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Physiological implications of preparing for a natural male bodybuilding competition

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Abstract
This study aimed to describe the body composition and physiological changes which take place during the in-season and recovery periods of a group of natural bodybuilders. Natural male bodybuilders (n = 9) were assessed 16 (PRE16), 8 (PRE8), and 1 (PRE1) week(s) before, and 4 (POST4) weeks after a bodybuilding competition. Assessments included body composition, resting metabolic rate (RMR), serum hormones, and 7-day weighed food and training diaries. Change in parameters was assessed using repeated-measures analysis of variance. Dietary protein intake remained high throughout the study period (2.8 – 3.1 g kg⁻¹ d⁻¹). Fat mass (FM) was significantly reduced from PRE16 to PRE1 (8.8 ± 3.1 vs. 5.3 ± 2.4 kg, P < .01). There was a small decrease in lean mass (LM) from PRE8 to PRE1 (71.8 ± 9.1 vs. 70.9 ± 9.1 kg, P < .05). No changes in RMR were observed (P > .05). Large reductions in total and free testosterone (16.4 ± 4.4 vs. 10.1 ± 3.6 nmol L⁻¹, P < .05; 229.3 ± 72.4 vs. 116.8 ± 76.9 pmol L⁻¹, P < .05), and insulin-like growth factor-1 (IGF-1) (27.0 ± 7.7 vs. 19.9 ± 7.6 nmol L⁻¹, P < .05) occurred between PRE16 and PRE1. LM and IGF-1 increased from PRE1 to POST4 (70.9 ± 9.1 vs. 72.5 ± 8.5 kg, P < .05; 19.9 ± 7.6 vs. 25.4 ± 9.3 nmol L⁻¹, P < .05). Despite substantial reductions in FM, participants maintained almost all of their LM. The reduction in anabolic hormone concentration is likely attributable to the prolonged negative energy balance, despite a high dietary protein intake.

Keywords: Lean mass, fat mass, resting metabolic rate, protein, hormones, body composition

Highlights
• Bodybuilders maintained a very high dietary protein intake and a high resistance training volume during the 16 week competition preparation period, while no changes in dietary carbohydrate and fat intake occurred.
• Large reductions in fat mass and small reductions in lean mass were observed, likely attributable to an ongoing negative energy balance. Both fat and lean mass were increased 4 weeks after competition.
• The relative maintenance of lean mass occurred despite reductions in serum testosterone and IGF-1 concentrations during competition preparation.

Introduction
Athlete physique traits have been associated with success in a variety of sports, including swimming (Siders, Lukaski, & Bolonchuk, 1993), track and field (Claessens, Hlatky, Lefevre, & Holdhaus, 1994), and rugby (Olds, 2001; Sedeaud et al., 2012), as well as aesthetically judged sports such as gymnastics (Claessens, Lefevre, Beunen, & Malina, 1999) and bodybuilding (Fry, Ryan, Schwab, Powell, & Kraemer, 1991). Competitive bodybuilders are judged on muscular size, symmetry, and leanness, and employ a long-term approach to competition preparation (Hackett, Johnson, & Chow, 2013). In doing so, bodybuilders achieve the pinnacle of body composition translation for physique-based athletes: extreme leaness and hypermuscularity (Rossow, Fukuda, Fabs, Loenneke, & Stout, 2013). Rigorous diet and training practices are followed, and a range of dietary supplements are utilised (Hackett et al., 2013; Spendlove et al., 2015). The off-season period, lasting months to years, targets hypertrophy and is characterised by an energy-dense, high-protein diet, plus large volumes of high-
intensity resistance training (Hackett et al., 2013; Mitchell, Hackett, Gifford, Estermann, & O’Connor, 2017; Spendlove et al., 2015). The in-season focuses on reductions in fat mass (FM) while maintaining lean mass (LM) through manipulation of diet and exercise variables (Hackett et al., 2013; Spendlove et al., 2015). In-season duration varies between athletes, typically lasting 12–26 weeks (Mitchell et al., 2017).

Given the extreme outcomes achieved, efforts have been made to describe the diet and training programmes employed by bodybuilders, along with physiological adaptations that occur during the in-season. Early evidence from longitudinal research using small cohorts of males and females suggested bodybuilders make progressive reductions in energy intake, and increases in aerobic training volume, which are associated with the desired decreases in FM during this phase (Bamman, Hunter, Newton, Roney, & Khaled, 1993; van der Ploeg et al., 2001). More recent evidence has corroborated this and further shown that significant changes in anabolic hormone concentrations occur (Maestu, Eliakim, Jurimae, Valter, & Jurimae, 2010). However, numerous studies have also suggested that bodybuilders may experience a significant loss of LM during the in-season period (van der Ploeg et al., 2001), which is an undesirable outcome considering that they are judged on muscularity as well as leanness. On the basis of case study observations, there appears to be large associated reductions in resting metabolic rate (RMR; Robinson, Lambeth-Mansell, Gillibrand, Smith-Ryan, & Bannock, 2015), which is likely a compensatory physiological response to reduce energy expenditure and mitigate the energy deficit, ultimately preventing further reductions in body mass (Friedl et al., 2000). From a bodybuilding perspective, this may limit FM loss, while potentially impeding muscle mass maintenance.

Although behavioural changes of bodybuilders, and their physiological associations, have individually been described, comprehensive longitudinal data in natural bodybuilders are currently limited to small cohorts and case studies (Bamman et al., 1993; Kistler, Fitschen, Ranadive, Fernhall, & Wilund, 2014; Maestu et al., 2010; Robinson et al., 2015; Rossov et al., 2013; van der Ploeg et al., 2001). Given the increasing popularity of competitive bodybuilding (Helms, Aragon, & Fitschen, 2014), and the success of bodybuilders in achieving high degrees of muscularity and leanness, gaining more data to inform and potentially better understand bodybuilding practices and the physiological implications is warranted.

Taking current evidence into account, there is a need to document longitudinal physiological responses of male, natural bodybuilders to competition preparation. Thus, utilising a cohort of high-calibre competitors, this prospective study aimed to describe the body composition and physiological changes experienced by male, natural bodybuilders during the in-season and recovery periods of a bodybuilding contest. Based on documented changes associated with long-term energy restriction and high energy expenditure, we hypothesised that the bodybuilders would experience large reductions in FM with concomitant reductions in LM, RMR, and anabolic hormones during the in-season period.

**Methods**

**Participants**

To be eligible for inclusion, participants had to be male, drug-free bodybuilders, ≥18 years of age, and preparing for a competition in a natural federation. Recruitment methods included advertisements on the website and social media page of the Australasian Natural Bodybuilding and other social media pages. Advertisements were distributed at the Australasian Natural Bodybuilding national contest in October 2015, and to a database of bodybuilders held by the researchers from previous studies. Written informed consent was provided by all participants. Ethics approval was obtained from the University of Sydney Human Ethics Committee, project number 2015/425.

**Procedures**

Four testing sessions were conducted over a 20-week period. Three tests occurred during competition preparation (16, 8, and 1 week(s) pre-competition), and one occurred during competition recovery (4 weeks post-competition). The 16-week pre-competition testing duration was selected based on previous reports indicating average in-season preparation periods of 16 weeks in natural bodybuilders (Mitchell et al., 2017). Participants presented to the laboratory between 0600 and 0800 hours after a 12-h food and fluid fast and having been instructed to abstain from caffeine, alcohol, and exercise for 12 h. Participants were advised to avoid physical activity, such as walking, jogging, and cycling, the morning of assessment. A urine sample was collected upon arrival. All participants presented in a euhydrated state, confirmed via urinary specific gravity assessment (UG-α, Atago, Japan). Stature (WS220S stadiometer, Wedderburn, Sydney, Australia) and mass (Wildcat, Mettler Toledo, Ohio, USA) in swimwear were measured according to standardised protocols (Stewart, Marfell-Jones, Olds, & de Ridder, 2011),
before a battery of examinations was performed in the following order.

**Bioelectrical impedance analysis (BIA).** After 10 min rest in a supine position, bioimpedance spectroscopy was used to estimate total body water (TBW), intracellular fluid (ICF), and extracellular fluid (ECF) content. According to manufacturer recommendations (IMP SFB7, ImpediMed, Queensland, Australia), dual tab electrodes were placed on the hand and foot on the right side of the body. The device scans 256 frequencies and utilises Cole modelling with Hanai mixture theory. The average of three trials was used to calculate TBW, ICF, and ECF. Values were calculated internal to the BIA device.

**RMR (resting energy expenditure).** Resting energy expenditure was estimated using indirect calorimetry with a metabolic cart (Quark CPET, COSMED, Rome, Italy). Participants remained rested after BIA measurement in the same position. Expired respiratory gas analysis began with the participant instructed to breathe normally. Expired air was collected using a face mask for 30 min, measured at 30-s intervals. A 5-min period with VO₂ and VCO₂ coefficient of variation ≤10% during the second 15 min was used to quantify the resting energy expenditure and respiratory exchange ratio (Compher, Frankenfield, Keim, & Roth-Yousey, 2006). Participants were instructed to lie still but not fall asleep. The gas analyser was calibrated immediately prior to testing with a known gas concentration (5% CO₂, 16% O₂, 79% N₂), and a 3-L calibration syringe (Hans Rudolf, USA) was used to calibrate the volume transducer. Testing took place in a quiet, dimly lit, thermo-neutral room.

**Dual-energy X-ray absorptiometry (DXA).** A whole-body DXA scanner (Lunar Prodigy, GE Medical Systems, Madison, WI, USA) was used to estimate body composition. Total FM and LM were determined using the system’s software package (enCORE 2011 version 13.60.033; GE Healthcare). The DXA was calibrated with phantoms as per manufacturer guidelines each day prior to measurement. Participants were placed in a standardised position on the scanning bed (feet neutral, ankles strapped together, arms straight, palms down and isolated from the body, face up with neutral chin; Hangartner, Warner, Braillon, Jankowski, & Shepherd, 2013), wearing only swimwear. Measurements were performed by a licenced operator, with excellent test–retest reliability for FM (ICC: 0.998; CV: 3.7%) and LM (ICC: 0.999; CV: 3.7%). The typical error of measurement for a Lunar Prodigy established by repeat measurements has been reported as 0.4% and 1.9% for LM and FM, respectively (Nana, Slater, Hopkins, & Burke, 2012).

**Anthropometry.** An accredited anthropometrist (level 1 ISAK) with a technical error of measurement of 2.4% used surface anthropometry (Harpenden skinfold callipers, Baty International, West Sussex, UK) to quantify subcutaneous fat thickness according to the ISAK level 1 protocol which includes eight skinfolds (triceps, subscapular, biceps, iliac crest, supraspinale, mid-abdominal, front thigh, and medial calf sites; Stewart et al., 2011). Measurements were made in duplicate, with the mean value reported if within 5% variation. In the case of greater than 5% variation between measures, a third measurement was taken, and the median measure was reported.

**Blood parameters.** Venous blood samples were obtained by venepuncture from the antecubital vein. Samples were centrifuged, then serum separated and stored at −80°C for later analysis at a NATA-accredited hospital laboratory. Testosterone, sex hormone binding globulin, and cortisol were measured using a competitive electrochemiluminescence immunoassay on a Cobas 8000 analyser (Roche, Manheim, Germany). Free testosterone was calculated using the measured testosterone and sex hormone binding globulin values. Insulin-like growth factor-1 (IGF-1) was measured using a sandwich chemiluminescence immunoassay on a Liaison XL analyser (DiaSorin, Italy). Leptin and adiponectin were analysed by commercially available radioimmunoassay kits (EMD Millipore, Billerica, MA, USA). Insulin was analysed by chemiluminescent microparticle immunoassay using an Architect System (Abbott Laboratories, Abbott Park, IL, USA). Blood lipids were analysed by an enzymatic colorimetric assay on a Cobas 8000 analyser (Roche).

**Diet and exercise.** Seven-day weighed food and training diaries were completed at each time point. Participants documented all food, fluid, and supplements consumed during the 7-day period. All resistance and aerobic exercises were documented in the training diary. Food diaries were analysed using the FoodWorks program (Version 8; Xyris Software, Brisbane, Australia) and included analysis of reported dietary supplement consumption. Macronutrient intake distribution was calculated as reported elsewhere (MacKenzie, Slater, King, & Byrne, 2015). Resistance training volume (repetitions×weight×sets) was determined for the total body, upper body (exercises using predominantly...
upper body muscles), and lower body (exercises using predominantly lower body muscles).

Analysis

Means and standard deviations were calculated for all test parameters. Normality of data was assessed using the Shapiro–Wilk test. Independent-samples t-tests were performed to test for differences between participants who commenced their in-season diet prior to baseline testing, and those who had not. For normally distributed data, repeated-measures analysis of variance were performed to test for changes between time points, with Greenhouse–Geisser corrections used when the assumption of sphericity was violated. Where significant change was detected, post hoc pairwise comparisons with Bonferroni correction were performed. Where data were not normally distributed, Friedman analyses of variance by ranks were run, and Wilcoxon sign-rank test with Bonferroni correction was performed where significant differences were detected. Relative effect sizes (Cohen’s $d$) were calculated for all significant findings using the following formula: \[ \frac{\text{mean value}_1 - \text{mean value}_2}{\text{pooled } SD} \]. Effect sizes were considered small (0.2), medium (0.5), or large (0.8) (Cohen, 1992). Missing data were imputed using the last result carried forward method. Analyses were conducted using IBM SPSS statistics version 22 (IBM SPSS; Chicago, IL, USA). Significance was set at $P < .05$.

Results

Eleven bodybuilders consented to participate in the study. Two withdrew after baseline testing due to withdrawal from competition, with the remaining nine (29.0 ± 9.5 years, 177.9 ± 2.5 cm, 83.7 ± 8.9 kg, 6.0 ± 6.6 years bodybuilding participation) included in analyses. Results are displayed with zero values include contributions from supplements. There were no significant differences in energy intake across measurement points ($P = .071$). No significant changes in total (g d$^{-1}$) or relative (g kg$^{-1}$) protein intake were detected ($P = .506$ and $P = .625$, respectively). There were no significant differences in carbohydrate or fat intake during pre-competition; however, significant differences were detected between PRE8 and POST4 time points for total ($P = .035$, $d = -0.8$) and relative ($P = .032$, $d = -0.8$) carbohydrate values.

Energy and macronutrient distribution results are presented in Table I. Throughout in-season testing, participants consumed 5.2 ± 1 meals d$^{-1}$. Across all participants and meals consumed during testing, 81.3 ± 19.8% of meals were above the 0.25 g kg$^{-1}$ of protein threshold (Moore et al., 2015). Dietary supplements were used during the pre- ($n = 7$) and post-competition ($n = 8$) periods. Dietary supplement contribution to total daily intake is presented in Table I. The most commonly used dietary supplements were whey protein ($n = 7$), creatine ($n = 5$), branched chain amino acids ($n = 4$), and glutamine ($n = 3$).

Four participants reported implementing a “re-feed” day or meal during the PRE16 andPRE8 testing weeks. On these days, there was a 46 ± 21% increase in energy, a 114 ± 41% increase in carbohydrate, and a 63 ± 66% increase in fat, while protein was reduced by 4 ± 11%.

Reported training volumes are presented in Table I. No significant differences in resistance training volume were found between testing points ($P > .10$). A significant difference in aerobic training volume was found ($P = .01$); however, post hoc analysis with Bonferroni correction failed to reach significance ($P > .10$).

Body composition

Body composition results are presented in Table II and Figure 1. On average, 85 ± 38% of mass lost during pre-competition testing was FM (range 29–136%). Medium, large, and small reductions in subcutaneous adiposity estimated by anthropometry occurred between PRE16 and PRE8, PRE16 and PRE1, and PRE8 and PRE1 ($P = .018$, $d = 0.5$; $P = .004$, $d = 0.9$; $P = .01$, $d = 0.4$, respectively). No
significantly changes were found for TBW, ECF, or ICF ($P > .1$). There were no differences in FM, LM, percentage change in FM or LM, or proportion of mass lost as FM, between participants who commenced their in-season diet before versus during or after PRE16 ($P > .05$).

**Resting metabolic rate**

No significant changes in RMR were detected across the study period when assessed absolute ($P = .87$) or relative to LM ($P = .91$; Table II, Figure 1). No differences were found for RMR or percentage change in RMR between participants who commenced their in-season diet before versus during or after PRE16 ($P > .05$).

**Blood parameters**

Blood parameter results are presented in Table II and Figure 2. Five, four, and one participant dropped below reference ranges for serum testosterone, free testosterone, and IGF-1 concentrations during pre-competition testing, respectively. No differences were found in blood parameters or percentage change in blood parameters between participants who commenced their in-season diet before versus during or after PRE16 ($P > .05$).

**Discussion**

This prospective study aimed to describe body composition and physiological changes in male, natural bodybuilders during competition preparation and recovery. We hypothesised large reductions in FM, with concomitant reductions in LM, RMR, and anabolic hormones during the in-season period. Bodybuilders in this study lost significant amounts of FM, with only small losses in LM, and no change in RMR. During the 4 months of pre-competition measurement, all participants reduced FM to low levels, in some cases to the lower limits of human FM (Friedl et al., 1994). There was a large variability in the proportion of body mass lost as fat, although the average ratio was high (85 ± 38%, range 31–136%). Despite these body composition changes, RMR remained unchanged throughout the competition preparation period, while serum testosterone and IGF-1 concentrations were significantly reduced.

These findings are valuable, given the paucity of longitudinal research in natural bodybuilders.

**Body composition**

As hypothesised, there were significant reductions in FM measured via DXA (mean reduction = 3.5 kg). Similarly, a moderate reduction in the sum of eight skinfolds occurred (mean reduction = 10.7 mm).
The FM loss documented in this study was small relative to those previously reported, likely resulting from the shorter assessment period. In case reports, natural bodybuilders have been shown to lose up to 10.4 kg of FM during competition preparation (Kistler et al., 2014; Robinson et al., 2015; Rossow et al., 2013). The bodybuilders in our study were at a moderately low FM at PRE16, which may also account for the smaller reductions (8.8 ± 3.1 kg compared with 11.7–15.9 kg in case studies). Furthermore, four participants had commenced their in-season dieting at PRE16 which would in part explain the low initial FM and smaller reduction in FM.

A common and undesired side-effect of prolonged energy restriction is a loss of LM. This is particularly evident in lean individuals, including natural bodybuilders (Kistler et al., 2014; Robinson et al., 2015). Indeed, among lean individuals in an energy deficit, the ratio of lean to total mass lost typically increases (Heymsfield, Gonzalez, Shen, Redman, & Thomas, 2014). However, the bodybuilders in this cohort were mostly successful at maintaining LM. Fat loss accounted for 85% of total mass lost, although this varied widely between participants (range 29–136%). There were no statistical changes in LM seen between PRE16 and PRE8, and only a small reduction between PRE8 and PRE1 (mean difference = 0.9 kg, $d = 0.1$). Reductions in LM in the previously cited natural bodybuilder case studies ranged from 2.8 to 6.6 kg (Kistler et al., 2014; Robinson et al., 2015; Rossow et al., 2013). The success of the bodybuilders in our study in maintaining LM may be attributed to a small energy deficit used throughout the in-season. A smaller energy deficit during a period of weight reduction has been demonstrated as an effective mechanism for maintaining LM (Garthe, Raastad, Refsnes, Koivisto, & Sundgot-Borgen, 2011). The maintenance of LM is even more significant considering the low FM observed at PRE16, given previous research demonstrates leaner individuals lose a proportionately greater amount of LM during an energy deficit (Friedl et al., 1994).

### Table II. Body composition, RMR, and blood parameters during competition preparation and recovery.

<table>
<thead>
<tr>
<th>Reference range</th>
<th>PRE16</th>
<th>PRE8</th>
<th>PRE1</th>
<th>POST4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXA Total mass (kg)</td>
<td>83.7 ± 8.9</td>
<td>81.8 ± 9.1</td>
<td>79.6 ± 9.0</td>
<td>83.0 ± 7.7</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>8.8 ± 3.1</td>
<td>6.6 ± 2.4</td>
<td>5.3 ± 2.4</td>
<td>7.1 ± 3.0</td>
</tr>
<tr>
<td>LM (kg)</td>
<td>74.2 ± 8.9</td>
<td>71.8 ± 9.1</td>
<td>71.2 ± 9.1</td>
<td>72.5 ± 8.5</td>
</tr>
<tr>
<td>BIA TBW (L)</td>
<td>54.3 ± 6.9</td>
<td>54.6 ± 7.0</td>
<td>53.7 ± 6.7</td>
<td>54.8 ± 6.3</td>
</tr>
<tr>
<td>ECF (L)</td>
<td>21.4 ± 2.5</td>
<td>21.4 ± 2.8</td>
<td>21.0 ± 2.5</td>
<td>21.7 ± 2.3</td>
</tr>
<tr>
<td>ICF (L)</td>
<td>32.9 ± 4.5</td>
<td>33.2 ± 4.4</td>
<td>32.7 ± 4.3</td>
<td>33.1 ± 4.2</td>
</tr>
<tr>
<td>Skinfolds Sum of 8 sites (mm)</td>
<td>47.7 ± 12.7</td>
<td>42.0 ± 11.4</td>
<td>37.3 ± 11.1</td>
<td>43.3 ± 18.8</td>
</tr>
<tr>
<td>RMR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kJ d$^{-1}$</td>
<td>10,036.3 ± 1592.0</td>
<td>9706.4 ± 1728.4</td>
<td>9805.1 ± 1800.6</td>
<td>10,160.0 ± 1313.8</td>
</tr>
<tr>
<td>kJ kg$^{-1}$ d$^{-1}$</td>
<td>120.4 ± 18.7</td>
<td>119.5 ± 23.6</td>
<td>123.5 ± 19.1</td>
<td>123.1 ± 19.0</td>
</tr>
<tr>
<td>kJ kg$^{-1}$ LM$^{-1}$ d$^{-1}$</td>
<td>141.2 ± 20.2</td>
<td>136.2 ± 25.0</td>
<td>139.2 ± 22.4</td>
<td>141.5 ± 21.3</td>
</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (nmol L$^{-1}$)</td>
<td>10.0–30.0</td>
<td>16.4 ± 4.4</td>
<td>11.5 ± 5.3</td>
<td>10.1 ± 3.6</td>
</tr>
<tr>
<td>Free testosterone (pmol L$^{-1}$)</td>
<td>80–370</td>
<td>229.3 ± 72.4</td>
<td>153.9 ± 85.4</td>
<td>116.8 ± 76.9</td>
</tr>
<tr>
<td>IGF-1 (nmol L$^{-1}$)</td>
<td>14.2–58.8</td>
<td>27.0 ± 7.7</td>
<td>23.4 ± 7.4</td>
<td>19.9 ± 7.6</td>
</tr>
<tr>
<td>Cortisol (nmol L$^{-1}$)</td>
<td>170–500</td>
<td>358.0 ± 107.8</td>
<td>328.7 ± 71.7</td>
<td>364.8 ± 74.0</td>
</tr>
<tr>
<td>Insulin (pmol L$^{-1}$)</td>
<td>10–96</td>
<td>24.1 ± 7.4</td>
<td>20.7 ± 5.5</td>
<td>18.0 ± 7.0</td>
</tr>
<tr>
<td>Leptin (ng mL$^{-1}$)</td>
<td>2.0–5.6</td>
<td>2.8 ± 1.9</td>
<td>2.8 ± 1.6</td>
<td>3.2 ± 2.0</td>
</tr>
<tr>
<td>Adiponectin (µg mL$^{-1}$)</td>
<td>3.0–30.0</td>
<td>13.8 ± 5.0</td>
<td>14.3 ± 4.6</td>
<td>19.0 ± 12.6</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mmol L$^{-1}$)</td>
<td>≤ 5.2</td>
<td>4.0 ± 0.8</td>
<td>3.9 ± 0.8</td>
<td>4.0 ± 0.9</td>
</tr>
<tr>
<td>HDL (mmol L$^{-1}$)</td>
<td>1.0–2.5</td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>LDL (mmol L$^{-1}$)</td>
<td>≤ 3.5</td>
<td>2.2 ± 0.6</td>
<td>2.2 ± 0.9</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>Triglycerides (mmol L$^{-1}$)</td>
<td>≤ 2.5</td>
<td>0.7 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

Notes Mean ± SD for all values. Total mass, FM, LM measured by DXA; TBW, ECF and ICF measured by BIA. RMR presented as total and relative (total mass, LM).

*Significantly different to PRE16.

**Significantly different to PRE8.

†Significantly different to PRE1.

\* $P < .05$; \*\* $P < .01$.

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
A second possible explanation for the LM maintenance is the high dietary protein intake. A higher protein intake has been demonstrated as an effective mechanism for limiting LM loss during energy restriction in resistance-trained individuals (Mettler, Mitchell, & Tipton, 2010). In athletes, to optimise the ratio of LM to FM loss during an energy deficit, a protein intake of 1.8–2.7 g kg\(^{-1}\) d\(^{-1}\) has been suggested (Phillips & Van Loon, 2011). In already lean individuals, a protein intake dependent on fat-free mass has been proposed: 2.3–3.1 g kg\(^{-1}\) fat-free mass\(^{-1}\) d\(^{-1}\) may be effective in achieving LM maintenance during an energy deficit (Helms, Zinn, Rowlands, & Brown, 2014). Throughout this study, participants consumed 2.8–3.1 g kg\(^{-1}\) d\(^{-1}\), and 3.3–3.6 g kg LM\(^{-1}\) day\(^{-1}\), thus met or exceeded these recommendations. This high-protein intake and smaller overall energy deficit would help negate physiological adaptations associated with weight loss which drive a reduction in LM.

In conjunction with an increased total protein intake, distribution of protein is reported to be an effective means of maximising muscle protein synthesis (Areta et al., 2013). Participants in this study ate 5.2 ± 1 meals d\(^{-1}\), with 81.3 ± 19.8% of meals surpassing the 0.25 g kg\(^{-1}\) dose recommended (Moore et al., 2015), facilitating conditions for building and maintaining LM, despite remaining in negative energy balance. The inclusion of a high-protein post-exercise meal would also assist in increasing muscle protein synthesis (Burd, Tang, Moore, & Phillips, 2009).

Regular high-intensity resistance training would aid in attenuation of LM reduction in these bodybuilders. Study participants maintained a high volume of resistance training (Table I). The muscle protein synthesis response to protein is reduced during an energy deficit. However, resistance exercise during the energy deficit has been demonstrated to stimulate protein synthesis to rates similar to those during energy balance (Areta et al., 2014). This uninhibited muscle protein synthesis response to protein ingestion associated with resistance training would counter the catabolic effects of a negative energy balance, and hence assist in the maintenance of LM.

### Resting metabolic rate

Reductions in RMR are typically seen during periods of energy restriction and weight loss (Schwartz &
Doucet, 2010), which is attributed to changes in LM and FM. Our results showed no change in RMR during the pre-competition period (mean difference 231 kJ d$^{-1}$). This result contrasts those found in previous bodybuilder case studies, where small (752 kJ d$^{-1}$) and large reductions (4746 kJ d$^{-1}$) have been reported (Robinson et al., 2015; Rossow et al., 2013). Maintenance of RMR in the current study is likely attributable to the very small reductions in LM observed, and the high-intensity resistance training performed throughout the pre-competition period (Bryner et al., 1999). Resistance training during a period of negative energy balance has been shown to alleviate reductions in 24-h resting energy expenditure (Bryner et al., 1999). By maintaining LM, and subsequently resting energy expenditure, the bodybuilders in this study required smaller reductions in energy intake to maintain an overall negative energy balance. This smaller energy deficit would result in continued FM reductions, while limiting reductions in LM and subsequently RMR, thereby producing a positive feedback cycle allowing the achievement of body composition modification.

**Blood parameters**

Circulating anabolic hormone concentrations are sensitive to energy status. Periods of short-term energy deficit may produce acute reductions in testosterone, which are accentuated when the energy deficit is prolonged (Friedl et al., 2000; Henning, Margolis, McClung, Young, & Pasiakos, 2014). This anti-anabolic response aids in reducing protein synthesis and energy expenditure (Mauras et al., 1998) and may correspond with a loss of LM (Friedl et al., 2000). During the pre-competition period, total and free testosterone reduced by 38% and 49%, respectively, while IGF-1 reduced by 26%. These reductions compare to the reduction in testosterone measured during a 6-month competition preparation of a male bodybuilder (75% reduction; Rossow et al., 2013), while a 15% mean reduction in testosterone was found in seven male bodybuilders during the final 11 weeks of competition preparation (Maestu et al., 2010).

The hormonal response to energy restriction is likely attributable to low energy availability (Dolan et al., 2012). Similar reductions in serum

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**Figure 2. Serum hormone changes.** Enclosed dots indicate individual data; bars indicate mean. Effect sizes indicate changes in mean. $d$ indicates the effect size between time points.
testosterone to those found in this study are evident in competitive jockeys, who undertake periods of energy restriction resulting in low energy availability in order to make weight (Dolan et al., 2011). As no significant reductions in energy intake were found, and exercise energy expenditure was unable to be accurately evaluated, low energy availability cannot be confirmed in this study.

Despite reductions in anabolic hormone concentrations, bodybuilders in this study were still able to prevent large losses of LM, indicating that the LM response to a continual energy deficit was not associated with changes in testosterone or IGF-1 concentrations. It also suggests that the high-protein intake and resistance training programme employed by participants was sufficient to counteract the anti-anabolic effects of these hormonal changes.

The rapid return to baseline values for testosterone and IGF-1 concentrations post-competition is also of significance. This may reflect energy deficit cessation. One case study has examined hormonal changes after a bodybuilding competition, finding testosterone increased to 94% of baseline concentrations after 3 months of increased energy intake (Rossow et al., 2013). A similar restoration of testosterone concentration was found among army rangers during 2–6 weeks of recovery from an 8-week period of high energy expenditure and low energy intake (Henning, Scofield, et al., 2014). The rapid increase of anabolic hormone concentrations after competition observed in our study suggests there may be no significant physiological detriment associated with a short-term reduction in anabolic hormones when protein intake and resistance training are maintained.

Limitations of this study include a modest sample size (n = 9) which requires consideration when interpreting the non-significant findings. A 12-h exercise-free period in preparation for testing was implemented, due to the high-frequency exercise regimen employed by the participants. This limited time frame relative to current guidance (Compher et al., 2006) may have inflated RMR results, as metabolic rate may remain elevated for up to 48 h following resistance exercise (Compher et al., 2006). Additionally, a face mask was used to collect expired gas for RMR assessment, rather than a ventilated hood, although this may not significantly affect the results (Compher et al., 2006). The lack of statistically significant change in dietary intake during pre-competition testing may be attributed to the testing timeline. Strategies used by participants during the PRE1 testing week incorporate an increased carbohydrate and hence energy intake. Rather than observing a decrease in energy intake between PRE8 and PRE1 as predicted, a small, insignificant increase was observed. One may speculate that a modified testing timeline, including testing 2 weeks before the contest, would observe significant reductions in energy, carbohydrate, and fat intake compared to PRE16 values. More frequent testing, for example every 2–4 weeks leading to competition as used in previous case studies (Kistler et al., 2014), may allow closer observation of changes. Several participants in this study had commenced in-season dieting before PRE16; therefore, this time point does not reflect a true off-season status in these participants, thus changes observed may not encompass total changes typically occurring from off-season to competition.

**Conclusion**

These bodybuilders demonstrated significant reductions in FM with only small reductions in LM. We suggest that the maintenance of resistance training volume and an evenly distributed, high-protein intake during the competition preparation may have provided a stimulus to maintain LM while reducing FM. A subsequent outcome of maintaining LM was maintenance of RMR, likely enabling participants to continue with only small reductions in energy intake. Assessing the effect of preparation strategies employed by these bodybuilders in other athlete populations may help identify recommendations that assist in modification of body composition.

**Disclosure statement**

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