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

**Ville Isola** [a], **Juha J. Hulmi** [a], **Pirita Petäjä**[b], **Eric R. Helms**[c,d], **Jari E. Karppinen** [e], and **Juha P. Ahtiainen** [a]

[a]Faculty of Sport and Health Sciences, Neuromuscular Research Center, University of Jyväskylä. P.O. Box 35, Jyväskylä 40014, Finland; [b]Department of Food and Nutrition, University of Helsinki, Helsinki, Finland; [c]Sports Performance Research Institute New Zealand (SPRINZ), Auckland University of Technology, Auckland, New Zealand; [d]Department of Exercise Science and Health Promotion, Muscle Physiology Research Laboratory, Florida Atlantic University, Boca Raton, FL, 33431, USA; [e]Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland

Corresponding author: **Ville Isola** (email: ville.isola@gmail.com)

## 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 nondieting 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 dual-energy X-ray absorptiometry, 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).

**Key words:** energy restriction, metabolic rate, competition preparation, bodybuilding, sex differences, fitness

## 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 (REE) and associated hormonal alternations (Trexler et al. 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% lower than 5% (Rossow et al. 2013; Pardue et al. 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 REE due to weight loss beyond what would be predicted from the loss of body weight (BW) and corresponding changes in FM or LM alone (Rosenbaum et al. 2008). In general, a decrease in REE has been observed during weight loss in obese and nonobese 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).

Appl. Physiol. Nutr. Metab. **48**: 307–320 (2023) | dx.doi.org/10.1139/apnm-2022-0372

307

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. 2019). 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 et al. 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 REE, 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 REE 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 REE 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 World Antidoping Agency (WADA), or those competing in the junior (below 19 years of age) or master (over 40 years of age) categories or in a nondrug 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 FM 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 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 prestudy questionnaire. The participants selected for the study completed a health questionnaire subsequently reviewed by the study physician. As a result, 10 female and 13 male COMP and 12 female and 10 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.

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) (Fig. 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 h). 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.

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 h 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 (DXA), and REE 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

**Fig. 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.
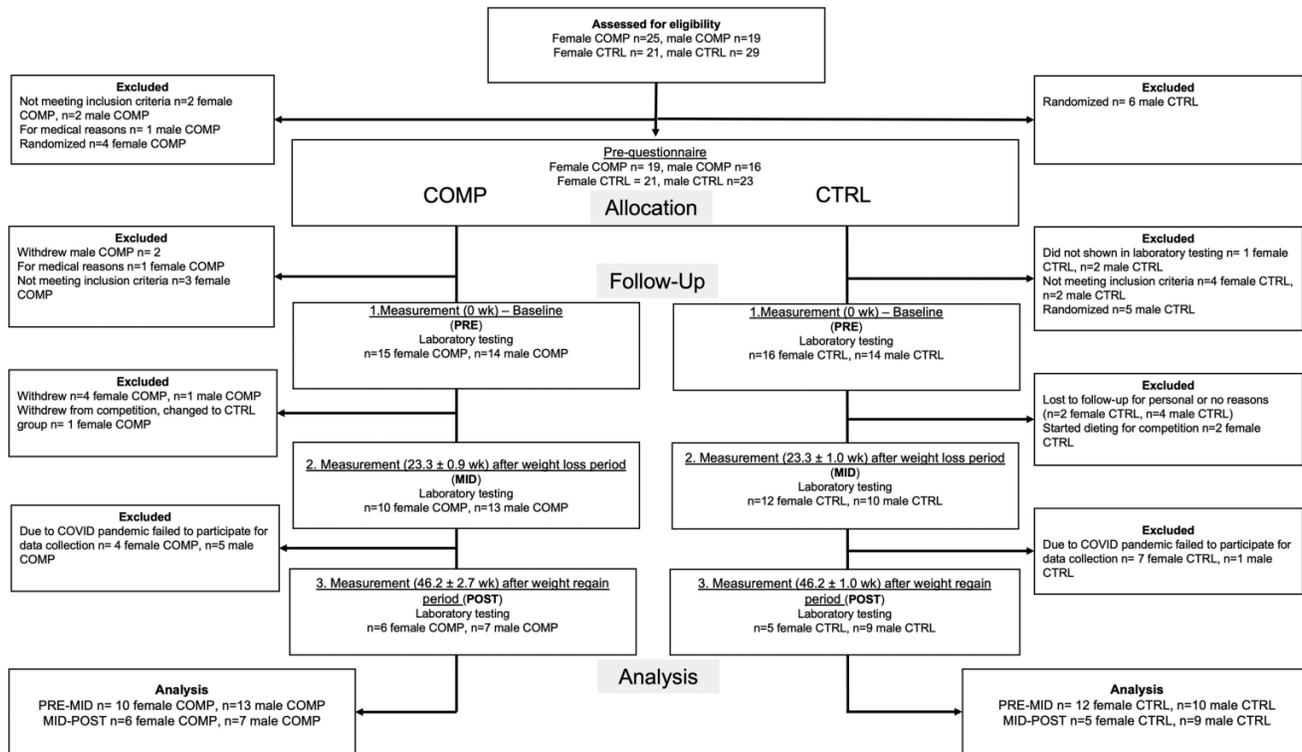


**Table 1.** Participants' characteristics at baseline (mean ± SD).

| | Age (year) | Height (cm) | Body mass (kg) | Body fat (%) | Training history (year) | Diet period (week) |
|---|---|---|---|---|---|---|
| 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 | |

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

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 7 female and 12 male COMP group participants, and 12 female and 7 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 9 male CTRL group participants from PRE to MID, and 6 female and 10 male COMP group participants and 8 female and 8 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

Appl. Physiol. Nutr. Metab. **48:** 307–320 (2023) | dx.doi.org/10.1139/apnm-2022-0372

309

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 10 male COMP group participants, and 10 female and 8 male CTRL group participants from PRE to MID, and 5 female and 5 male COMP group participants and 5 female and 8 male CTRL group participants from MID to POST period.

## Body composition

Body composition was estimated by 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). Nonelastic 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 et al. 2012*a*), and half-body scans produced no significant differences in body composition compared to whole-body scans (Nana et al. 2012*b*).

## Resting metabolism and heart rate

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 and 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) recommended using a regression equation created based on the sample baseline information. 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 ($R^2$ = 0.83, adjusted $R^2$ = 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 composi-

tion assessment and indirect calorimetry (Müller et al. 2018). The figure of adjusted REE with regression line and 95% confidence intervals is in Supplementary Information (Fig. 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 2022).

## 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-$\alpha$10, 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 SFA were measured by the same device. The scanning head was overlaid 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*b*). 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, USA). 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 min 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 DS2 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 analysis software (SPSS version 27; Chicago, IL, USA). The normality of the data was analyzed 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's 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, REE, 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 $2609.7 \pm 475.9$ kcal/day and $3254.1 \pm 429.0$ kcal/day in the female and male COMP groups, respectively (Table 2). Energy intake decreased in both the female—$889.2 \pm 322.7$ kcal/day ($p < 0.01$)—and male—$1248.8 \pm 462.6$ 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 ($p < 0.01$).

## 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 ($p < 0.01$ and $p < 0.05$), 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 ($p < 0.001$; Fig. 2). The BF% differed between the sexes ($p < 0.05$). BF% decreased from $26.1 \pm 5.4\%$ to $12.6 \pm 6.0\%$ in the female 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 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 ($r = 0.66$ and $p < 0.05$).

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.

## 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 (Fig. 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 ($p < 0.05$).

## 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 $p < 0.001$, Fig. 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

**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/week)** | | | | | | | | |
| 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/week)** | | | | | | | | |
| 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 |

**Note:** *, **, 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.

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

## 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 $p < 0.001$, Fig. 5). A statistically significant difference ($p < 0.001$) was observed between the sexes in the absolute change in serum leptin (Fig.

5I) but not in the relative changes ($71.3 ± 74.2\%$ in females versus $77.9 ± 84.5\%$ 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 ($r = 0.78$ and $p < 0.05$) and carbohydrate intake ($r = 0.92$ and $p < 0.001$) in the female COMP group. Changes in T3 were positively associated with changes in LM ($r = 0.68$ and $p < 0.01$) in the male COMP group. Changes in ghrelin were positively associated with the change in RER ($r = 0.67$ and $p < 0.05$), and changes in leptin were positively associated with the change in FM ($r = 0.57$ and $p < 0.05$) and protein intake ($r = 0.71$ and $p < 0.05$) 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 ($p < 0.001$).

**Fig. 2.** (A–D) Absolute body composition changes in COMP and CTRL groups from PRE to MID and MID to POST. Baseline pre-test (PRE) was 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 *** ($p < 0.05$–$0.001$) and ### ($p < 0.001$). (E–H) 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 percentage is not depicted. FM, fat mass; LM, lean mass.
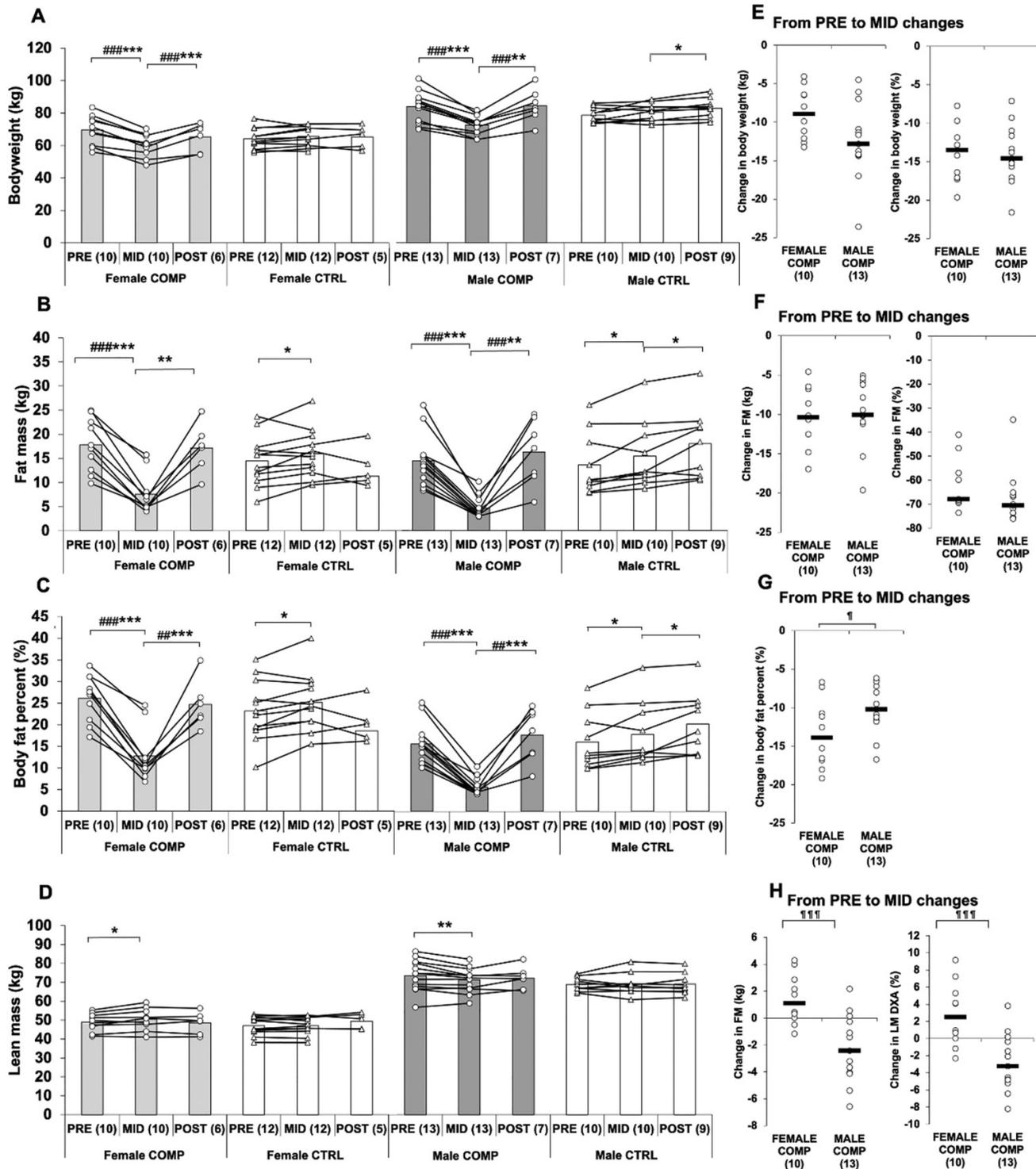
Appl. Physiol. Nutr. Metab. **48:** 307–320 (2023) | dx.doi.org/10.1139/apnm-2022-0372

313

**Fig. 3.** (A–D) 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) was 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–H) 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 ¶¶¶ are a statistically significant ($p < 0.01$–0.001) difference between the sexes. CSA, cross-sectional area; VL, vastus lateralis muscle.
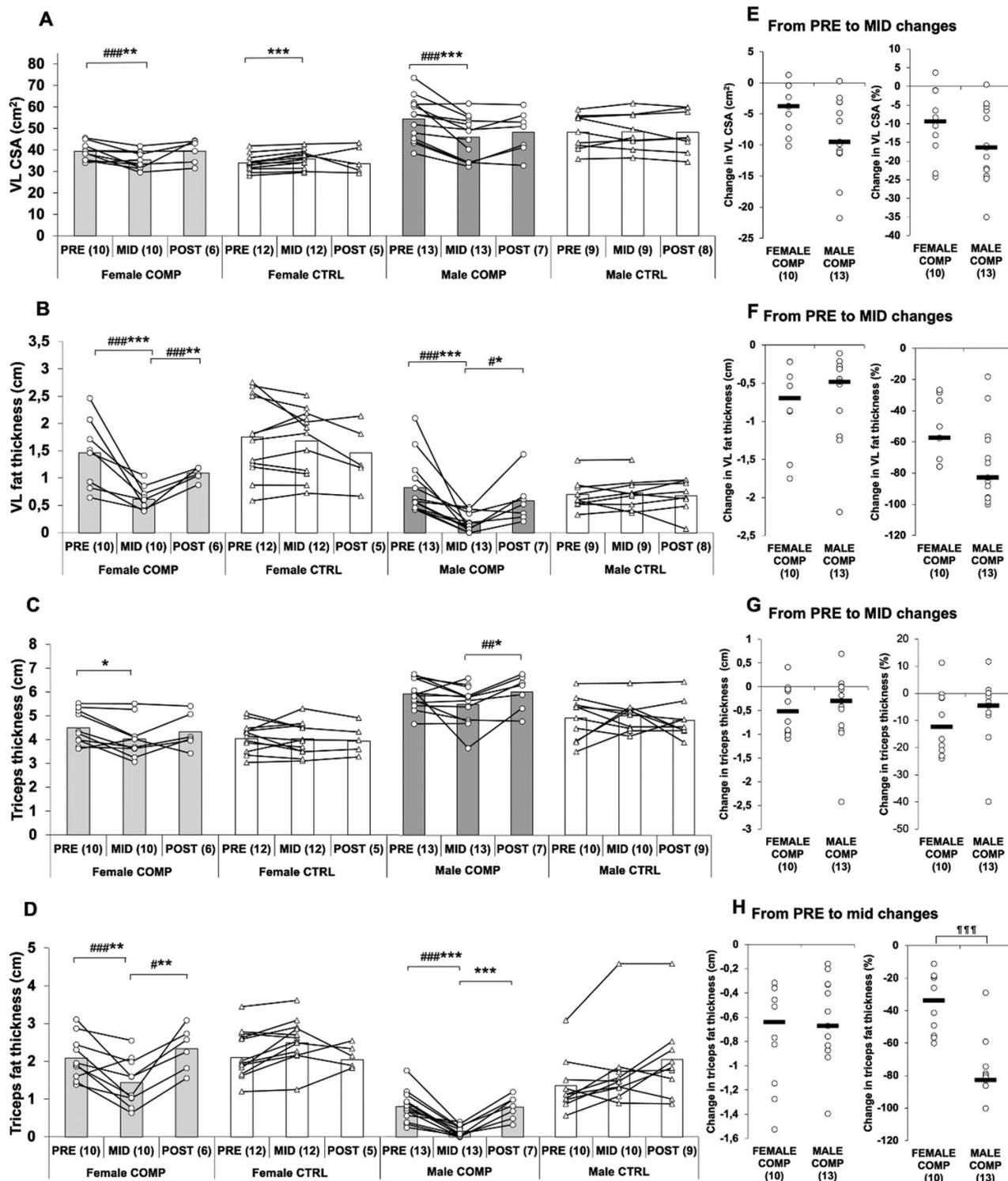
**Fig. 4.** (A–D) Absolute changes in resting metabolism and heart rate in COMP and CTRL groups from PRE to MID and MID to POST. Baseline pre-test (PRE) was obtained before the dieting phase for the competition, MID one week before the competition, and POST after the recovery period. Numbers in parentheses 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 ***###, ($p < 0.05$–$0.001$). The black dotted line represent bradycardia limit-value. (E–H) 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 and LM, calculated measured and predicted resting energy expenditure adjusted with fat mass and lean mass.
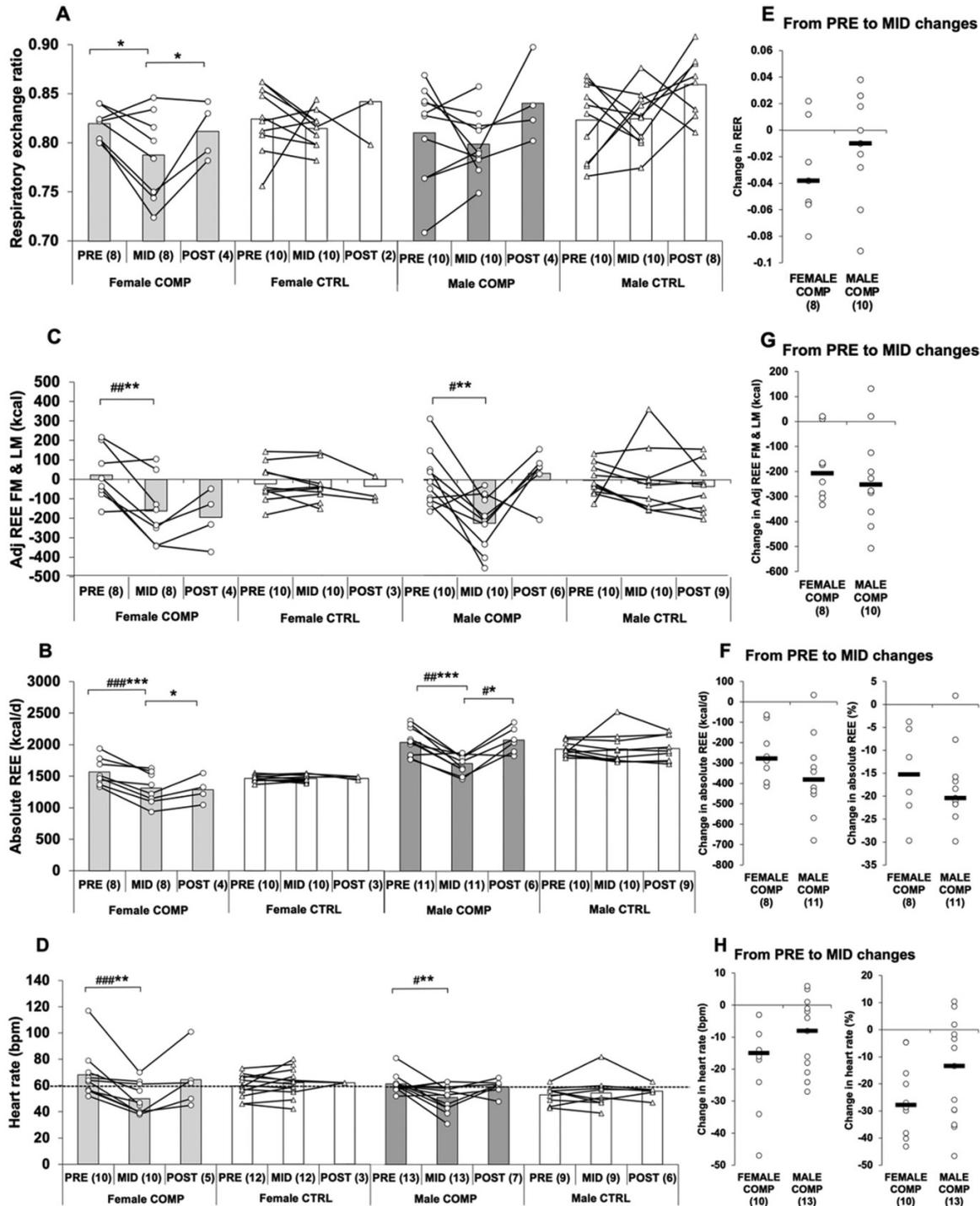
**Fig. 5.** (A–E) Absolute changes in appetite-regulating and thyroid hormones in COMP and CTRL groups from PRE to MID and MID to POST. Baseline pre-test (PRE) was obtained before the dieting phase for the competition, MID one week before the competition, and POST after the recovery period. Numbers in 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 ***###, ($p < 0.05$–$0.001$). The black dotted lines represents the reference values in normal-weight individuals. (F–J) 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.
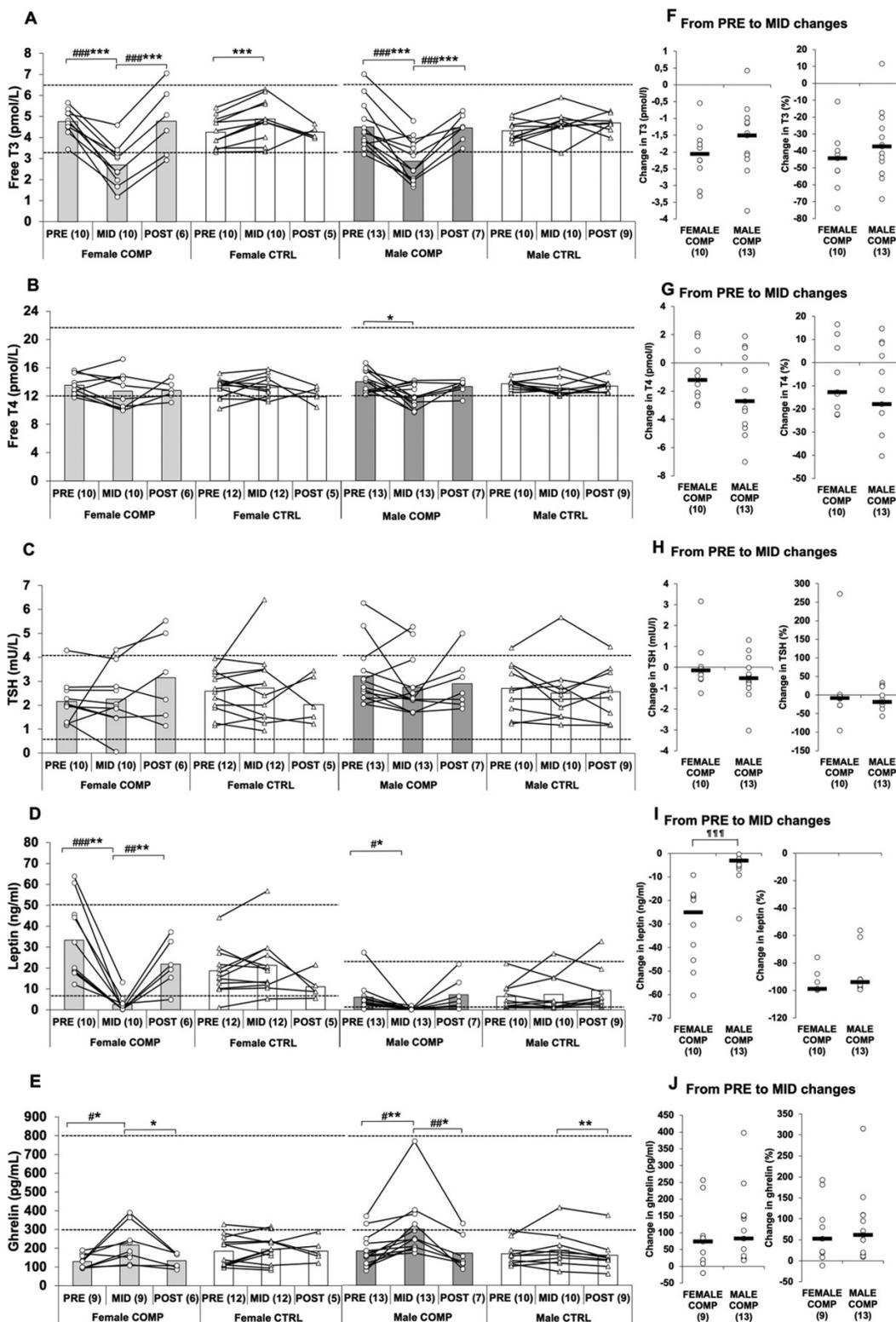
# Discussion

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. 2019). 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. 2016b). 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 et al. 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 autonomic nervous system during adaptive thermogenesis (Rosenbaum and Leibel 2010). Moreover, we reported a temporary suppression of the immune (Sarin et al. 2019a) and reproductive (Hulmi et al. 2017) systems and decreased levels of systemic inflammation (Sarin et al. 2019b), 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 et al. 2019a, 2019b, 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 et al. 2021). Accordingly, Müller et al. (2016a) 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 et al. 2016a), but further studies are warranted to investigate the physiological and behavioral 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

Appl. Physiol. Nutr. Metab. **48**: 307–320 (2023) | dx.doi.org/10.1139/apnm-2022-0372

317

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 nondieting 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 FM, 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.

## Acknowledgements

## Article information

### Copyright

### Data availability

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

## Author information

### Author ORCIDs
Ville Isola https://orcid.org/0000-0002-7170-158X
Juha J. Hulmi https://orcid.org/0000-0003-3813-2124
Jari E. Karppinen https://orcid.org/0000-0002-9886-9067
Juha P. Ahtiainen https://orcid.org/0000-0003-2305-4741

### Author contributions

Conceptualization: VI, JJH, ERH, JPA
Data curation: VI, JJH, PP, ERH, JEK, JPA
Formal analysis: VI, JPA
Funding acquisition: VI, ERH, JPA
Investigation: VI, PP, JEK
Methodology: VI, JJH, JEK, JPA
Project administration: VI, JPA
Resources: VI, JPA
Software: VI, JEK
Supervision: VI, JJH, ERH, JPA
Validation: VI, JJH, JPA
Visualization: VI, JPA
Writing – original draft: VI, JJH, JEK, JPA
Writing – review & editing: VI, JJH, ERH, JPA

### Competing interests

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

## Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/apnm-2022-0372.

## References

Ahtiainen, J.P., Hoffren, M., Hulmi, J.J., Pietikäinen, M., Mero, A.A., Avela, J., and Häkkinen, K. 2010. Panoramic ultrasonography is a valid method to measure changes in skeletal muscle cross-sectional area. Eur. J. Appl. Physiol. **108**(2): 273–279. doi:10.1007/s00421-009-1211-6. PMID: 19777252.

Alwan, N., Moss, S.L., Elliott-Sale, K.J., Davies, I.G., and Enright, K. 2019. A narrative review on female physique athletes: the physiological and psychological implications of weight management practices. Int. J. Sport Nutr. Exercise Metab. **29**(6): 682–689. doi:10.1123/ijsnem.2019-0037. PMID: 31141414.

Christen, T., Trompet, S., Noordam, R., van Klinken, J.B., van Dijk, K.W., and Lamb, H.J., 2018. Sex differences in body fat distribution are related to sex differences in serum leptin and adiponectin. Peptides, **107**: 25–31. doi:10.1016/j.peptides.2018.07.008. PMID: 30076861.

Considine, R.V., Sinha, M.K., Heiman, M.L., Kriauciunas, A., Stephens, T.W., and Nyce, M.R., 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. New Engl. J.

Med. **334**(5): 292–295. doi:10.1056/NEJM199602013340503. PMID: 8532024.

Couillard, C., Mauriege, P., Prud'Homme, D., Nadeau, A., Tremblay, A., Bouchard, C., and Després, J.P. 1997. Plasma leptin concentrations: gender differences and associations with metabolic risk factors for cardiovascular disease. Diabetologia, **40**(10): 1178–1184. doi:10.1007/s001250050804. PMID: 9349599.

di Munno, C., Busiello, R.A., Calonne, J., Salzano, A.M., Miles-Chan, J., and Scaloni, A., 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. Front. Endocrinol. 27.

Fagerberg, P. 2018. Negative consequences of low energy availability in natural male bodybuilding: a review. Int. J. Sport Nutr. Exercise Metab. **28**(4): 385–402. doi:10.1123/ijsnem.2016-0332. PMID: 28530498.

Friedl, K.E., Moore, R.J., Martinez-Lopez, L.E., Vogel, J.A., Askew, E.W., and Marchitelli, L.J., 1994. Lower limit of body fat in healthy active men. J. Appl. Physiol. **77**(2): 933–940. doi:10.1152/jappl.1994.77.2.933. PMID: 8002550.

Fullmer, S., Benson-Davies, S., Earthman, C.P., Frankenfield, D.C., Gradwell, E., and Lee, P.S., 2015. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. J. Acad. Nutr. Diet. **115**(9): 1417–1446. doi:10.1016/j.jand.2015.04.003. PMID: 26038298.

Helms, E.R., Aragon, A.A., and Fitschen, P.J. 2014. Evidence-based recommendations for natural bodybuilding contest preparation: nutrition and supplementation. J. Int. Soc. Sports Nutr. **11**(1): 20. doi:10.1186/1550-2783-11-20. PMID: 24864135.

Hubal, M.J., Gordish-Dressman, H., Thompson, P.D., Price, T.B., Hoffman, E.P., and Angelopoulos, T.J., 2005. Variability in muscle size and strength gain after unilateral resistance training. Med. Sci. Sports Exercise, **37**(6): 964–972.

Hulmi, J.J., Isola, V., Suonpää, M., Järvinen, N.J., Kokkonen, M., and Wennerström, A., 2017. The effects of intensive weight reduction on body composition and serum hormones in female fitness competitors. Front. Physiol. **7**: 689.

Ivey, F.M., Roth, S.M., Ferrell, R.E., Tracy, B.L., Lemmer, J.T., and Hurlbut, D.E., 2000. Effects of age, gender, and myostatin genotype on the hypertrophic response to heavy resistance strength training. J. Gerontol. Ser. A Biol. Sci. Med. Sci. **55**(11): M641–M648. doi:10.1093/gerona/55.11.M641.

Jagim, A.R., Camic, C.L., Askow, A., Luedke, J., Erickson, J., and Kerksick, C.M., 2019. Sex differences in resting metabolic rate among athletes. J. Strength Cond. Res. **33**(11): 3008–3014.

Janssen, I., Heymsfield, S.B., Wang, Z., and Ross, R. 2000. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. J. Appl. Physiol. **89**(1): 81–88. doi:10.1152/jappl.2000.89.1.81.

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. Nutr. Rev. **80**(7): 1811–1825. doi:10.1093/nutrit/nuac006.

Martin, A., Fox, D., Murphy, C.A., Hofmann, H., and Koehler, K. 2022. Tissue losses and metabolic adaptations both contribute to the reduction in resting metabolic rate following weight loss. Int. J. Obesity, **46**(6): 1168–1175. doi:10.1038/s41366-022-01090-7.

Martins, C., Roekenes, J., Salamati, S., Gower, B.A., and Hunter, G.R. 2020. Metabolic adaptation is an illusion, only present when participants are in negative energy balance. Am. J. Clin. Nutr. **112**(5): 1212–1218. doi:10.1093/ajcn/nqaa220.

Mitchell, L., Slater, G., Hackett, D., Johnson, N., and O'connor, H. 2018. Physiological implications of preparing for a natural male bodybuilding competition. European journal of sport science, **18**(5): 619–629. doi:10.1080/17461391.2018.1444095.

Müller, M.J., Enderle, J., and Bosy-Westphal, A. 2016a. Changes in energy expenditure with weight gain and weight loss in humans. Current Obesity Rep. **5**(4): 413–423. doi:10.1007/s13679-016-0237-4.

Müller, M.J., Geisler, C., Hübers, M., Pourhassan, M., Braun, W., and Bosy-Westphal, A. 2018. Normalizing resting energy expenditure across the life course in humans: challenges and hopes. Eur. J. Clin. Nutr. **72**(5): 628–637. doi:10.1038/s41430-018-0151-9.

Müller, M.J., Heymsfield, S.B., and Bosy-Westphal, A. 2021. Are metabolic adaptations to weight changes an artefact? Am. J. Clin. Nutr. **114**(4): 1386–1395. doi:10.1093/ajcn/nqab184.

Müller, W., Lohman, T.G., Stewart, A.D., Maughan, R.J., Meyer, N.L., and Sardinha, L.B., 2016b. 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. Br. J. Sports Med. **50**(1): 45–54. doi:10.1136/bjsports-2015-095641.

Nana, A., Slater, G.J., Hopkins, W.G., and Burke, L.M. 2012a. Effects of daily activities on dual-energy X-ray absorptiometry measurements of body composition in active people. Med. Sci. Sports Exercise, **44**(1): 180–189. doi:10.1249/MSS.0b013e318228b60e.

Nana, A., Slater, G.J., Hopkins, W.G., and Burke, L.M. 2012b. Techniques for undertaking dual-energy X-ray absorptiometry whole-body scans to estimate body composition in tall and /or broad subjects. Int. J. Sport Nutr. Exercise Metab. **22**(5): 313–322. doi:10.1123/ijsnem.22.5.313.

Nijholt, W., Jager-Wittenaar, H., Raj, I.S., van der Schans, C.P., and Hobbelen, H. 2020. Reliability and validity of ultrasound to estimate muscles: a comparison between different transducers and parameters. Clin. Nutr. ESPEN, **35**: 146–152. doi:10.1016/j.clnesp.2019.10.009.

Nunes, C.L., Jesus, F., Francisco, R., Matias, C.N., Heo, M., and Heymsfield, S.B., 2022. Adaptive thermogenesis after moderate weight loss: magnitude and methodological issues. Eur. J. Nutr. **61**(3): 1405–1416. doi:10.1007/s00394-021-02742-6.

Pardue, A., Trexler, E.T., and Sprod, L.K. 2017. Case study: unfavorable but transient physiological changes during contest preparation in a drug-free male bodybuilder. Int. J. Sport Nutr. Exercise Metab. **27**(6): 550–559. doi:10.1123/ijsnem.2017-0064.

Pontzer, H. 2018. Energy constraint as a novel mechanism linking exercise and health. Physiology, **33**(6): 384–393. doi:10.1152/physiol.00027.2018.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL https://www.R-project.org/.

Roberts, B.M., Nuckols, G., and Krieger, J.W. 2020. Sex differences in resistance training: a systematic review and meta-analysis. J. Strength Cond. Res. **34**(5): 1448–1460.

Rohrig, B.J., Pettitt, R.W., Pettitt, C.D., and Kanzenbach, T.L. 2017. Psychophysiological tracking of a female physique competitor through competition preparation. Int. J. Exercise Sci. **10**(2): 301.

Rosenbaum, M., and Leibel, R.L. 2010. Adaptive thermogenesis in humans. Int. J. Obesity, **34**(1): S47–S55. doi:10.1038/ijo.2010.184.

Rosenbaum, M., Hirsch, J., Gallagher, D.A., and Leibel, R.L. 2008. Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight. Am. J. Clin. Nutr. **88**(4): 906–912. doi:10.1093/ajcn/88.4.906.

Rossow, L.M., Fukuda, D.H., Fahs, C.A., Loenneke, J.P., and Stout, J.R. 2013. Natural bodybuilding competition preparation and recovery: a 12-month case study. Int. J. Sports Physiol. Perform. **8**(5): 582–592.

Sarin, H.V., Gudelj, I., Honkanen, J., Ihalainen, J.K., Vuorela, A., and Lee, J.H., 2019a. Molecular pathways mediating immunosuppression in response to prolonged intensive physical training, low-energy availability, and intensive weight loss. Front. Immunol. **10**: 907. doi:10.3389/fimmu.2019.00907.

Sarin, H.V., Lee, J.H., Jauhiainen, M., Joensuu, A., Borodulin, K., and Männistö, S., 2019b. Substantial fat mass loss reduces low-grade inflammation and induces positive alteration in cardiometabolic factors in normal-weight individuals. Sci. Rep. **9**(1): 1–14. doi:10.1038/s41598-019-40107-6.

Sarin, H.V., Pirinen, E., Pietiläinen, K.H., Isola, V., Häkkinen, K., Perola, M., and 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. FASEB J. **35**(4): e21484. doi:10.1096/fj.202002029R.

Tinsley, G.M., Trexler, E.T., Smith-Ryan, A.E., Paoli, A., Graybeal, A.J., Campbell, B.I., and Schoenfeld, B.J. 2019. Changes in body composition and neuromuscular performance through preparation, 2 competitions, and a recovery period in an experienced female physique athlete. J Strength Cond Res. **33(7)**: 1823–1839.

Trexler, E.T., Smith-Ryan, A.E., and Norton, L.E. 2014. Metabolic adaptation to weight loss: implications for the athlete. J. Int. Soc. Sports Nutr. **11**(1): 7. doi:10.1186/1550-2783-11-7.

Warren, M.P. 2011. Endocrine manifestations of eating disorders. J. Clin. Endocrinol. Metab. **96**(2): 333–343.

Weir, J.D.V. 1949. New methods for calculating metabolic rate with special reference to protein metabolism. J. Physiol. **109**(1–2): 1. doi:10.1113/jphysiol.1949.sp004363.