue masses. The central stimulus for adaptive thermogenesis is often proposed
395 as the presence of a negative energy balance (Müller *et al.*, 2016). However, increased aerobic
396 exercise volume during contest preparation may have further contributed to its development in this
397 study, although we have no further data to support this hypothesis. It has been previously reported
398 that long-term increases in exercise energy expenditure may suppress other energy expenditure
399 components, even while in energy balance (Pontzer, 2018).

400 Previous studies in obese and non-obese individuals demonstrated that REE is
401 inevitably reduced after weight loss and that this reduction occurs through metabolic adaptations and
402 the loss of energy-expending tissues such as adipose tissue, skeletal muscle, and other organs (Müller,
403 Heymsfield and Bosy-Westphal, 2021; Martin *et al.*, 2022). For instance, Martin *et al.* (2022) found
404 that ~40 % of the reduction in REE after weight loss was attributed to metabolic adaptations.

405 Metabolic adaptations may reflect, for example, a decline in immunity, reproduction, and stress
406 response (Pontzer, 2018). In the present study, the LM of female athletes increased, and the LM of
407 male athletes decreased during weight loss. Although we did not measure organ masses, we assume
408 that the decrease in adjusted REE with FM and LM reflects metabolic adaptation. In support of this
409 contention, we observed a decline in resting heart rate in both sexes, which has previously been
410 connected to changes in the autonomous nervous system during adaptive thermogenesis (Rosenbaum
411 and Leibel, 2010). Moreover, we reported a temporary suppression of the immune (Sarin, Gudelj, *et*
412 *al.*, 2019) and reproductive (Hulmi *et al.*, 2017) systems and decreased levels of systemic
413 inflammation (Sarin, Lee, *et al.*, 2019), and using leukocyte transcriptomics, repressed mitochondrial
414 oxidative function and protein translation (Sarin *et al.*, 2021) during contest preparation in our earlier
415 female physique athlete study. It has also been questioned whether adaptive thermogenesis only exists
416 during an energy deficit (Martins *et al.*, 2020), but in the aforementioned studies, the participants had
417 already been in energy balance due to the last week of competition preparation, and still many of the
418 fat-loss -induced physiological and molecular mechanisms behind adaptive thermogenesis were still
419 present (Hulmi *et al.*, 2017; Sarin, Gudelj, *et al.*, 2019; Sarin, Lee, *et al.*, 2019; Sarin *et al.*, 2021).
420 Future studies should investigate the time course in adaptive thermogenesis and other physiological
421 changes after the restoration of energy deficit.

422 In this study, leptin decreased, and ghrelin increased in both sexes, reflecting reduced
423 FM and energy intake. We also observed changes in hypothalamic-pituitary-thyroid axis function, as
424 T3 levels decreased during weight loss in both sexes. Leptin and thyroid hormones are among the
425 main drivers of adaptive thermogenesis in humans (Müller, Heymsfield and Bosy-Westphal, 2021).
426 Accordingly, (Müller, Enderle and Bosy-Westphal, 2016) reported that an association between
427 adaptive thermogenesis and changes in plasma leptin and T3 concentrations occurred at the end of
428 weight loss. Similar to the present reductions in leptin and T3 have been previously reported in
429 healthy mixed-sex cohorts during weight loss (Warren, 2011) as well as in female (Hulmi *et al.*, 2017)

430 and male physique athletes (Rossow *et al.*, 2013). The statistical sex difference in leptin resulted from
431 a naturally higher leptin level in females due to their higher fat percentage (Couillard *et al.*, 1997).
432 The higher initial BF% may also explain why leptin levels decreased more in females than males
433 during the dietary phase (Christen *et al.*, 2018). Low leptin levels associated with low T3 status may
434 increase the risk of weight regain after dieting (Müller, Enderle and Bosy-Westphal, 2016), but further
435 studies are warranted to investigate the physiological and behavioural importance of these changes.

436 Taken together, we found that intensive weight loss through energy restriction
437 combined with aerobic and resistance training causes adaptive thermogenesis similarly in female and
438 male physique athletes. Adaptive thermogenesis was observed in both sexes, with concurrent changes
439 in crucial hormone levels regulating energy metabolism, nervous system function, and REE. These
440 responses to weight loss seemed to be temporary and related to energy intake and body composition
441 as they returned toward baseline levels after the recovery phase.

442 Our study has some limitations, such as the lack of data on the total daily energy
443 expenditure of the participants, which would have provided important information when looking at
444 metabolic adaptation. In addition, the timing of the measurement may have affected the rate of
445 metabolic adaptation as athletes were in an energy deficit at the time of measurement. In addition,
446 due to the SARS-CoV-2 pandemic, interruptions affect the study design and may, therefore, also
447 weaken the analytical methods. Nevertheless, even with these limitations, this is the first study to
448 provide detailed data following a long period of competition preparation in both sexes compared to
449 non-dieting controls.

450 There were no significant sex differences in the effects of physique competition
451 preparation for BW, FM, REE, and selected serum hormones or muscle size. However, adaptive
452 thermogenesis resulting from competition preparation may further complicate weight loss, creating
453 challenges despite low energy intakes and high training volumes during physique competition
454 preparation. Therefore, to achieve the optimal body composition for competition, some individuals

455 may need to reach a very low energy intake which may be required for competitive success. Also,
456 adaptive thermogenesis during weight loss may predispose physique athletes to rapidly regain weight
457 following competition preparation. There is some evidence that refeeding after energy restriction may
458 induce weight regains and a disproportionate increase in fat mass, which is associated with a higher
459 risk of chronic metabolic disorders (di Munno et *al.*, 2021). However, more studies are warranted to
460 investigate this phenomenon.

461

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467

468 **Conflict of Interest**

469 V.I. conducts part-time work at the Finnish Fitness Sports Association as an executive manager of
470 the Finnish Fitness Sports Association, which also supported a small part of the research. The grant
471 was allocated directly towards the costs of the research, such as paying for the hotel accommodation
472 of the participants who travelled far and needed accommodation before the fasted measurements next
473 morning. The funders had no role in the design of the study; in the collection, analyses, or
474 interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

475

476 **Data Availability statement**

477 Data generated or analyzed during this study are provided in full within the published article.

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642 Table 1. Participants' characteristics at baseline (mean \pm SD)

	Age (yr)	Height (cm)	Body mass (kg)	Body fat (%)	Training history (yr)	Diet period (wk)
Female COMP	27 \pm 4	167.7 \pm 7.4 ^{***}	69.5 \pm 9.2 ^{***}	26.1 \pm 5.5 ^{***}	7.0 \pm 2.0	20.5 \pm 2.0
Female CTRL	28 \pm 4	164.1 \pm 4.0	64.1 \pm 6.3	23.2 \pm 7.0	5.5 \pm 2.0	
Male COMP	29 \pm 6	180.2 \pm 4.2	91.6 \pm 10.0	15.7 \pm 4.7	6.5 \pm 2.0	20.5 \pm 4.5
Male CTRL	31 \pm 5	180.7 \pm 1.9	86.0 \pm 5.2	16.2 \pm 6.6	6.5 \pm 3.5	

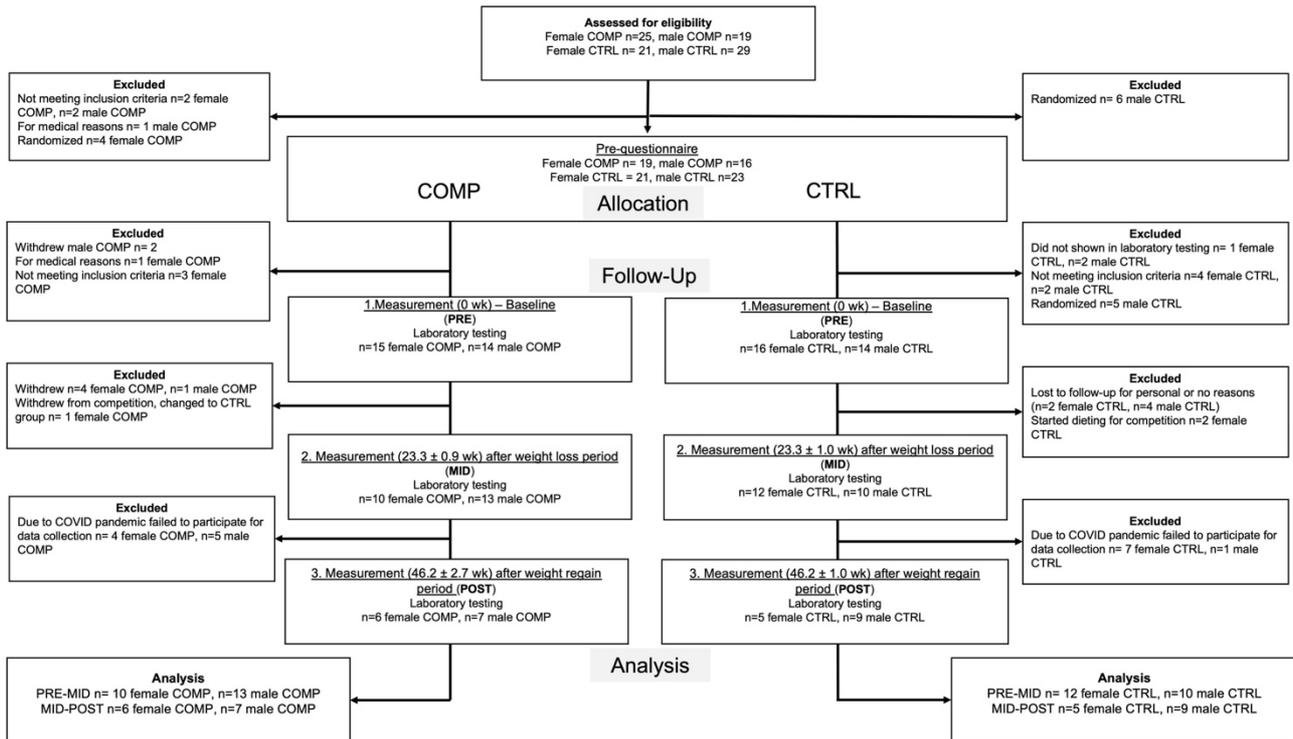
.***, the statistically significant difference in the COMP group ($p < 0.05-0.001$) from the male COMP group.

644 Table 2. Nutrition intake and exercise training. Data are presented in daily values as mean \pm SD.

	n	Pre	Mid	Change	n	Mid	Post	Change
Energy (kcal / kg bw)								
Female COMP	9	38.5 \pm 7.8	28.7 \pm 4.0 **	-9.8 \pm 4.4 ††	5	28.6 \pm 3.7	38.6 \pm 6.9*	+10.0 \pm 6.7
Female CTRL	10	35.4 \pm 6.4	33.1 \pm 6.1	-2.4 \pm 5.4	5	32.8 \pm 4.9	34.7 \pm 4.1	+1.9 \pm 4.9
Male COMP	10	34.8 \pm 4.5	24.4 \pm 4.4 ***	-10.4 \pm 4.4 †††	5	24.0 \pm 5.4	37.6 \pm 5.7*	+13.1 \pm 7.3 ††
Male CTRL	8	34.0 \pm 3.9	32.8 \pm 5.9	+0.11 \pm 6.2	8	32.8 \pm 5.9	32.7 \pm 7.4	-0.03 \pm 4.3
Protein (g/kg bw)								
Female COMP	9	2.9 \pm 0.6	2.9 \pm 0.5	-0.03 \pm 0.3	5	2.9 \pm 0.7	2.7 \pm 0.5	-0.17 \pm 0.5
Female CTRL	10	2.7 \pm 0.7	2.4 \pm 0.5 *	-0.3 \pm 0.5	5	2.5 \pm 0.5	2.5 \pm 0.7	+0.1 \pm 0.3
Male COMP	10	2.7 \pm 0.4	2.4 \pm 0.3 *	-0.3 \pm 0.4	5	2.5 \pm 0.5	2.5 \pm 0.6	+0.05 \pm 0.9
Male CTRL	8	2.4 \pm 0.4	2.4 \pm 0.5	+0.01 \pm 0.3	8	2.4 \pm 0.5	2.5 \pm 0.6	-0.03 \pm 0.4
Carbohydrate (g/kg bw)								
Female COMP	9	4.0 \pm 1.1	2.2 \pm 1.0 ***	-1.8 \pm 1.0 †††	5	2.3 \pm 0.8	4.1 \pm 1.4 *	+2.0 \pm 1.0 †
Female CTRL	10	3.3 \pm 0.9	3.2 \pm 1.2	-0.2 \pm 0.8	5	3.2 \pm 1.2	3.4 \pm 1.5	+0.03 \pm 1.3
Male COMP	10	3.8 \pm 1.1	2.3 \pm 0.7 **	-1.5 \pm 1.1 ††	5	2.2 \pm 0.7	4.2 \pm 0.9 **	+1.9 \pm 1.0 ††
Male CTRL	8	3.5 \pm 0.9	3.5 \pm 0.9	-0.04 \pm 1.4	8	3.5 \pm 1.0	3.5 \pm 1.0	+0.05 \pm 0.7
Fat (g/kg bw)								
Female COMP	9	1.1 \pm 0.4	0.8 \pm 0.2 **	-0.3 \pm 0.2 †	5	0.8 \pm 0.3	1.1 \pm 0.2	+0.3 \pm 0.3
Female CTRL	10	1.1 \pm 0.5	1.0 \pm 0.4	-0.1 \pm 0.2	5	0.9 \pm 0.2	1.0 \pm 0.2	+0.1 \pm 0.2
Male COMP	10	0.8 \pm 0.2	0.5 \pm 0.1 ***	-0.3 \pm 0.2	5	0.5 \pm 0.1	1.0 \pm 0.2 *	+0.5 \pm 0.3 ††
Male CTRL	8	1.0 \pm 0.2	0.9 \pm 0.3	-0.1 \pm 0.2	8	0.9 \pm 0.3	0.9 \pm 0.2	-0.04 \pm 0.2
Resistance training volume (sets / muscle /wk)								
Female COMP	7	19.7 \pm 4.1	20.2 \pm 5.4	+0.5 \pm 4.7	5	18.9 \pm 4.6	18.7 \pm 6.9	-0.4 \pm 4.7
Female CTRL	9	15.6 \pm 5.3	14.6 \pm 9.2	-0.2 \pm 5.7	8	14.6 \pm 9.2	16.4 \pm 7.4	+1.4 \pm 9.3
Male COMP	12	20.1 \pm 5.7	21.2 \pm 5.7	+0.5 \pm 2.5	9	19.8 \pm 4.6	18.9 \pm 7.0	-0.4 \pm 4.7
Male CTRL	7	12.7 \pm 4.0	13.2 \pm 7.3	+0.5 \pm 4.7	7	13.2 \pm 7.3	15.1 \pm 4.3	+2.0 \pm 7.4
Aerobic training volume (min / wk)								
Female COMP	8	153.6 \pm 116.3	343.6 \pm 143.8 **	+188.3 \pm 114.7 ††	5	343.6 \pm 143.8	66.0 \pm 71.0**	-235.8 \pm 164.9 †††
Female CTRL	8	106.6 \pm 90.1	109.6 \pm 131.5	+3.0 \pm 58.8	8	109.6 \pm 131.5	140.6 \pm 138.4	+31.0 \pm 65.1
Male COMP	12	71.8 \pm 114.0	176.6 \pm 187.3 **	+104.8 \pm 115.3 ††	10	199.5 \pm 198.2	16.0 \pm 47.2 *	-183.5 \pm 207 †
Male CTRL	8	124.4 \pm 176.2	53.1 \pm 95.5	-71.3 \pm 148.5	8	53.1 \pm 95.5	50.6 \pm 100.4	-2.5 \pm 21.9

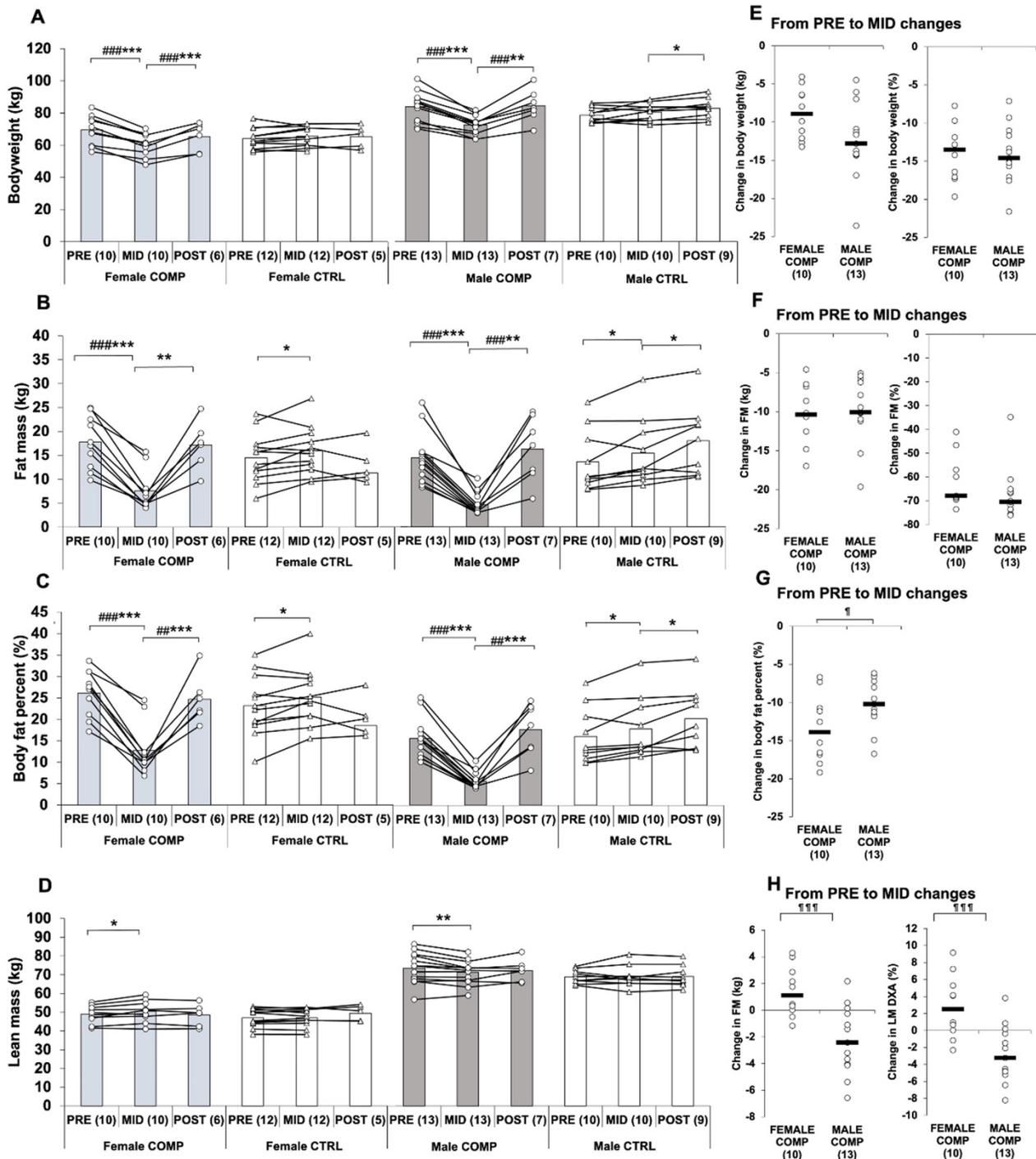
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*** and ** are statistically significant (p<0.05–0.001) changes within the group, †, †† and ††† are statistically significant (p<0.05–0.001) differences in the change (pre to mid, or mid to post) between the COMP and the CTRL groups, and ¶ is a significant p<0.05 difference between sexes at MID

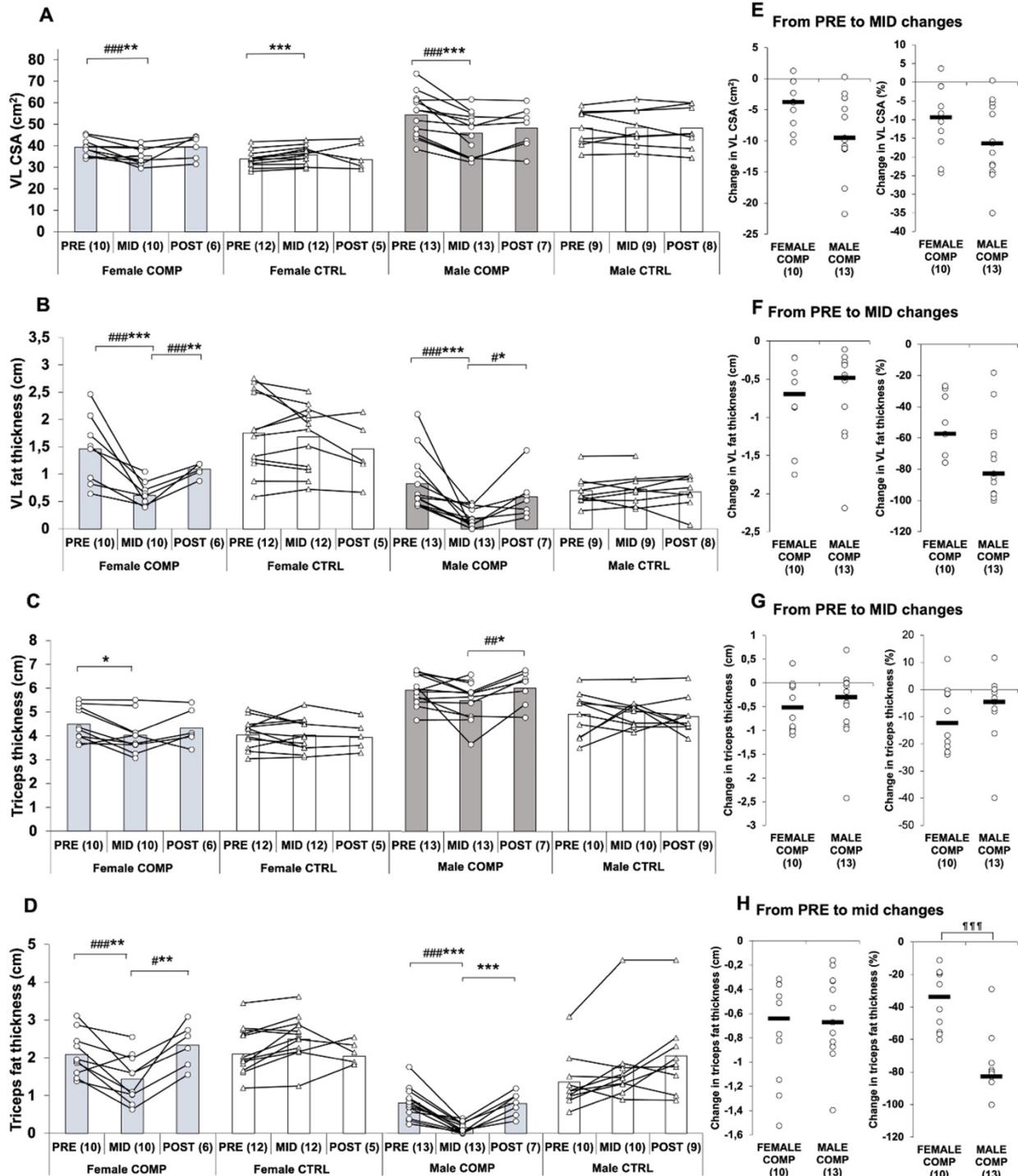


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Figure 1. The experimental design of the study. Flowchart illustrating the study protocol. COMP groups study protocol on the left, and CTRL groups study protocol on the right. From PRE to MID, the female and male COMP participants decreased their energy intake and increased the amount of aerobic exercise. In contrast, their CTRL groups maintained their activity levels and nutrient intake. The MID to POST period was a recovery period with increased energy intake and decreased aerobic exercise back toward the baseline levels in the COMP groups, whereas the CTRL groups maintained their energy intake and exercise levels.



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 659 Figure 2. A, B, C and D show absolute body composition changes in COMP and CTRL groups
 660 from PRE to MID and MID to POST. Baseline pre-test (PRE) were obtained before the dieting
 661 phase for the competition, MID one week before the competition, and POST after the recovery
 662 period. Numbers in parentheses indicate the number of participants. Circles and triangles indicate
 663 individual data of COMP and CTRL group, respectively, and bars indicate means. * is a statistically
 664 significant change within the group. # is a statistically significant difference between the COMP
 665 and CTRL groups in the change. *, **, and ***, ($p < 0.05 - 0.001$), ### ($p < 0.001$). E, F, G and H show
 666 absolute and percentage changes in female and male COMP groups from PRE to MID. Circles
 667 indicate individual data, and black lines indicate the mean. ¶¶¶ is a statistically significant
 668 ($p < 0.001$) difference between sexes. The percentage changes for body fat percent is not depicted.
 669 FM, fat mass; LM, lean mass.



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672 Figure 3 A, B, C, and D show absolute changes in muscle CSA, and muscle and fat thickness in

673 COMP and CTRL groups from PRE to MID and MID to POST. Baseline pre-test (PRE) were

674 obtained before the dieting phase for the competition, MID one week before the competition, and

675 POST after the recovery period. Numbers in parentheses indicate the number of participants.

676 Circles and triangles indicate individual data of COMP and CTRL group, respectively, and bars

677 indicate means. * is a statistically significant change within the group. # is a statistically significant

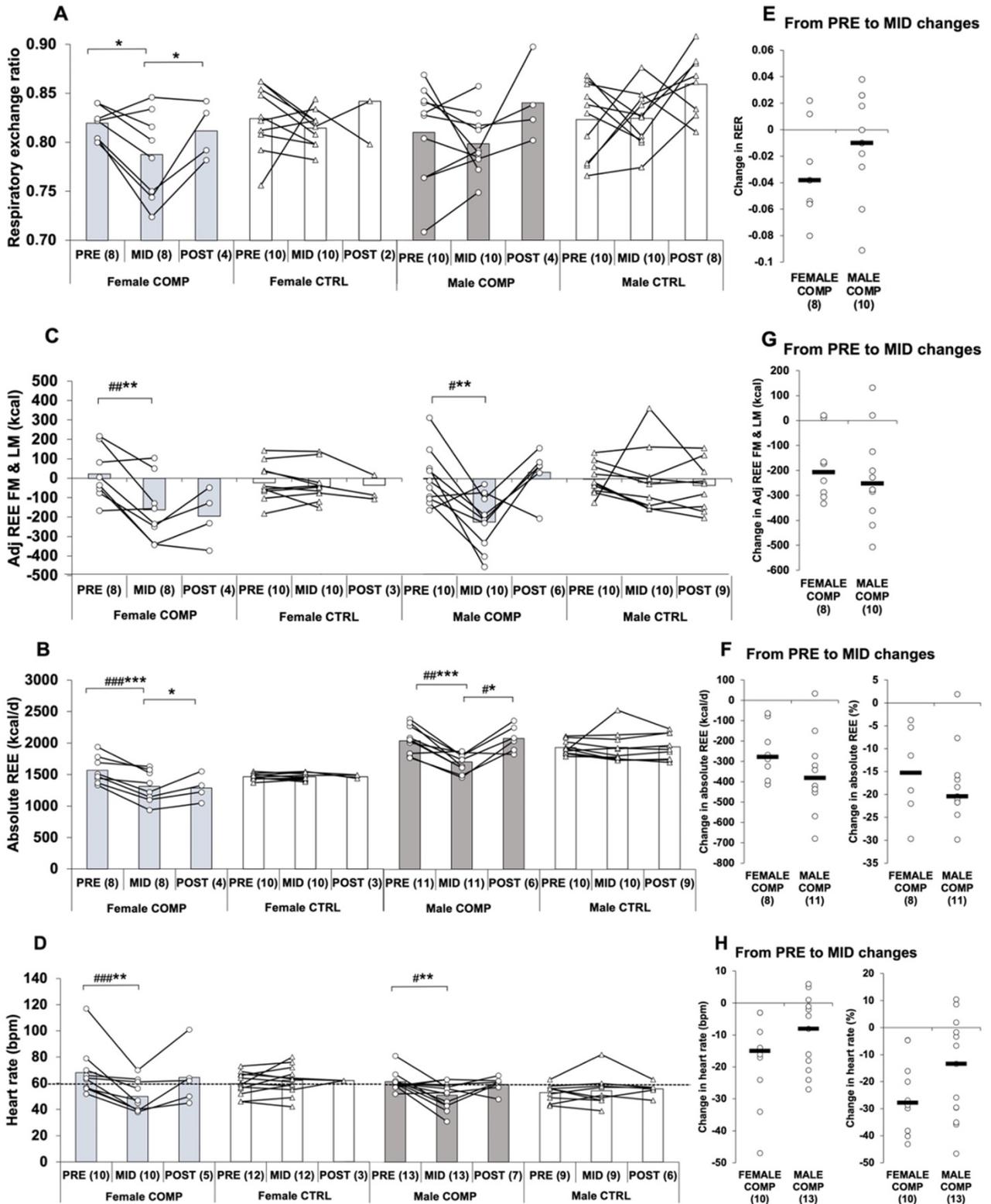
678 difference between the COMP and CTRL groups in the change. #, **###, and ***###, (p<0.05–

679 0.001). E, F, G, and H show absolute and percentage changes in female and male COMP groups

680 from PRE to MID. Circles indicate individual data, and black lines indicate the mean. ¶¶ and ¶¶¶ is

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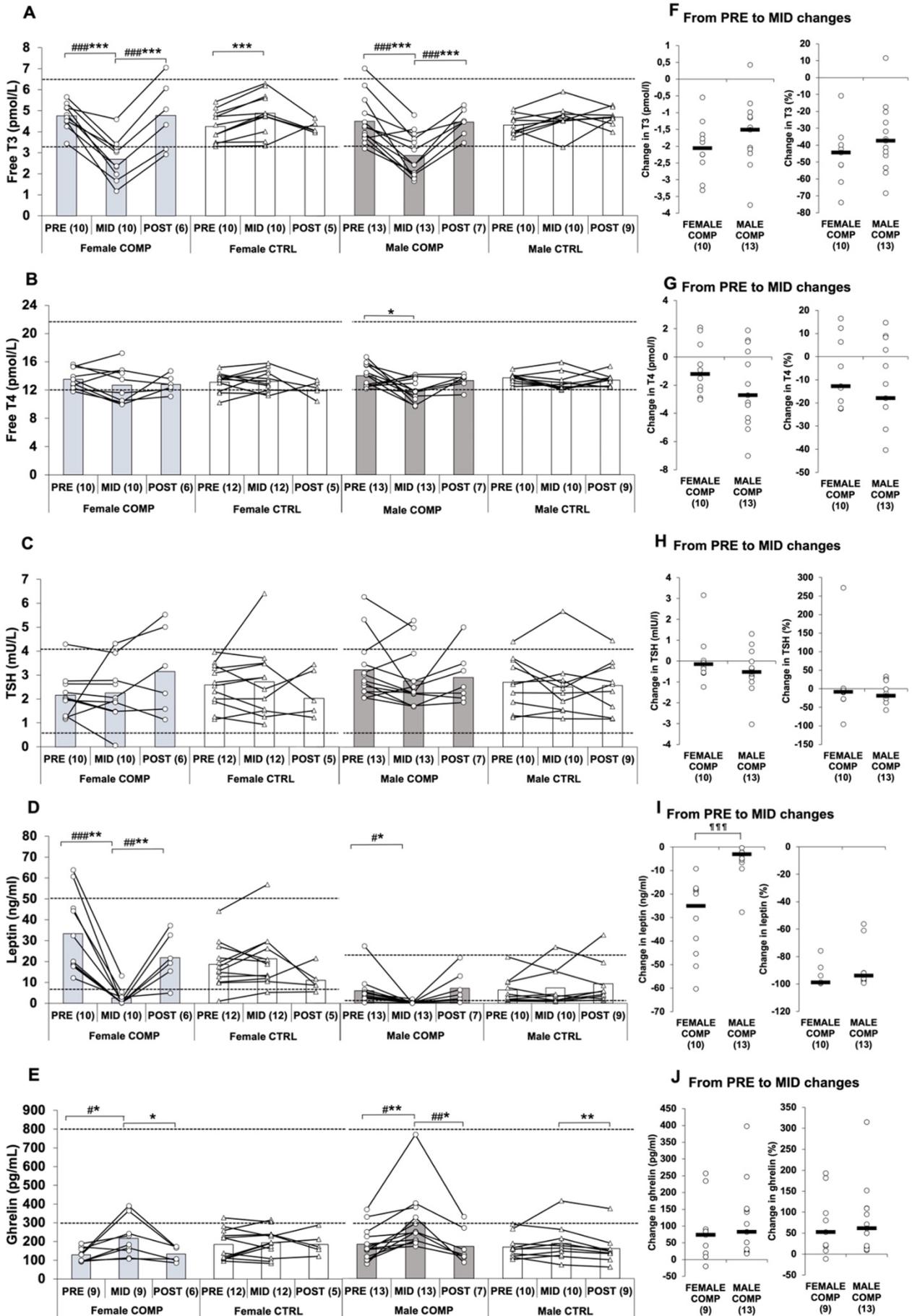
a statistically significant ($p < 0.01-0.001$) difference between the sexes. CSA, cross-sectional area; VL, vastus lateralis muscle.



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Figure 4 A, B, C, and D show absolute changes in resting metabolism and heart rate in COMP and CTRL groups from PRE to MID and MID to POST. Baseline pre-test (PRE) were obtained before the dieting phase for the competition, MID one week before the competition, and POST after the recovery period. Numbers in parentheses indicate the number of participants. Circles and triangles

688 indicate individual data of COMP and CTRL group, respectively, and bars indicate means.* is a
689 statistically significant change within the group. # is a statistically significant difference between
690 the COMP and CTRL groups in the change. *#,**###, and ***##, (p<0.05–0.001). E, F, G, and H
691 shows absolute and percentage changes in female and male COMP groups from PRE to MID.
692 Circles indicate individual data, and black lines indicate the mean. Values of RER and Adj REE FM
693 and LM are relative and body composition adjusted values, respectively; thus, percentage changes
694 for them are not depicted. RER, respiratory exchange ratio. Adj REE FM & LM, calculated
695 measured and predicted resting energy expenditure adjusted with fat mass and lean mass.
696



698 Figure 5 A, B, C, D and E show absolute changes in appetite-regulating and thyroid hormones in
699 COMP and CTRL groups from PRE to MID and MID to POST. Baseline pre-test (PRE), were
700 obtained before the dieting phase for the competition, MID one week before the competition, and
701 POST after the recovery period. Numbers in parentheses indicate the number of participants.
702 Circles and triangles indicate individual data of COMP and CTRL group, respectively, and bars
703 indicate means. * is a statistically significant change within the group. # is a statistically significant
704 difference between the COMP and CTRL groups in the change. *#, **###, and ***###, (p<0.05–
705 0.001). F, G, H, I and J shows absolute and percentage changes in female and male COMP groups
706 from PRE to MID. Circles indicate individual data, and black lines indicate the mean. ¶¶¶ is a
707 statistically significant (p<0.001) difference between sexes. Free T3, free triiodothyronine; Free T4,
708 free thyroxine; TSH, thyroid-stimulating hormone.