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

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ABSTRACT

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

INTRODUCTION

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

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

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

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

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

MATERIALS AND METHODS

PARTICIPANTS

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

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

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

INSERT TABLE 1 HERE

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

Study design

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

INSERT FIGURE 1 HERE

If participants traveled over 50 km to the laboratory, they were provided a hotel room for the night before the measurement day. Participants were advised to avoid physical activity such as walking, jogging and cycling on the morning of the assessment day. The participants from the hotel were transported to the laboratory by car. The participants arrived for testing after an 8-hour food and fluid fast and after instruction to sleep for 8 h and abstain from alcohol and caffeine for 12 h, and exercise for 24 h prior to the first measurements (i.e., blood sampling, Dual-energy X-ray absorptiometry, and resting energy expenditure measurements, see details below). After that, participants were provided a standardized breakfast, including a protein drink and bar (Celcius Finland Ltd) and a medium-sized banana, containing a total of 36 g protein, 53 g carbohydrates, and 11 g fat. After breakfast, ultrasound and skinfold measurements were conducted (see details below).

Resistance and aerobic training

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

Nutrition intake and supplementation

Daily energy and macronutrient intake and dietary supplementation guidance were provided to the athletes by their coaches throughout the study. To estimate energy intake, the COMP groups provided nutrition logs throughout the study period, where they recorded the nutrition information of their diets. When athletes changed their nutrition, they reported these adjustments to the investigators. To obtain nutrition information from the CTRL groups, they completed 4-day food records for three weekdays and one weekend day, which were analyzed at PRE, MID, and POST-equivalent measurement time-points using nutrient analysis software (Aivodiet, Flow-team Oy, Oulu, Finland). Nutrients in food supplements were included in the analysis. Daily energy, carbohydrate, protein, and fat intake were adjusted to BW. Complete dietary information was available from 9 female and ten male COMP group participants, and ten female and eight male CTRL group participants from PRE to MID, and five female and five male COMP group participants, and five female and eight male CTRL group participants from MID to POST period.

Body composition

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

Resting metabolism and heart rate

Resting energy expenditure (REE) was assessed with a Vmax Encore 29 metabolic cart (Sensormedics, Yorba Linda, CA, USA) using the canopy method. The manufacturer's recommendations for gas and flow calibrations were followed. The measurements were performed for 20 min in a dimmed, thermoneutral and quiet laboratory. If necessary, the measurement time was extended to ensure that each participant reached a steady state. Resting heart rate was measured simultaneously with a heart rate monitor (Polar V800, Kempele, Finland). The first 5 min of gas exchange data were discharged. Then a 5 min steady-state period with a coefficient of variation for VO2 and VCO2 <10% with a respiratory exchange ratio (RER) between 0.7–0.91 was located (Fullmer *et al.*, 2015). REE was calculated with the modified Weir equation (Weir, 1949). To investigate the presence of adaptive thermogenesis, Nunes et *al.* (2022) recommend using a regression equation created based on the sample baseline information. (Nunes *et al.*, 2022) Therefore, a multiple linear regression model with FM and LM as covariates were first used to produce a prediction equation in the PRE measurement data: REE (kcal/day) = 449.5 + 9.3 FM (kg) + 19.0 LM (kg). Sex was not included as a covariate because it did not improve model fit, and it does not change in time. The model explained measured REE (mREE) at the PRE measurement well (R2 = 0.83, adjusted R2 = 0.82, p<0.001). The homogeneity of the sample explains the high coefficient of determination of the REE prediction model. Remaining unexplained variance likely results from intrinsic differences between individuals in REE and measurement error regarding body composition assessment and indirect calorimetry. (Müller et *al.,* 2018.) The figure of adjusted REE with regression line and 95% confidence intervals is in Supplementary Information (Figure S1). Predicted REE (pREE) was estimated for each participant at every measurement they participated in using the equation. REE residuals, termed as adjusted REE from herein, were calculated as mREE – pREE. Last, the adaptive thermogenesis at MID and POST was calculated by subtracting the adjusted REE at PRE from the respective estimation. REE calculations were performed with R version 4.0.5 (R Core Team, 2021).

Ultrasound for muscle cross-sectional area and subcutaneous fat thickness

Vastus lateralis (VL) muscle cross-sectional area (CSA) and subcutaneous fat thickness (SFA) were examined as earlier (Hulmi *et al.*, 2017) at the mid-thigh using a B-mode axial plane ultrasound (model SSD-α10, Aloka, Tokyo, Japan) with a 10 MHz linear-array probe (60 mm width) in extended-field-of-view mode (23 Hz sampling frequency). Triceps brachii muscle and subcutaneous fat thickness were measured by the same device. The scanning head was overlay with water-soluble transmission gel to provide acoustic contact. The reliability and validity of ultrasound to detect a change in resistance training-induced muscle CSA is reported as very high when compared with magnetic resonance imagining, e.g., ICC>0.9 (Ahtiainen *et al.*, 2010). Also, for muscle thickness, the reliability of ultrasound is high, with ICC> 0.87 (Nijholt *et al.*, 2020). Likewise, SFA standard error estimate and reliability for this method are also reported as high, 0.55 mm and ICC>0.998 (Müller *et al.*, 2016). The CSA of VL was measured from two points; the first measurement was 40 % from the superior point of the patella to the spina iliaca anterior superior, and the second measurement was 2 cm distally from the first. The thickness of subcutaneous fat in the thigh was measured from the 40 % line mentioned above but at the medial-lateral axis (Nijholt *et al.*, 2020). Three images were scanned at each measurement point. VL CSA and fat and muscle thickness were analyzed using ImageJ software (version 1.53a; National Institutes of Health, Bethesda, MD). The average values were used for statistical analyses. The same researcher performed all ultrasound measurements and analyses.

Blood parameters

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

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

Statistical analysis

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

RESULTS

Nutrition intake

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

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

INSERT TABLE 2 HERE

Exercise training

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

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

Body composition

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

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

INSERT FIGURE 2 HERE

Muscle CSA, muscle thickness and fat thickness

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

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

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

INSERT FIGURE 3 HERE

Resting metabolism and heart rate

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

INSERT FIGURE 4 HERE

Appetite-regulating and thyroid hormones

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

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

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

INSERT FIGURE 5 HERE

DISCUSSION

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

In this study, female physique athletes maintained their LM during competition preparation, while it decreased in male athletes. The loss of LM may have been greater in male physique athletes because they also substantially reduced BF% during the weight-loss period, finishing the dieting period with under 3 kg of FM (i.e., 4–6 % of body fat), which represents the lower limit for healthy males (Friedl *et al.*, 1994). However, in males, a very low body fat level may be a prerequisite for achieving an optimal physique for a competition (Rossow *et al.*, 2013). Therefore, greater LM loss in male athletes may be due to lower energy availability. At the end of the weight-loss period, the energy intake of the male COMP group was below the suggested levels (below 25 kcal/kg LM) necessary to maintain LM in male physique athletes (Fagerberg, 2018). Unlike the LM results, there were no significant differences between sexes in VL-muscle CSA or triceps muscle thickness changes. In contrast, VL-muscle CSA decreased in female and male athletes during competition preparation. These results suggest that male physique athletes may experience greater LM loss than females, but the difference between sexes in LM change may not be entirely due to skeletal muscle. Greater LM losses in male physique athletes may be partly explained by reaching lower body fat and energy availability levels by the end of the competition preparation period.

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

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

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

In this study, leptin decreased, and ghrelin increased in both sexes, reflecting reduced FM and energy intake. We also observed changes in hypothalamic-pituitary-thyroid axis function, as T3 levels decreased during weight loss in both sexes. Leptin and thyroid hormones are among the main drivers of adaptive thermogenesis in humans (Müller, Heymsfield and Bosy-Westphal, 2021). Accordingly, (Müller, Enderle and Bosy-Westphal, 2016) reported that an association between adaptive thermogenesis and changes in plasma leptin and T3 concentrations occurred at the end of weight loss. Similar to the present reductions in leptin and T3 have been previously reported in healthy mixed-sex cohorts during weight loss (Warren, 2011) as well as in female (Hulmi *et al.*, 2017) and male physique athletes (Rossow *et al.*, 2013). The statistical sex difference in leptin resulted from a naturally higher leptin level in females due to their higher fat percentage (Couillard *et al.*, 1997). The higher initial BF% may also explain why leptin levels decreased more in females than males during the dietary phase (Christen *et al.*, 2018). Low leptin levels associated with low T3 status may increase the risk of weight regain after dieting (Müller, Enderle and Bosy-Westphal, 2016), but further studies are warranted to investigate the physiological and behavioural importance of these changes.

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

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

There were no significant sex differences in the effects of physique competition preparation for BW, FM, REE, and selected serum hormones or muscle size. However, adaptive thermogenesis resulting from competition preparation may further complicate weight loss, creating challenges despite low energy intakes and high training volumes during physique competition preparation. Therefore, to achieve the optimal body composition for competition, some individuals may need to reach a very low energy intake which may be required for competitive success. Also, adaptive thermogenesis during weight loss may predispose physique athletes to rapidly regain weight following competition preparation. There is some evidence that refeeding after energy restriction may induce weight regains and a disproportionate increase in fat mass, which is associated with a higher risk of chronic metabolic disorders (di Munno et *al.,* 2021). However, more studies are warranted to investigate this phenomenon.

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Conflict of Interest

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

Data Availability statement

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

REFERENCES

Ahtiainen, J. P., Hoffren, M., Hulmi, J. J., Pietikäinen, M., Mero, A. A., Avela, J., & Häkkinen, K. (2010). Panoramic ultrasonography is a valid method to measure changes in skeletal muscle cross-sectional area. European journal of applied physiology, 108(2), 273-279.

Alwan, N., Moss, S. L., Elliott-Sale, K. J., Davies, I. G., & Enright, K. (2019). A narrative review on female physique athletes: The physiological and psychological implications of weight management practices. International journal of sport nutrition and exercise metabolism, 29(6), 682-689.

Christen, T., Trompet, S., Noordam, R., van Klinken, J. B., van Dijk, K. W., Lamb, H. J., ... & de Mutsert, R. (2018). Sex differences in body fat distribution are related to sex differences in serum leptin and adiponectin. Peptides, 107, 25-31.

Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., ... & Caro, J. F. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. New England Journal of Medicine, 334(5), 292-295.

Couillard, C., Mauriege, P., Prud'Homme, D., Nadeau, A., Tremblay, A., Bouchard, C., & Després, J. P. (1997). Plasma leptin concentrations: gender differences and associations with metabolic risk factors for cardiovascular disease. Diabetologia, 40(10), 1178-1184.

Fagerberg, P. (2018). Negative consequences of low energy availability in natural male bodybuilding: A review. International journal of sport nutrition and exercise metabolism, 28(4), 385-402.

Friedl, K. E., Moore, R. J., Martinez-Lopez, L. E., Vogel, J. A., Askew, E. W., Marchitelli, L. J., ... & Gordon, C. C. (1994). Lower limit of body fat in healthy active men. Journal of applied physiology, 77(2), 933-940.

Fullmer, S., Benson-Davies, S., Earthman, C. P., Frankenfield, D. C., Gradwell, E., Lee, P. S., ... & Trabulsi, J. (2015). Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non–critically ill individuals. Journal of the Academy of Nutrition and Dietetics, 115(9), 1417-1446.

Helms, E. R., Aragon, A. A., & Fitschen, P. J. (2014). Evidence-based recommendations for natural bodybuilding contest preparation: nutrition and supplementation. Journal of the International Society of Sports Nutrition, 11(1), 20.

Hubal, M. J., Gordish-Dressman, H., Thompson, P. D., Price, T. B., Hoffman, E. P., Angelopoulos, T. J., ... & Clarkson, P. M. (2005). Variability in muscle size and strength gain after unilateral resistance training. Medicine & science in sports & exercise, 37(6), 964-972.

Hulmi, J. J., Isola, V., Suonpää, M., Järvinen, N. J., Kokkonen, M., Wennerström, A., ... & Häkkinen, K. (2017). The effects of intensive weight reduction on body composition and serum hormones in female fitness competitors. Frontiers in physiology, 689.

Ivey, F. M., Roth, S. M., Ferrell, R. E., Tracy, B. L., Lemmer, J. T., Hurlbut, D. E., ... & Hurley, B. F. (2000). Effects of age, gender, and myostatin genotype on the hypertrophic response to heavy resistance strength training. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 55(11), M641-M648.

Jagim, A. R., Camic, C. L., Askow, A., Luedke, J., Erickson, J., Kerksick, C. M., ... & Oliver, J. M. (2019). Sex differences in resting metabolic rate among athletes. The Journal of Strength & Conditioning Research, 33(11), 3008-3014.

Janssen, I., Heymsfield, S. B., Wang, Z., & Ross, R. (2000). Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. Journal of applied physiology.

Magkos, F. (2022). Is calorie restriction beneficial for normal-weight individuals? A narrative review of the effects of weight loss in the presence and absence of obesity. Nutrition Reviews, 80(7), 1811-1825.

Martin, A., Fox, D., Murphy, C. A., Hofmann, H., & Koehler, K. (2022). Tissue losses and metabolic adaptations both contribute to the reduction in resting metabolic rate following weight loss. International Journal of Obesity, 46(6), 1168-1175.

Martins, C., Roekenes, J., Salamati, S., Gower, B. A., & Hunter, G. R. (2020). Metabolic adaptation is an illusion, only present when participants are in negative energy balance. The American journal of clinical nutrition, 112(5), 1212-1218.

Mitchell, L., Slater, G., Hackett, D., Johnson, N., & O'connor, H. (2018). Physiological implications of preparing for a natural male bodybuilding competition. European journal of sport science, 18(5), 619-629.

Müller, M. J., Enderle, J., & Bosy-Westphal, A. (2016). Changes in energy expenditure with weight gain and weight loss in humans. Current obesity reports, 5(4), 413-423.

Müller, M. J., Geisler, C., Hübers, M., Pourhassan, M., Braun, W., & Bosy-Westphal, A. (2018). Normalizing resting energy expenditure across the life course in humans: challenges and hopes. European Journal of Clinical Nutrition, 72(5), 628-637.

Müller, M. J., Heymsfield, S. B., & Bosy-Westphal, A. (2021). Are metabolic adaptations to weight changes an artefact?. The American Journal of Clinical Nutrition, 114(4), 1386-1395.

Müller, W., Lohman, T. G., Stewart, A. D., Maughan, R. J., Meyer, N. L., Sardinha, L. B., ... & Ackland, T. R. (2016). Subcutaneous fat patterning in athletes: selection of appropriate sites and standardisation of a novel ultrasound measurement technique: ad hoc working group on body composition, health and performance, under the auspices of the IOC Medical Commission. British journal of sports medicine, 50(1), 45-54.

Di Munno, C., Busiello, R. A., Calonne, J., Salzano, A. M., Miles-Chan, J., Scaloni, A., ... & Silvestri, E. (2021). Adaptive thermogenesis driving catch-up fat is associated with increased muscle type 3 and decreased hepatic type 1 iodothyronine deiodinase activities: a functional and proteomic study. Frontiers in Endocrinology, 27.

Nana, A., Slater, G. J., Hopkins, W. G., & Burke, L. M. (2012). Effects of daily activities on dual-energy X-ray absorptiometry measurements of body composition in active people. Med Sci Sports Exerc, 44(1), 180-189.

Nana, A., Slater, G. J., Hopkins, W. G., & Burke, L. M. (2012). Techniques for undertaking dual-energy X-ray absorptiometry whole-body scans to estimate body composition in tall and/or broad subjects. International Journal of Sport Nutrition and Exercise Metabolism, 22(5), 313-322.

Nijholt, W., Jager-Wittenaar, H., Raj, I. S., van der Schans, C. P., & Hobbelen, H. (2020). Reliability and validity of ultrasound to estimate muscles: a comparison between different transducers and parameters. Clinical nutrition ESPEN, 35, 146-152.

Nunes, C. L., Jesus, F., Francisco, R., Matias, C. N., Heo, M., Heymsfield, S. B., ... & Silva, A. M. (2022). Adaptive thermogenesis after moderate weight loss: magnitude and methodological issues. European Journal of Nutrition, 61(3), 1405-1416.

Pardue, A., Trexler, E. T., & Sprod, L. K. (2017). Case study: Unfavorable but transient physiological changes during contest preparation in a drug-free male bodybuilder. International journal of sport nutrition and exercise metabolism, 27(6), 550-559.

Pontzer, H. (2018). Energy constraint as a novel mechanism linking exercise and health. Physiology.

Roberts, B. M., Nuckols, G., & Krieger, J. W. (2020). Sex differences in resistance training: a systematic review and meta-analysis. The Journal of Strength & Conditioning Research, 34(5), 1448-1460.

Rohrig, B. J., Pettitt, R. W., Pettitt, C. D., & Kanzenbach, T. L. (2017). Psychophysiological tracking of a female physique competitor through competition preparation. International journal of exercise science, 10(2), 301.

Rosenbaum, M., Hirsch, J., Gallagher, D. A., & Leibel, R. L. (2008). Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight. The American journal of clinical nutrition, 88(4), 906-912.

Rosenbaum, M., & Leibel, R. L. (2010). Adaptive thermogenesis in humans. International journal of obesity, 34(1), S47-S55.

Rossow, L. M., Fukuda, D. H., Fahs, C. A., Loenneke, J. P., & Stout, J. R. (2013). Natural bodybuilding competition preparation and recovery: a 12-month case study. International Journal of Sports Physiology & Performance, 8(5).

Sarin, H. V., Gudelj, I., Honkanen, J., Ihalainen, J. K., Vuorela, A., Lee, J. H., ... & Perola, M. (2019). Molecular pathways mediating immunosuppression in response to prolonged intensive physical training, low-energy availability, and intensive weight loss. Frontiers in immunology, 10, 907.

Sarin, H. V., Lee, J. H., Jauhiainen, M., Joensuu, A., Borodulin, K., Männistö, S., ... & Perola, M. (2019). Substantial fat mass loss reduces low-grade inflammation and induces positive alteration in cardiometabolic factors in normal-weight individuals. Scientific reports, 9(1), 1-14.

Sarin, H. V., Pirinen, E., Pietiläinen, K. H., Isola, V., Häkkinen, K., Perola, M., & Hulmi, J. J. (2021). Mitochondrial bioenergetic pathways in blood leukocyte transcriptome decrease after intensive weight loss but are rescued following weight regain in female physique athletes. The FASEB Journal, 35(4), e21484.

Trexler, E. T., Smith-Ryan, A. E., & Norton, L. E. (2014). Metabolic adaptation to weight loss: implications for the athlete. Journal of the International Society of Sports Nutrition, 11(1), 7.

Warren, M. P. (2011). Endocrine manifestations of eating disorders. The Journal of Clinical Endocrinology & Metabolism, 96(2), 333-343.

Weir, J. D. V. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. The Journal of physiology, 109(1-2), 1.

Table 1. Participants' characteristics at baseline (mean ± SD)

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

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

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

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

*.** 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

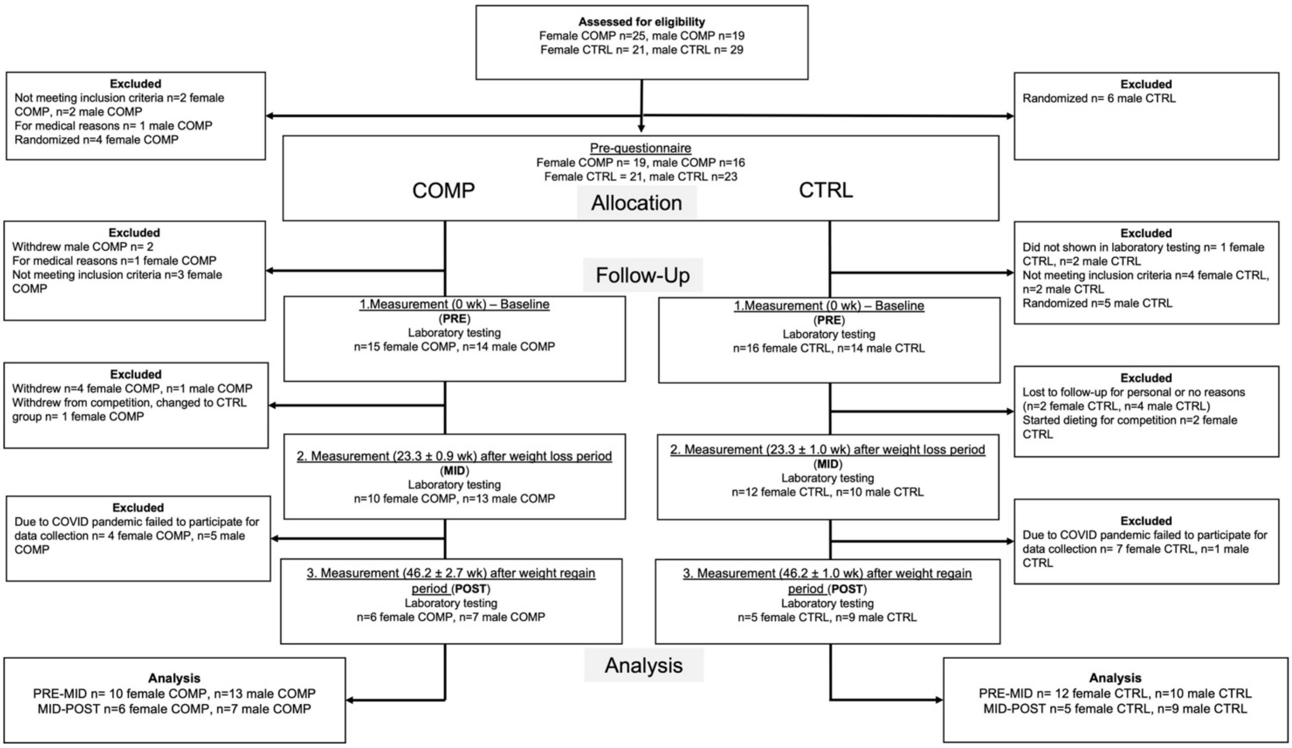


Figure 1. The experimental design of the study. Flowchart illustrating the study protocol. COMP groups study protocol on the left, and CTRL groups study protocol on the right. From PRE to MID, the female and male COMP participants decreased their energy intake and increased the amount of aerobic exercise. 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.

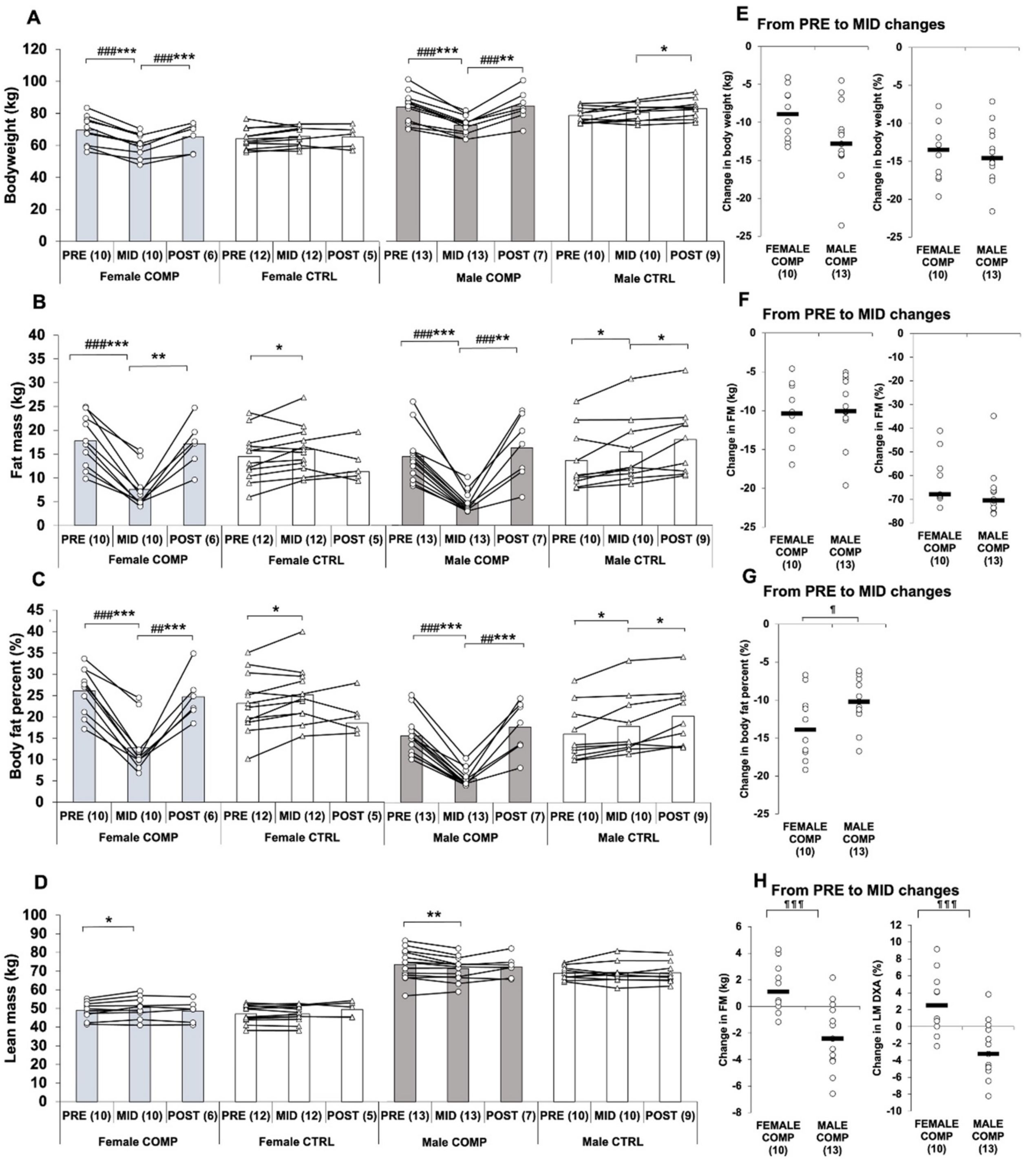


Figure 2. A, B, C and D show absolute body composition changes in COMP and CTRL groups from PRE to MID and MID to POST. Baseline pre-test (PRE) were obtained before the dieting phase for the competition, MID one week before the competition, and POST after the recovery period. Numbers in parentheses indicate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

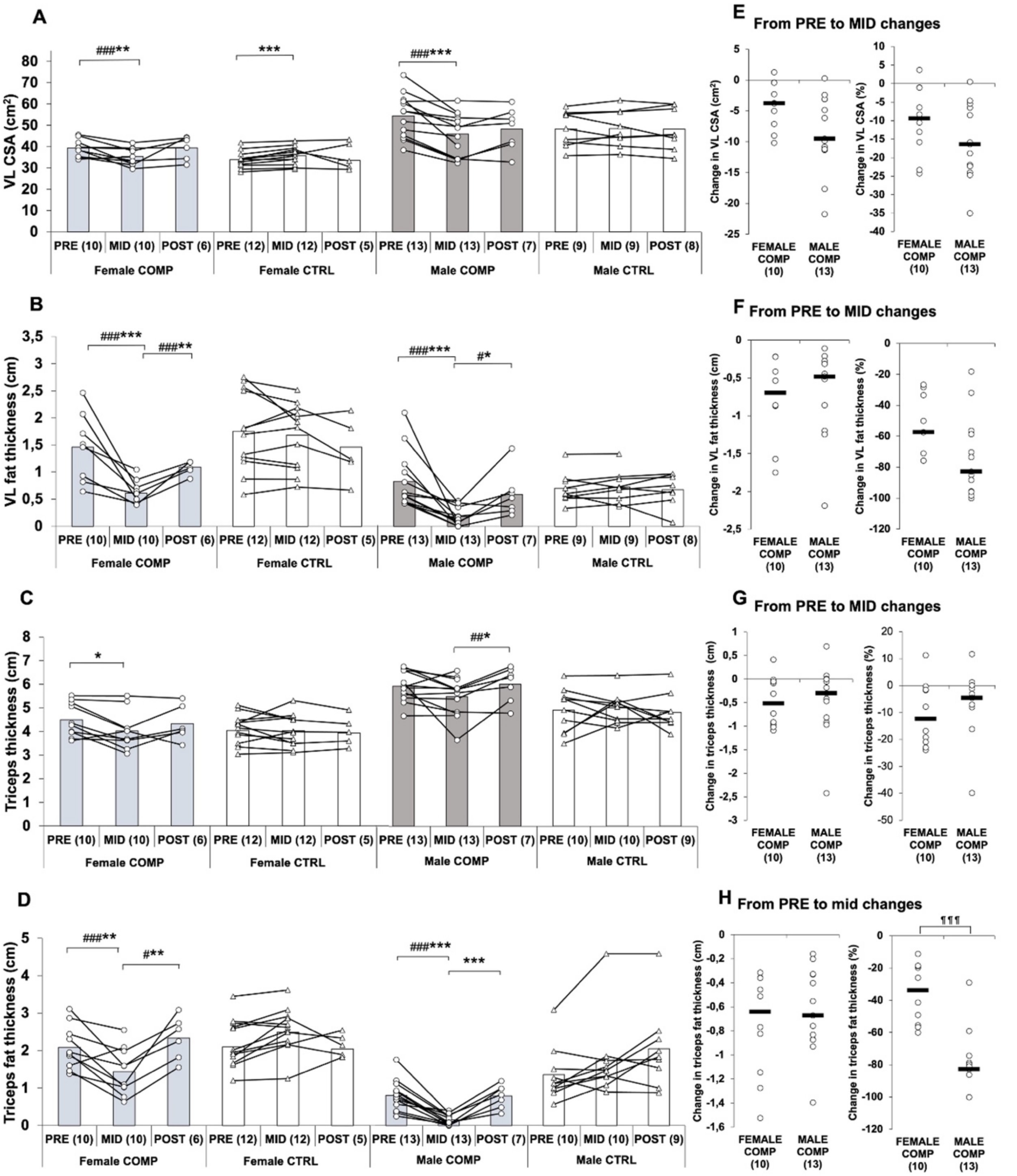


Figure 3 A, B, C, and D show absolute changes in muscle CSA, and muscle and fat thickness in COMP and CTRL groups from PRE to MID and MID to POST. Baseline pre-test (PRE) were obtained before the dieting phase for the competition, MID one week before the competition, and POST after the recovery period. Numbers in parentheses indicate the number of participants. Circles and triangles indicate individual data of COMP and CTRL group, respectively, and bars indicate means.* is a statistically significant change within the group. # is a statistically significant difference between the COMP and CTRL groups in the change. *#,**##, and ***##, ($p<0.05$–0.001). E, F, G, and H show absolute and percentage changes in female and male COMP groups from PRE to MID. Circles indicate individual data, and black lines indicate the mean. ¶¶ and ¶¶¶ is

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

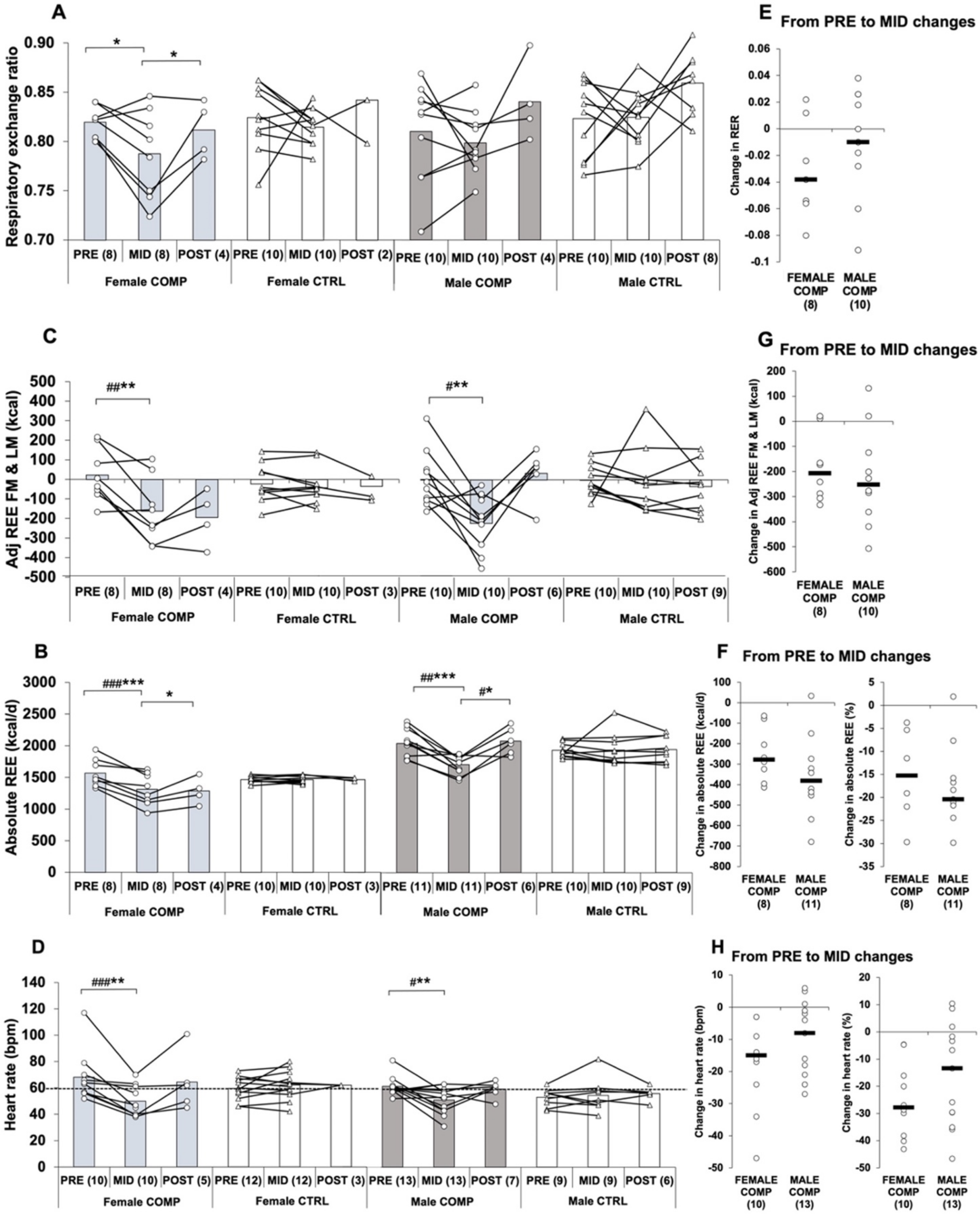
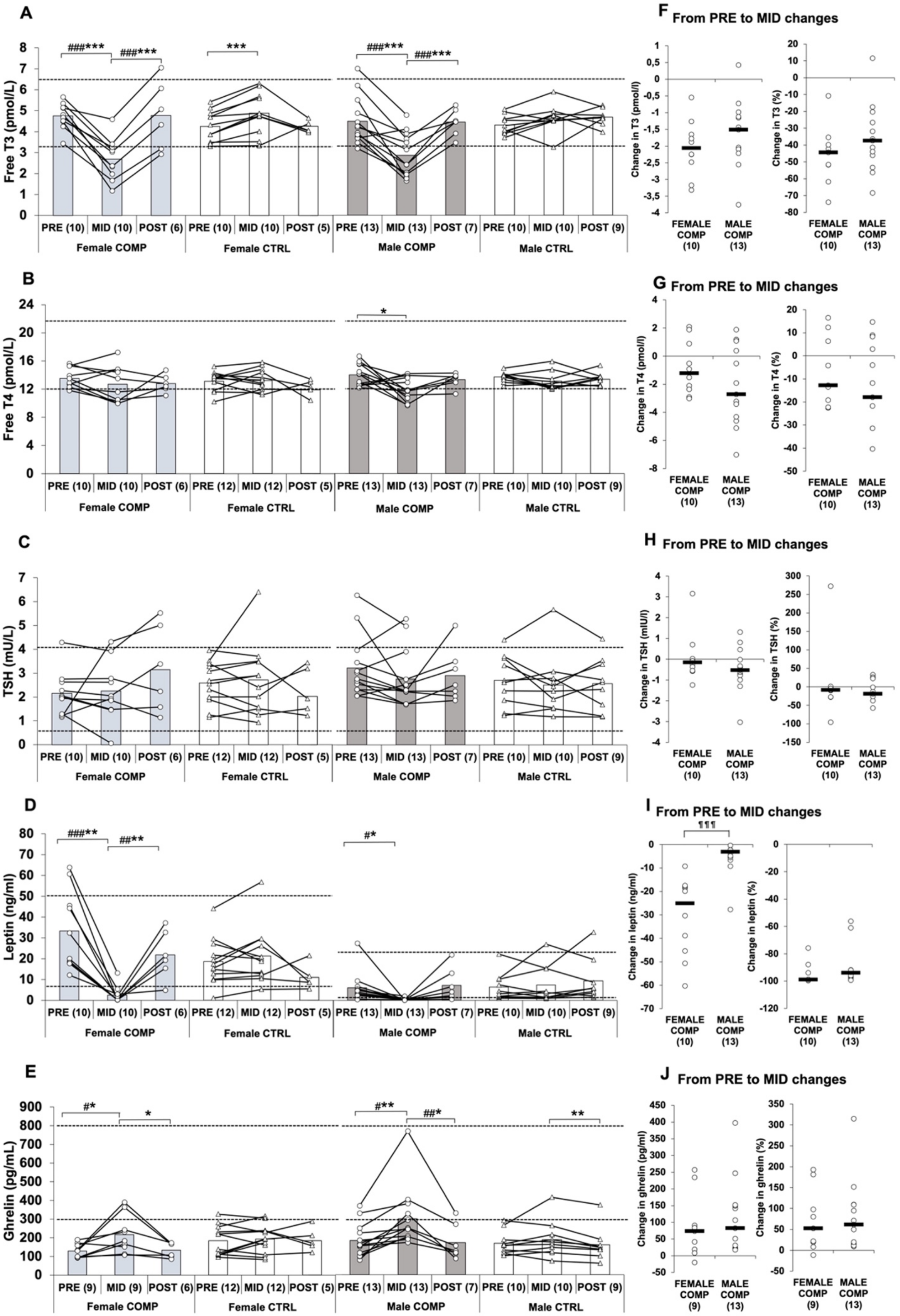


Figure 4 A, B, C, and D show absolute changes in resting metabolism and heart rate in COMP and CTRL groups from PRE to MID and MID to POST. Baseline pre-test (PRE) were obtained before the dieting phase for the competition, MID one week before the competition, and POST after the recovery period. Numbers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

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



A
Free T3 (pmol/L)
###*** ###*** *** ###*** ###***
PRE (10) MID (10) POST (6) PRE (10) MID (10) POST (5) PRE (13) MID (13) POST (7) PRE (10) MID (10) POST (9)
Female COMP Female CTRL Male COMP Male CTRL
F From PRE to MID changes
Change in T3 (pmol/l)
Change in T3 (%)
FEMALE COMP (10) MALE COMP (13)
B
Free T4 (pmol/L)
*
PRE (10) MID (10) POST (6) PRE (12) MID (12) POST (5) PRE (13) MID (13) POST (7) PRE (10) MID (10) POST (9)
Female COMP Female CTRL Male COMP Male CTRL
G From PRE to MID changes
Change in T4 (pmol/l)
Change in T4 (%)
FEMALE COMP (10) MALE COMP (13)
C
TSH (mU/L)
PRE (10) MID (10) POST (6) PRE (12) MID (12) POST (5) PRE (13) MID (13) POST (7) PRE (10) MID (10) POST (9)
Female COMP Female CTRL Male COMP Male CTRL
H From PRE to MID changes
Change in TSH (mIU/l)
Change in TSH (%)
FEMALE COMP (10) MALE COMP (13)
D
Leptin (ng/ml)
###** ##** #*
PRE (10) MID (10) POST (6) PRE (12) MID (12) POST (5) PRE (13) MID (13) POST (7) PRE (10) MID (10) POST (9)
Female COMP Female CTRL Male COMP Male CTRL
I From PRE to MID changes
¶¶¶
Change in leptin (ng/ml)
Change in leptin (%)
FEMALE COMP (10) MALE COMP (13)
E
Ghrelin (pg/mL)
#* * #** ##* **
PRE (9) MID (9) POST (6) PRE (12) MID (12) POST (5) PRE (13) MID (13) POST (7) PRE (10) MID (10) POST (9)
Female COMP Female CTRL Male COMP Male CTRL
J From PRE to MID changes
Change in ghrelin (pg/ml)
Change in ghrelin (%)
FEMALE COMP (9) MALE COMP (13)

Figure 5 A, B, C, D and E show absolute changes in appetite-regulating and thyroid hormones 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 indicate individual data of COMP and CTRL group, respectively, and bars indicate means.* is a statistically significant change within the group. # is a statistically significant difference between the COMP and CTRL groups in the change. *#,**##, and ***##, ($p<0.05$–0.001). F, G, H, I and J shows absolute and percentage changes in female and male COMP groups from PRE to MID. Circles indicate individual data, and black lines indicate the mean. ¶¶¶ is a statistically significant ($p<0.001$) difference between sexes. Free T3, free triiodothyronine; Free T4, free thyroxine; TSH, thyroid-stimulating hormone.