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ABSTRACT

Purpose: To evaluate the effects of low energy availability (EA) on health and performance indices associated with the Male Athlete Triad and Relative Energy Deficiency in Sports (RED-S) models. **Methods:** Over an 8 week period, a male combat sport athlete adhered to a phased body mass (BM) loss plan consisting of 7 weeks energy intake (EI) equating to resting metabolic rate (RMR) (1700 kcal·day^{-1}) (Phase 1), 5 days of reduced EI (1200-300 kcal·day^{-1}) prior to weigh-in (Phase 2) and one week of ad libitum EI post-competition (Phase 3). EA fluctuated day by day due to variations in exercise energy expenditure. Regular assessments of body composition, RMR, cardiac function, cardiorespiratory capacity, strength & power, psychological state and blood clinical chemistry for endocrine, bone turnover, hydration, electrolyte, renal, liver and lipid profiles were performed. **Results:** BM was reduced over the 8 week period by 13.5% (72.5 to 62.7 kg). No consequences of Male Athlete Triad or RED-S were evident during phase 1, where mean daily EA equated to 20 kcal·kg·FFM·day^{-1} (range: 7 to 31 kcal·kg·FFM·day^{-1}) and BM and fat mass (FM) losses were 6.5 and 4.4 kg, respectively. However, consequences did present in phase 2 when mean daily EA was consistently <10 kcal·kg·FFM·day^{-1}, as evidenced by alterations to endocrine hormones (e.g. testosterone: <5 nmol.L^{-1}) and reduced RMR (-257 kcal·day^{-1}). **Conclusion:** Data demonstrate that 7 weeks of daily fluctuations in EA equating to a mean value of 20 kcal·kg·FFM·day^{-1} permits reductions of BM and FM without perturbations to physiological systems associated with the Male Athlete Triad and RED-S. In contrast, a subsequent period of 5 consecutive days of EA <10 kcal·kg·FFM·day^{-1} induced consequences of Male Athlete Triad and RED-S. **Key Words:** MALE ATHLETE TRIAD, RELATIVE ENERGY DEFICIENCY IN SPORTS, WITHIN DAY ENERGY BALANCE, REBOUND HYPERPHAGIA

INTRODUCTION

Combat sport athletes compete within designated weight categories that are intended to promote fair competition by matching opponents of equal stature and body mass (BM) (1). In an attempt to qualify for lowest weight category possible (often referred to as "making weight"), these athletes typically engage in practices associated with acute and chronic BM loss, many of which are detrimental to health and performance (2). In the acute phase (i.e. within hours to days prior to official weigh in), combat sport athletes often employ combinations of active and passive dehydration techniques, where 5-10% reductions in BM have been observed (3). These practices may cause acute kidney injury and in extreme situations, can even lead to death (4, 5). As such, there is a definitive need for these athletes to adopt a more gradual approach to making weight, where BM is manipulated across longer timescales (i.e. weeks to months), therefore reducing the requirement to engage in methods of extreme dehydration.

Combat sport athletes often complete a training camp prior to competition, ranging in duration from 4 to 12 weeks. Such timescales allow for the opportunity to reduce BM through an accumulated energy deficit, though it has been reported that these athletes may resort to dietary practices limiting energy intake (EI) to only one meal per day (6). These types of feeding strategies are likely to result in low energy availability (EA), thereby manifesting as consequences associated with the Male Athlete Triad or Relative Energy Deficiency in Sports (RED-S) models (7, 8). In the Triad model, low EA is associated with a conservation of energy metabolism (e.g. via hypothalamic pituitary thyroid axis disturbance, reductions in insulin-like growth factor-1, leptin), suppression of reproductive function (e.g. via hypothalamic pituitary gonadal axis disturbance, poor semen quality) and impaired bone health (9). In the RED-S

model, these consequences are purported to further extend to impairments of metabolic rate, immunity, protein synthesis and cardiovascular function, all of which can be causative of reduced health and performance outcomes (10). Laboratory studies on females have demonstrated that some of these systems are perturbed at a daily EA <30 kcal·kgFFM·day^{-1}, yet it is not yet clear whether the same threshold applies for males (11, 12). Furthermore, whilst experimental modulation of daily low EA often involves a consistent absolute value, it is possible that fluctuations in daily low EA status (i.e. via manipulation of EI and/or exercise energy expenditure) may reduce the risk of developing consequences associated with the Male Athlete Triad and RED-S (6, 13). This approach may be particularly beneficial in male athletes given that impairments in the hypothalamic pituitary gonadal axis can be restored within days of increased energy availability (14).

On this basis, the aim of the present case report was to evaluate the effects of incorporating daily fluctuations in low EA on health and performance indices associated with the Male Athlete Triad and RED-S models. To this end, we monitored a male combat sport athlete for an eight week period whilst making weight for competition and for a one week recovery period post competition. Importantly, we performed regular assessments of body composition, resting metabolic rate, cardiac function, maximal dynamic strength & power, cardiorespiratory capacity, psychological state and blood clinical chemistry to assess for biomarkers related to endocrine, bone turnover, hydration, electrolyte, renal, liver and lipid profiles.

METHODS

Athlete Overview and Case Report Design

The athlete (age: 19 years; stature: 1.66 metres; BM: 72.5 kg; Body Mass Index (BMI): 26.3 $kg \cdot m^{-2}$) was a male international standard (>5 years' experience) Taekwondo competitor, who typically competed 10 times per year in the <68 kg feather weight category, habitually losing 4-5 kg across periods of 3-4 weeks prior to competition. For the present case report, the athlete elected to further reduce his BM, to compete in the <63 kg bantam weight category at the 2018 British University Championships, therefore requiring a BM loss of >9.5 kg (>13%) over a period of 8 weeks. The implementation of an intervention to achieve this aim, as well as the assessment of health and performance consequences associated with the Male Athlete Triad and RED-S (15), were completed via a multi methodological approach, as described in the subsequent sections and highlighted in the supplemental content (see Figure, Supplemental Digital Content 1, Diagram of measurements taken throughout phases 1 & 2 intervention and phase 3 post competitive recovery periods, http://links.lww.com/MSS/C148). Measurements were taken daily and also at set intervals inclusive of 8 weeks, 4 weeks and 1 week prior to competition (-8 WK, -4 WK and -1 WK) as well as the day before the official competition weigh in (-1 D), the official competition weigh in day (WI), 24 hours post competition (+1 D) and 1 week post competition (+1 WK). The athlete gave written informed consent and this case report was approved by the Liverpool John Moores University research ethics committee.

Anthropometric Assessment

Daily body mass (BM) was measured post void of the bladder/bowels between 9.00 – 9.30 hours after a 12 hour fast and determined to the nearest 0.01 kg on a calibrated digital scale, with

measures of stature established to the nearest 0.1 cm using a free standing stadiometer (Seca 702 & 123; Seca GmbH, Hamburg, Germany). Body composition was assessed via Dual X-ray Absorptiometry (DXA- QDR Series Discovery A; Hologic Inc., Bedford, MA, USA - software version 12:4:3) to generate fat free mass (FFM), fat mass (FM), percentage body fat (BF%), total body (minus head) and lumbar spine bone mineral content (BMC) and density (BMD) Z-scores, alongside anthropometric sum of 8 skinfolds ($\sum_{8SKf}$ – Harpenden, Baty Int., West Sussex, Great Britain). Measurements were collected according to the DXA Best Practice Protocol (16), BMD and BMC as previously described (17) and $\sum_{8SKf}$ adhering to the guidelines of the International Society for the Advancement of Kinanthropometry. Laboratory technical error of measurement (TEM) and coefficient of variation (CV) for DXA derived measures of FFM, FM and BF% are 0.44 kg:1.0%, 0.37 kg:1.9%, 0.41:1.9%, respectively and for BMD measures CV is <1.5%.

Resting Metabolic Rate (RMR) and RMR_{ratio}

Measured RMR (RMR_{meas}) was assessed utilising indirect calorimetry (GEM Open Circuit Indirect Calorimeter; GEMNutrition Ltd., Warrington, UK), calibrated via known concentrations of O_2/CO_2, a zero span gas and an ethanol burn, to confirm an established respiratory exchange ratio (RER) of 0.67. Laboratory based TEM and CV for this system is 42 kcal·day^{-1} and <2%, respectively. Application of measurement and subsequent analyses were conducted according to the recommendations of Bone and colleagues (18) and predicted RMR (RMR_{pred}) was calculated via the Cunningham equation (19). RMR_{ratio} was established by the division of RMR_{meas} and RMR_{pred}, where a values of <0.90 were classified to define instances of potential energy deficiency (20). RMR_{meas} values below those of RMR_{pred} were also used to calculate instances of adaptive thermogenesis (21).

Blood Clinical Chemistry and Hydration Status

All blood samples were drawn from the antecubital vein of the left arm, which were collected and stored as previously described (22). Measures of endocrine, bone turnover, electrolyte, renal, liver and lipid based biomarkers were conducted via immunoassay with chemiluminescence detection on Roche Cobas e601/602 and c701/702 modular analysers, utilising Rate A, 1 and 2 point end assays and potentiometry ion selective electrode on a Roche Cobas ISE analyser (Roche Diagnostics Ltd. Burgess Hill, Great Britain). Plasma (P_{osm}) osmolality was examined to establish hydration status via freezing point depression (Advanced Micro-Osmometer 3320, Advanced Instruments, Norwood Massachusetts, USA) as previously described (23). Laboratory individual inter/intra assay CV and sensitivity (replicates of the zero standard) for all biomarkers are described in the supplemental content (see Table, Supplemental Digital Content 2, CV%, range and sensitivity of measurement for all assessed blood clinical chemistry biomarkers, http://links.lww.com/MSS/C149). Measures for bone formation and reabsorption were divided to calculate a ratio, with values above or below one being indicative of positive or negative bone turnover responses, respectively.

Cardiac Screening

Cardiac scanning and subsequent analyses were conducted by a registered clinical cardiac physiologist prior to exercise testing via 12-lead electrocardiogram (ECG) and echocardiography (24). Indices of heart rate (HR), cardiac output (CO) and deformation of the left and right ventricles were established through alterations in the structure of the left ventricular end-diastolic volume (LVEDV) and right ventricular diastolic area (RVD_{Area}), in parallel to function from left

ventricular ejection fraction (LVEF) and right ventricular fractional area change (RVFAC). Intra measurement CV for all assessments was <15%.

Cardiorespiratory Capacity and Substrate Utilisation Assessment

Combined peak oxygen uptake ($\dot{V}O_{2peak}$) and fat oxidation rate (FAT_{peak}) were measured via an online indirect calorimetry system (CPX Ultima Series; Medgraphics, Saint Paul, MN, USA) during an incremental exercise test performed on a motorized treadmill (h/p/cosmos Pulsar; h/p/cosmos Sports & Medical gmbh, Nussdorf-Trainstein, Germany) The test began with 3 minute stages at speeds of 6 to 11 $km \cdot hr^{-1}$, followed by 2 minute stages at 12, 14 and 16 $km \cdot hr^{-1}$. The treadmill was then inclined by 1 degree each minute, until volitional exhaustion despite strong verbal encouragement. Raw $\dot{V}O_2$, $\dot{V}CO_2$ ($L.min^{-1}$) and RER data were then averaged and converted into kilocalories (kcal) (13) and FAT_{peak} was calculated as the highest fat oxidation rate, utilising the following equation: $g \cdot min^{-1} = (1.695 \times \dot{V}O_2) - (1.701 \times \dot{V}CO_2)$ (25). Laboratory test–retest reliability of this system using 95% limits of agreement is $0.29 \pm 2.4\ mL \cdot kg^{-1} \cdot min^{-1}$. All tests took place in the same order as presented, under standard laboratory conditions (room temperature 20.0 ± 1.5°C; humidity 38.5 ± 4.0%; barometric pressure 750.2 ± 6.5mmHg) and were performed after a 12 hour fast between 9.00-11.00 hours.

Maximal Dynamic Strength and Power Assessment

Maximal Dynamic Strength (MDS) was assessed via one repetition maximum (1 RM) upper and lower bench press and back squat exercises. The athlete performed a readiness set of 10 repetitions with a 20 kg barbell, then 10 repetitions at 50%, 5 repetitions at 75% and 1-2 repetitions at 90% of predicted 1 RM. All subsequent 1 RM attempts were interspersed with 3

minutes recovery and the load was exponentially increased until failure occurred. Maximal Dynamic Power (MDP) was examined via the same exercises for both upper and lower force velocity profile as previously described (26) by performing 3 maximal attempts at 20, 40, 60, and 80% of measured 1 RM load. Concentric average power (W), force (N) and velocity ($m \cdot s^{-1}$) were recorded by a linear encoder (MuscleLab version 4010, Ergotest, Porsgrunn, Norway – software version 8.31) mounted perpendicular to the line of exercise movement. MDS tests were performed on the same day as the anthropometric and physiological assessments between 17.00-18.00 hours, whereas MDP tests were performed at the same time on the following day. Both assessments were administered under standard gymnasium conditions (room temperature 21.0 ± 1.5°C; humidity 40.5 ± 5.0%; barometric pressure 755.5 ± 3.5mmHg) after a minimum period of 3 hours postprandial feeding.

Psychological Profile

Psychological profile was assessed by both a Profile of Moods States (POMS) (27) and semi structured interviews. The POMS vigour subscale was subtracted from the other combined subscales to generate a total mood disturbance (TMD) score. Semi structured interviews and subsequent questions were generated, conducted and analysed in the same manner as previous investigations in combat sport athletes (28) in order to reflect the athlete's perceptions, thoughts, attitudes, emotions and behaviours throughout each time phase.

Overview of Nutritional and Training Intervention

Phase 1 -8 WK to -1 WK: All meals were provided for the athlete in pre-prepared packages with known energy and macronutrient contents equivalent to RMR_{meas} (1700 $kcal \cdot day^{-1}$ – see

Table 1) and consisting of 3.4 g·kgFFM^{-1} carbohydrate (CHO) (748 kcal·day^{-1}), 2.3 g·kgFFM^{-1} protein (506 kcal·day^{-1}) and 0.9 g·kgFFM^{-1} fat (446 kcal·day^{-1}). Subsequent feedings followed a typical daily distribution of four meals periodised in line with daily training sessions (see Table 2). The athlete's weekly training schedule consisted of three aerobic continuous running (CR), two high-intensity interval training (HIT), two resistance training (RT), three technical/tactical and one sparring based sessions totalling 12-15 hours·wk^{-1}. Aerobic CR was conducted after a 12 hour fasting period, for 45-60 minutes at a running speed equating to FAT_{peak}. HIT was completed on a motorized treadmill in two differing activity:recovery ratios consisting of 10 x 1:1 minutes at 120% $\dot{V}O_{2peak}$ and 6 x 3:1 minutes at 90% of $\dot{V}O_{2peak}$, where the recovery period in both protocols was 1 minute of walking. RT was structured into whole body bi/unilateral general strength/speed exercises, concurrently performed in superset, with speed/strength, ballistic and reactive strength modalities, where volume loads and intensities were established from MDS 1 RM testing.

Phase 2 -1 WK to WI: Daily EI was reduced exponentially until WI (see Table 1), which consisted of low fibre, residue and sodium based foods. At -5 and -4 D, macronutrient intake equated to 0.5 g·kgFFM^{-1} CHO (110 kcal·day^{-1}), 1.8 g·kgFFM^{-1} protein (385 kcal·day^{-1}), 0.9 g·kgFFM^{-1} fat (420 kcal·day^{-1}) and at -3 and -2 D, 0.3 g·kgFFM^{-1} CHO (67 kcal·day^{-1}), 1.8 g·kgFFM^{-1} protein (400 kcal·day^{-1}) and 0.8 g·kgFFM^{-1} fat (396 kcal·day^{-1}), respectively. Finally, at -1 D the athlete ate only one meal containing 1.4 g·kgFFM^{-1} protein (300 kcal·day^{-1}). All meal timings corresponded to a periodised taper of training volume, consisting of aerobic CR and sport specific technical/tactical non-contact sessions only. From 12.00 hours on -1 D until the official competition weigh in at 13.00 hours, the athlete refrained from ingesting any food or

fluids. During this time period the athlete also engaged in self-directed active and passive dehydration techniques, inclusive of a 1 hour sauna session at 18.00 hours and a CR at 20.00 hours on -1 D, then a final 1 hour sauna session at 09.00 hours on WI.

Phase 3 WI to + 1 WK: In the post competitive period, the athlete consumed food and fluids ad libitum and reported these via the remote food photography method (29). This data was assessed utilising dietary analysis software (Nutritics V5. Nutritics Ltd., Swords, Co. Dublin, Ireland) as previously described (30), to establish respective energy and macronutrient values and with systematic bias of measurements via independent t-tests highlighting no significant differences ($p < 0.05$). This resulted in varying daily EI intakes (see Table 1) with macronutrient contents equating to averages of 7.9 $g \cdot kgFFM^{-1}$ CHO, 2.8 $g \cdot kgFFM^{-1}$ protein and 3.1 $g \cdot kgFFM^{-1}$ fat, respectively. Other than the competition day, exercise was completely ceased during this time phase. No dietary supplements were implemented or allowed throughout any time phase, to limit potential ergogenic effects on subsequent performance based testing. Fluid consumption throughout all time phases equated to an average of 2 $L \cdot day^{-1}$ and on -1 D was reduced to 500 ml until 12.00 hours.

Assessment of Daily Wellbeing, Training Load, Energy Availability, Within Day Energy Balance and Weekly Accumulated Energy Deficit

The athlete was requested to report perception of wellness via a Daily Well Being Score (DWBS) (31). Training load was assessed internally via the session rating of perceived exertion (s-RPE) method (32) and externally via HR chest strap (Polar V800; Polar Electro UK Ltd., Warwick, Great Britain), with specific profiles and zones created for each type of training

modality, based on percentages of $\dot{V}O2_{peak}$. The HR wristwatch tri-axial accelerometer unit also acted as an activity monitor and was worn continuously for estimations of non exercise activity thermogenesis (NEAT). Estimated exercise energy expenditure (EEE) was assessed via an Actiheart combined HR and accelerometry actigraphy unit (CamNtech Ltd. Papworth, Great Britain) as previously described (33). Within day energy balance (WDEB) was calculated to examine the total daily fluctuations in 24 hour energy balance, utilising the methodology of Torstveit and colleagues (20), which includes additional estimations of diet induced thermogenesis (DIT) and excess post oxygen consumption (EPOC), respectively (see Table 2). Each daily WDEB measurement was added to the subsequent day, to calculate an estimated weekly accumulated energy deficit (WAED) across all intervention and recovery periods (see Figure 1 A.). Daily EA was also calculated as described by Loucks (34), where measures of EI were subtracted from EEE values (EEE Actiheart – NEAT + RMR_{meas}/60 x training in minutes) and divided by FFM established by DXA (see Table 1).

Statistical Analysis

In Table 1, descriptive statistics are presented as mean ± *SD*, where appropriate. Time phase combined averages for EI, DIT, NEAT, EEE, EPOC, 24 hour energy balance (24-hr EB) and EA were assessed via a within participant one way ANOVA utilising SPSS 26 (PASW, Chicago, USA). Bonferroni post hoc analysis was employed for pairwise comparisons and statistical significance was set at an alpha of $p < 0.05$.

RESULTS

Overview of Intervention and Recovery periods on Athlete Wellbeing, Training Load, Energy Availability, Within Day Energy Balance and Weekly Accumulated Energy Deficit

Throughout phases 1 and 2 of the intervention period, the athlete completed all assigned sport specific, aerobic CR, HIT and RT sessions to meet the assigned training load and DWBS did not fall below a minimum threshold. Time phase assessments of energy intake, expenditure, balance and availability are presented in Table 1, highlighting main effects between phases when comparing measurements of EI (p = 0.04), DIT (p = 0.02), EEE (p = 0.02), EPOC (p = 0.02), 24-hr EB and EA (p < 0.001). Low EA status was established throughout phase 1, with values ranging 7-31 kcal·kgFFM·day^{-1} equating to an average of 20 kcal·kgFFM·day^{-1}. During phase 2, low EA status was further evident with values ranging from -4 to 9 kcal·kgFFM·day^{-1} and considerably lower than all other time periods (p = 0.03), due to reductions in EI (p = 0.03) which in turn was causative of reductions in DIT (p = 0.03). In phase 3, EA was increased substantially in comparison to all other time phases (p <0.001) with daily values ranging between 53-98 kcal·kgFFM·day^{-1}, accompanied by significant increases in measures of EI, DIT, and 24-hr EB and decreases in EEE and EPOC (p <0.001). Figure 1 A highlights estimated WAED, demonstrating a net energy deficit of -105,757 kcal throughout phases 1 and 2, with weekly EI ranging from 11,900 to 3,856 kcal·wk^{-1} in parallel to mean total energy expenditures of 25,100 kcal·wk^{-1}, resulting in average deficits of -13,200 kcal·wk^{-1}. In phase 3, estimated WAED is only rescued to a -95,984 kcal deficit, despite a considerable increase in EI totalling 36,477 kcal·wk^{-1} and following a cessation of all training activity.

Changes in Anthropometric Measurements

Time phase changes of BM, Σ_{8SKf}, FM, FFM and BF% are shown in in Figure 1 B-F. The athlete recorded an official weight of 62.7 kg, successfully achieving the limit for the elected weight category. This represented an overall BM reduction of 9.8 kg (<13.5%), consisting of a loss of 4.9 kg (<6.8%) FM and 3.6 kg (<5.0%) FFM across phase 1 and 2 intervention periods. In the phase 3 post competitive recovery period, the athlete increased BM by 8.3 kg (>13.2%), FM by 1.0 kg (>1.6%) and FFM by 7.0 kg (>11.2%). Σ_{8SKf} were reduced by 28.5 mm across phases 1 and 2 with a 10.4 mm increase throughout phase 3. Finally, at the cessation of phase 2 BMI was reduced to 22.8 $kg \cdot m^{-2}$, with an increase to 25.8 $kg \cdot m^{-2}$ at the end of the phase 3 post competitive recovery period.

Assessment of low Energy Availability on Markers of Male Athlete Triad and RED-S

Assessment of the athlete's RMR is presented in Figure 2. During phases 1 and 2, there was a gradual reduction in RMR_{meas} values by -36 $kcal \cdot day^{-1}$ at -4 WK, -72 $kcal \cdot day^{-1}$ at -1 WK and a -149 $kcal \cdot day^{-1}$ at -1 D, representing an overall reduction from baseline of -257 $kcal \cdot day^{-1}$. This recovers within the phase 3 post competitive recovery period by an increase of 648 $kcal \cdot day^{-1}$ at +1 WK. Adaptive thermogenesis occurred at a rate of -36 $kcal \cdot day^{-1}$ at -4 WK, -99 $kcal \cdot day^{-1}$ at -1 WK and a larger decrease of -213 $kcal \cdot day^{-1}$ at -1 D. In relation to RMR_{ratio}, most values remained within an acceptable range, with a gradual decrease across phases 1 and 2, followed by a substantial increase at +1 WK in phase 3. However, there was a marked reduction in RMR_{ratio} at -1 D of phase 2, indicating RMR suppression and potential energy deficiency.

Blood clinical chemistry biomarkers and BMD/BMC measurements are presented in Table 3. Hypothalamic pituitary gonadal axis hormones remained relatively stable throughout phase 1, though markers were outside of clinical reference ranges (testosterone <5 nmol·L^{-1}; luteinizing hormone <1.2 U·L^{-1}; follicle stimulating hormone <1.5 U·L^{-1}; sex hormone binding globulin >78 nmol·L^{-1}) in phase 2 at WI. However, these were all rescued within 48 hours by -1 D of the phase 3 post competitive recovery period. Fasting insulin was consistent within phases 1 and 2, but substantially increased above clinical reference ranges (>48 pmol·L^{-1}) throughout phase 3. Bone turnover markers procollagen type 1 N-terminal propeptide (P1NP) and β-carboxy-terminal cross-linked telopeptide (β-Ctx) were both above the highest clinical reference ranges across all phases (P1NP >80 ug·L^{-1}; β-Ctx >0.6 ug·L^{-1}), yet generating positive ratios (>1.0) at all time points. Additionally, DXA assessment of total body (minus head) and lumbar spine BMD were also within acceptable reference Z-scores, with no change across time phases as expected based on the time course of measurements. Predominantly all biomarkers of hydration, electrolyte, renal, liver and blood lipids remained stable throughout the phase 1, despite elevations in albumin above clinical reference ranges (>50 g·L^{-1}) across all measurement points. Within phase 2, P_{osm} examined in parallel with sodium, urea, creatinine and LDL was indicative of moderate hypohydration (>300 mOsmols·kgH_2O^{-1}) and hypernatremia (sodium >145 mmol·L^{-1}; urea >7.8 mmol·L^{-1}; creatinine >110 µmol·L^{-1}; LDL >3.0 mmol·L^{-1}) at WI, however, this was rescued to normative values by phase 3 at +1 D. Finally, total cholesterol values were elevated above clinical reference ranges (>5.0 mmol·L^{-1}) in phases 2 and 3 at WI and +1 WK.

Cardiac Assessment measured via ECG/echocardiography are presented in Figure 3. Despite an increased LVEDV response exhibited at -1 D, there were no major changes in either structure or

function of the left (Figure 3 A) or right (Figure 3 B) ventricles across all phases. As highlighted in Figure 3 C, both CO and HR reduced consistently across phases 1 and 2 with a large increase in both measures within 24 hours from -1 D to WI, which plateaued by the end of the phase 3 post competitive recovery period at +1 WK.

There were no major psychological fluctuations in POMS or TMD scores across the majority of all time phases as highlighted in Table 4. This is with the exception of WI, where reductions in vigour and increases in fatigue resulted in an elevated TMD, yet this remained well within normative values for athletic populations. In all semi structured interviews and despite relevant probing, the athlete made no comments in regards to any gastrointestinal distresses and reported no incidences of illness or injury. Excerpts ascertained from these interviews highlight throughout phase 1, the athlete displayed feelings of *'fear'* and *'perceived'* losing such a large volume of BM would negatively affect his health and performance. In phase 2, there were continued emotions of *'anxiety'* over his potential to make the required weight category, yet a *'realisation'* and *'confidence'* that he will achieve his goal. Finally, post WI the athlete describes his *'exhilaration'* and sense of *'accomplishment'* in meeting the targeted weight category, reinforced by a renewed sense of *'focus'* on competitive performance.

Physical performance assessments of both absolute and relative upper and lower MDS, MDP and cardiorespiratory capacity are highlighted in Figure 4. As demonstrated in Figure 4 A, across time phases both upper and lower MDS increased relatively by 18-19% and absolutely by 6-9% (n.b. bench press was not completed during phase 3 at +1 WK, due to the athlete fracturing his left hand in competition). Within phase 1 from -8 WK to -1 WK, MDP improved in both upper

absolute and lower relative force/velocity and power curve profiles (see Figure 4 B-C). Figure 4 D highlights that across phases, both relative and absolute $\dot{V}O2_{peak}$ values increased by 19% and 13% at -1 WK prior to competition, respectively. There was also an improvement in the athlete's FAT_{peak} oxidation profile throughout the phase 1 intervention period from 0.62 g·min^{-1} at 44% to 0.72 g·min^{-1} at 60% $\dot{V}O2_{peak}$. However, this is significantly reduced to 0.42 g·min^{-1} at 30% $\dot{V}O2_{peak}$ within the end of the post competitive recovery phase 3 at + 1 WK.

DISCUSSION

The aim of this case report was to evaluate the effects of incorporating daily fluctuations in low EA on health and performance indices associated with Male Athlete Triad and RED-S. In studying a male combat sport athlete making weight for competition, we provide novel data by demonstrating that 7 weeks of low EA (equating to a mean daily value of 20 kcal·kg·FFM·day^{-1}) permits a reduction of BM and FM without perturbations to physiological systems associated with Male Athlete Triad and RED-S. In contrast, a subsequent period of 5 consecutive days of EA <10 kcal·kg·FFM·day^{-1} induced Male Athlete Triad and RED-S consequences, as evidenced by disruptions to hormones of the hypothalamic pituitary gonadal axis, RMR_{meas} and RMR_{ratio}. Whilst such negative outcomes were quickly reversed during the rebound hyperphagic response that occurred in the 1-7 days post competition, this period of excessive EI also induced fasting hyperinsulinemia and hyperlipidaemia, thus suggesting impaired substrate handling and insulin resistance.

During the 7 weeks of phase 1, the athlete adhered to a daily EI that was equivalent to RMR and consumed a macronutrient intake representative of high protein (2.3 g·kgFFM^{-1}), low CHO (3.4

g·kgFFM^{-1}) and low fat (0.9 g·kgFFM^{-1}). However, given that absolute EEE fluctuated day by day during each weekly microcycle, this gave rise to a daily EA ranging from 7 to 31 kcal·kg·FFM·day^{-1} and a mean EA of 20 kcal·kg·FFM·day^{-1}. Despite the classification of low EA status during this phase, it is noteworthy that the athlete presented with none of the classical consequences associated with the Male Athlete Triad or RED-S. Indeed, we observed no reductions in RMR, impairments to cardiac function or clinically relevant changes in blood clinical chemistry. In agreement with previous case/cohort study accounts (5, 13) and randomised control trials (35), our data also suggest that the combination of resistance training and daily protein intake equivalent to 2-3 × recommended daily allowance is sufficient to maintain (or increase) FFM. Such findings are likely underpinned by the observation that the combination of increased protein intake and resistance training can maintain high rates of muscle protein synthesis despite low EA (36). Additionally, changes in bone turnover markers were consistent with normative ranges occurring in young athletic males performing high-intensity exercise (37), findings that also appear consistent with the observation that 5 days of EA equalling 15 kcal·kg·FFM·day^{-1} did not affect bone resorption or formation in healthy young males (38). Additionally, it is noteworthy that the athlete also presented with normal BMD values where in conjunction with markers of bone turnover, such data agree with previous observations from male amateur boxers and suggest that the physical loading associated with combat sport training may provide a sufficient osteogenic stimulus (39).

When taken together, our data therefore suggest that the classification of low EA as <30 kcal·kg·FFM·day^{-1}, a value commonly cited for females (40, 41), may not be representative of a threshold to induce health and performance consequences associated with both the Male Athlete

Triad and RED-S models, a view recently supported by De Souza and colleagues (9, 7). Whilst we obviously cannot ascertain such a threshold within the present case report, it is also tempting to speculate that it is the daily fluctuations in EA as opposed to a consistent absolute daily EA (subsequently giving rise to a mean daily EA of 20 kcal·kg·FFM·day^{-1}), which may have preserved physiological function during this phase. Indeed, such a hypothesis is supported by the observation that impairments in the hypothalamic pituitary gonadal axis can be restored within days of increased EA (14).

During phase 2, the athlete underwent a tapering of EEE and consumed a reduced EI (1200-300 kcal.d^{-1}) such that absolute EA was consistently <10 kcal·kg·FFM·day^{-1} for five consecutive days. It is noteworthy that during this short time, the athlete subsequently presented with classical consequences associated with low EA that are considered indicative of Male Athlete Triad and RED-S, as evidenced by clinically relevant reductions to hormones of the hypothalamic pituitary gonadal axis, reduced RMR_{meas} (-258 kcal·day^{-1}) and RMR_{ratio} (<0.90) (8). The combination of limited EI and likely associated changes to muscle glycogen concentration and acute dehydration, also presented as a reduction in FFM of approximately 3 kg when assessed via DXA. The acute dehydration period in the final 13 hours prior to the official competition weigh in also increased plasma osmolality, sodium, urea, creatinine and LDL. Whilst this magnitude of hypohydration and hypernatremia would not serve to cause acute kidney injury (5), these biomarkers are still markedly high considering the limited acute BM loss via dehydration (<3%). Additionally, in accordance with previous accounts of athletes engaging in low EA and acute dehydration, the final 24 hours before weigh in was associated with decreased vigour and increased tension and fatigue (42, 43). Nonetheless, although this increased

both resting cardiac output and heart rate, overall cardiac structure and function remain largely unchanged throughout all time phases, further indicating a minimal effect of acute short term low EA on cardiovascular function.

Despite daily low EA and the estimated WAED incurred across the intervention period (i.e. >105,000 kcal), it should be noted that the athlete was able to complete all prescribed training sessions and there were no reported incidences of illness or injury. Additionally, the athlete improved his MDS, MDP, $\dot{V}O2_{peak}$ and rates of lipid oxidation, whilst also not reporting any impairments to mood profile. The qualitative responses of the athlete during all time phases consistently highlighted how the intervention was deemed a major improvement on previous practice, whilst also reporting feeling more prepared than he had ever been in his competitive career. This was further supported by the athlete achieving the gold medal at the championships, after successfully winning 4 contests and qualifying for his elected weight category to represent the national team at the 2018 European University Games.

Despite the perceived success of the intervention, it is noteworthy that in the 7-day post competitive period (phase 3) the athlete still remained in an estimated WAED of >95,000 kcal when compared with baseline, despite consuming an ad libitum EI >36,000 kcal·wk^{-1} resulting in a BM gain of 8.3 kg. This level of EI is indicative of rebound hyperphagia (44) and whilst not resulting in FM overshoot (45), there were clear signs of impaired substrate handling. Indeed, this period of excessive EI induced fasting hyperinsulinemia and hyperlipidaemia, thus suggestive of insulin resistance (46). Additionally, the athlete's peak rate of fat oxidation during exercise decreased from 0.72 to 0.42 g.min^{-1} and occurred at a lower relative training intensity

(60 to 30% $\dot{V}O2_{peak}$). Such periods of rebound hyperphagia are of particular concern, given that "weight cycling" athletes typically gain more BM upon retirement when compared with non-weight cycling athletes (47, 48).

Whilst this examination highlights novel data in relation to the effects of low EA on the health and performance related consequences within the Male athlete Triad and RED-S models, it should be noted that this is only indicative of a single participant case report and cannot be immediately extrapolated to wider populations. Additionally, further studies examining this demographic need to focus on the effects of repeated exposure to these levels of EA, considering combat sport athletes consistently make weight numerous times per year across long spanning careers.

In summary, we provide novel data by demonstrating in a male combat sport athlete that 7 weeks of day to day fluctuations in EA (equating to a mean value 20 kcal·kg·FFM·day^{-1}) permits a reduction of BM and FM without perturbations to physiological systems associated with the Male Athlete Triad and RED-S. Importantly, the athlete was able to complete all prescribed training sessions during this time and improved his physical performance capacity without any impairment to mood profiles. In contrast, a subsequent period of five consecutive days of EA consistently <10 kcal·kg·FFM·day^{-1} and acute dehydration induced markers of Male Athlete Triad and RED-S, as evidenced by reductions in hormones of the hypothalamic pituitary gonadal axis and lowered RMR. Again these data are only indicative of a single participant case report and caution should be considered when extrapolating these conclusions to wider populations without the support of ongoing longitudinal and cohort based investigations. Additionally,

further studies are now required using randomised control trials to ascertain the threshold and time course of EA that induces impairments in physiological function of male athletes. Finally, we encourage all athletes who engage in BM reduction to do so with the guidance of qualified sport dieticians/nutritionists, to reduce the potential for health and performance consequences of low EA across both acute and chronic timeframes.

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CAPTIONS

FIGURE 1. Changes in estimated weekly accumulated energy deficit and energy intake (A.), body mass (B.), fat mass (C.), fat free mass (D.), body fat % (E.) and Σ_{8SKf} (F.) measurements throughout the intervention and post competitive recovery phases.

FIGURE 2. Changes in resting metabolic rate measurement, prediction and ratio assessments throughout the intervention and post competitive recovery phases.

FIGURE 3. Changes in left (A.) and right (B.) ventricular structure/function and cardiac output/heart rate (C.) throughout the intervention and post competitive recovery phases.

FIGURE 4. Changes in absolute and relative upper/lower maximal dynamic strength (A.), upper (B.) and lower (C.) maximal dynamic power and cardiorespiratory capacity (D.) throughout the intervention and post competitive recovery phases.

TABLE 1. Measurements of energy intake, expenditure and 24 hour within day energy balance/availability throughout the intervention and post competitive recovery phases.

TABLE 2. Within day energy balance calculations for a training day 5 during the Phase 1 -8 WK time period of the intervention.

TABLE 3. Changes in blood clinical chemistry and DXA bone mineral density and content measurement throughout the intervention and post competitive recovery phases.

TABLE 4. Changes in profile of mood states and total mood disturbance scores throughout the intervention and post competitive recovery phases.

Supplemental Digital Content 1. Diagram of measurements taken throughout phases 1 & 2 intervention and phase 3 post competitive recovery periods. tiff

Supplemental Digital Content 2. CV%, range and sensitivity of measurement for all assessed blood clinical chemistry biomarkers. pdf

Figure 1

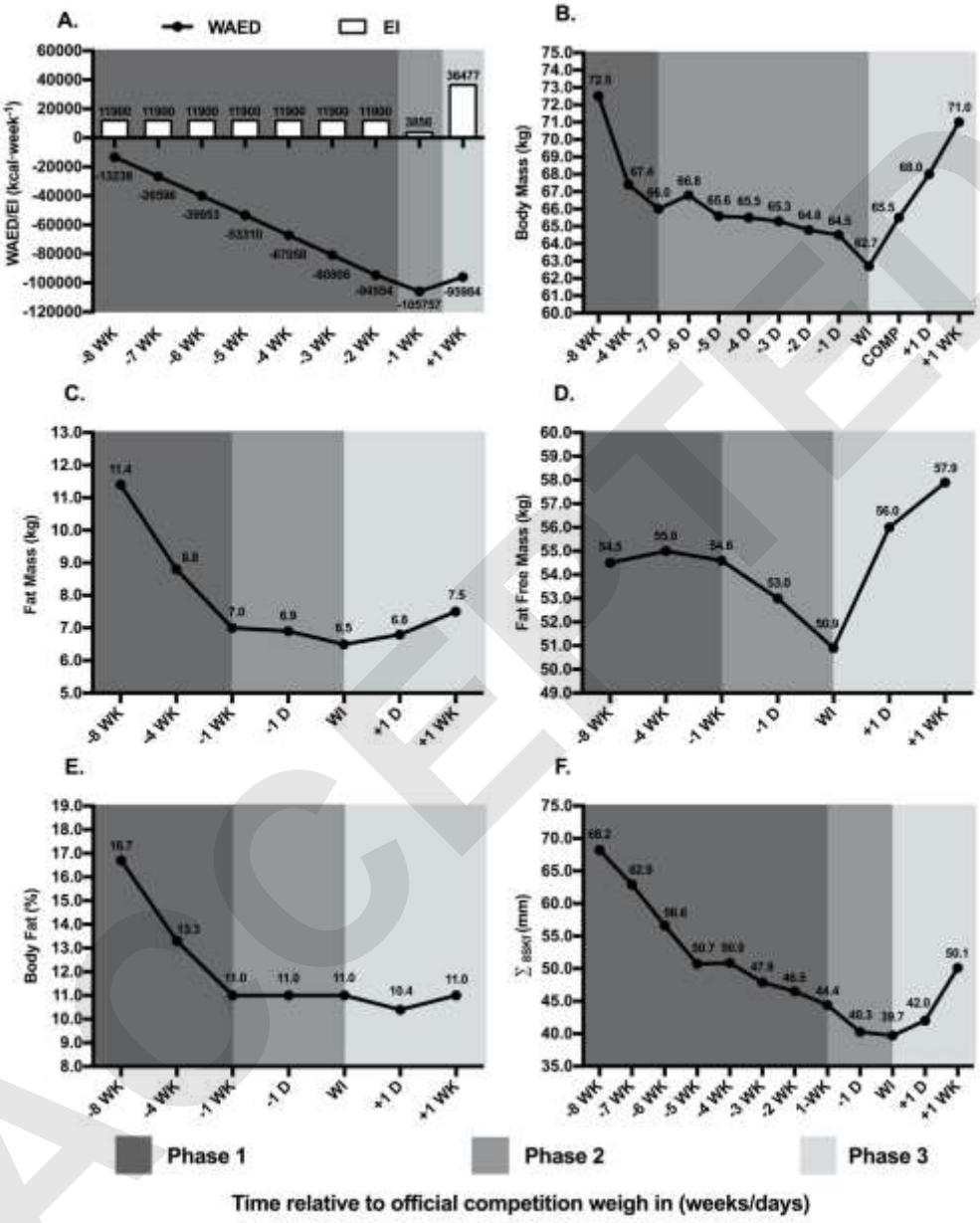


Figure 2

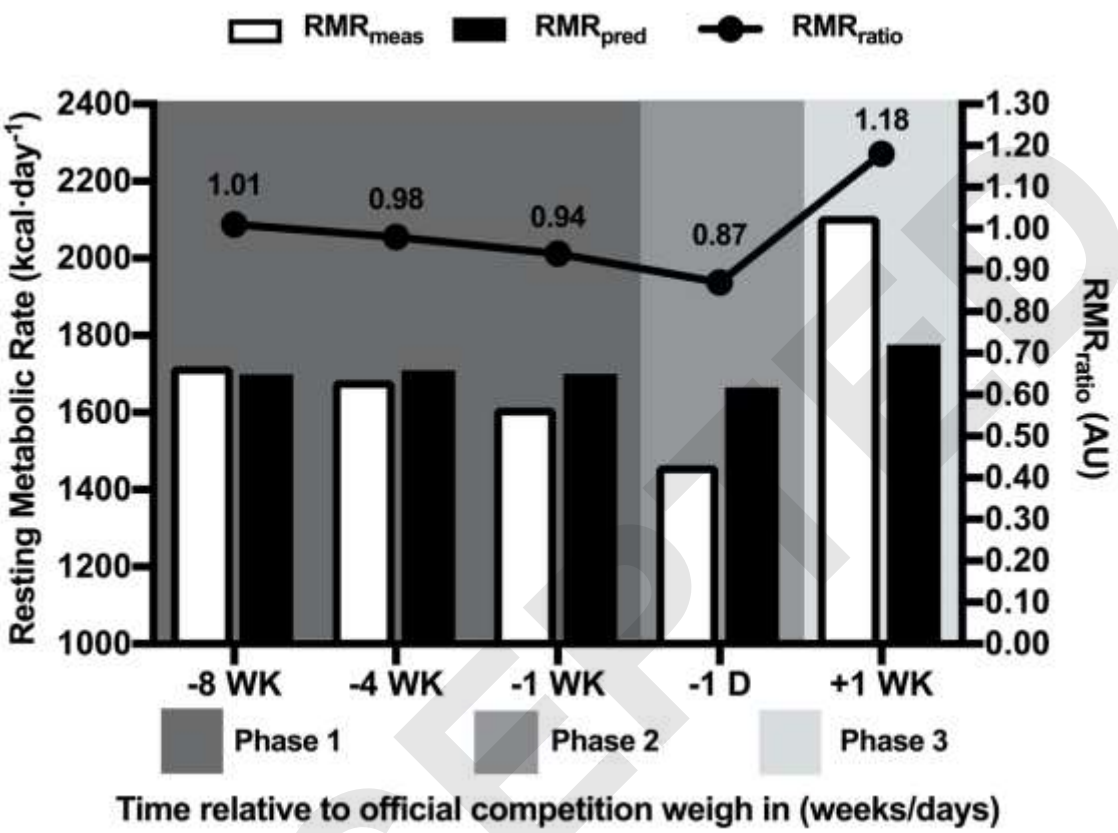


Figure 3

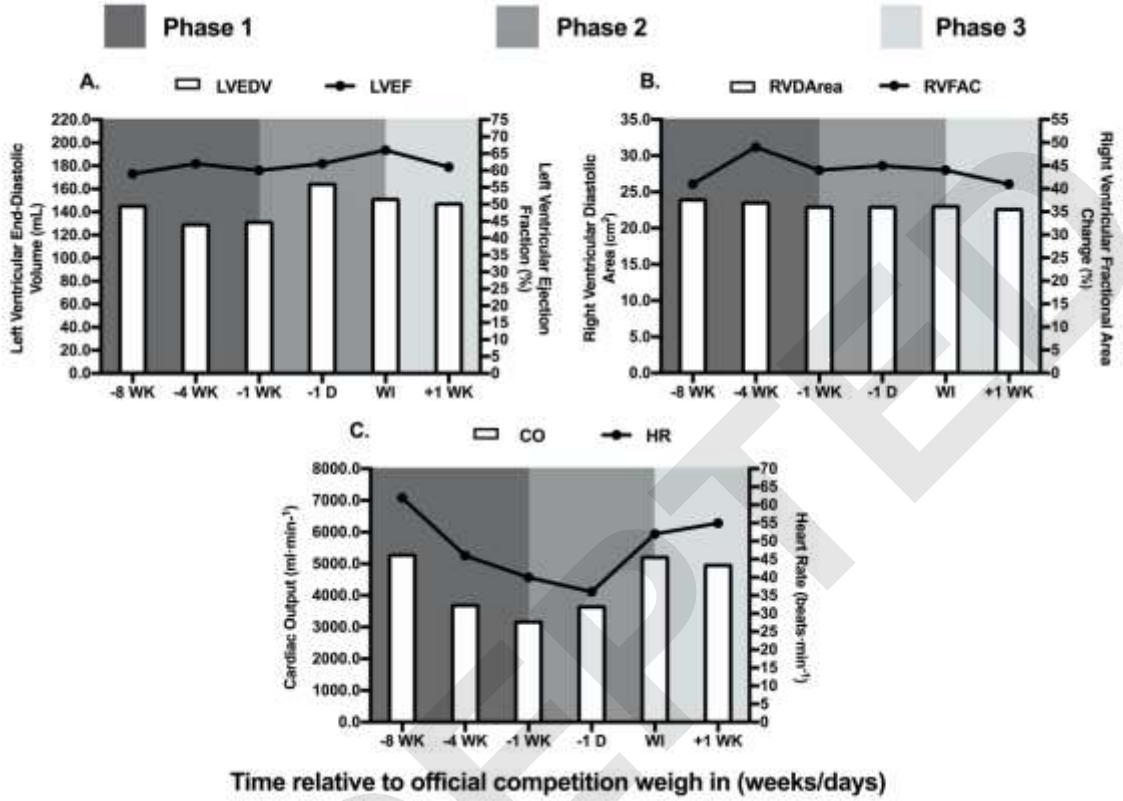


Figure 4

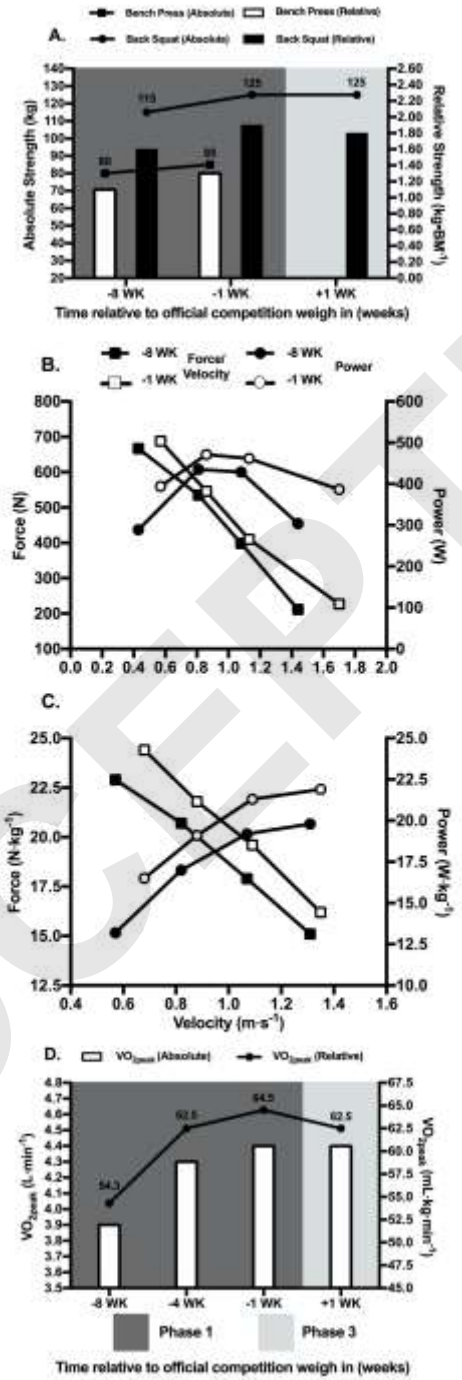


TABLE 1. Measurements of energy intake, expenditure and 24 hour within day energy balance/availability throughout the intervention and post competitive recovery phases.

TIME PHASE/ MEASUREMENT	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY	TIME PHASE COMBINED AVE.
PHASE 1 -8 WK to -4 WK								
EI (kcal·day^{-1})	1700 ± 0	1700 ± 0	1700 ± 0	1700 ± 0	1700 ± 0	1700 ± 0	1700 ± 0	**1700 ± 0**
DIT (kcal·day^{-1})	167 ± 0	170 ± 0	171 ± 0	170 ± 0	169 ± 0	171 ± 0	170 ± 0	**170 ± 2**
NEAT (kcal·day^{-1})	1794 ± 90	1270 ± 64	1235 ± 62	986 ± 49	682 ± 34	911 ± 46	1073 ± 54	**1136 ± 352**
EEE (kcal·day^{-1})	873 ± 47	584 ± 31	409 ± 22	617 ± 34	1209 ± 66	0 ± 0	376 ± 20	**559 ± 370**
EPOC (kcal·day^{-1})	70 ± 4	47 ± 2	33 ± 2	49 ± 3	97 ± 5	0 ± 0	30 ± 1	**45 ± 30**
24-hr WDEB (kcal·day^{-1})	-2843 ± 94	-2034 ± 66	-1824 ± 63	-1782 ± 54	-2069 ± 53	-1091 ± 46	-1077 ± 55	**-1919 ± 541**
EA (kcal·kgFFM·day^{-1})	15 ± 1	20 ± 1	24 ± 0	20 ± 1	9 ± 1	31 ± 0	24 ± 0	**20 ± 7**
PHASE 1 -4 WK to -1 WK								
EI (kcal·day^{-1})	1700 ± 0	1700 ± 0	1700 ± 0	1700 ± 0	1700 ± 0	1700 ± 0	1700 ± 0	**1700 ± 0**
DIT (kcal·day^{-1})	167 ± 0	170 ± 0	171 ± 0	170 ± 0	169 ± 0	171 ± 0	170 ± 0	**170 ± 2**
NEAT (kcal·day^{-1})	1503 ± 75	1176 ± 59	1173 ± 59	1436 ± 72	686 ± 34	1523 ± 76	944 ± 47	**1207 ± 313**
EEE (kcal·day^{-1})	968 ± 52	527 ± 29	340 ± 18	616 ± 34	1333 ± 72	0 ± 0	430 ± 23	**579 ± 418**
EPOC (kcal·day^{-1})	77 ± 4	43 ± 2	27 ± 1	49 ± 3	107 ± 6	0 ± 0	35 ± 2	**46 ± 34**
24-hr WDEB (kcal·day^{-1})	-2611 ± 82	-1846 ± 61	-1657 ± 60	-2194 ± 75	-2162 ± 57	-1667 ± 76	-1516 ± 49	**-1974 ± 401**
EA (kcal·kgFFM·day^{-1})	13 ± 1	22 ± 1	25 ± 0	20 ± 1	7 ± 1	31 ± 0	23 ± 0	**20 ± 8**
PHASE 2 -1 WK to COMP						**OCWI**	**COMP**	†
EI (kcal·day^{-1})	915	915	863	863	300	3490	5185	**794 ± 538***
DIT (kcal·day^{-1})	92	92	421	86	30	330	464	**79 ± 39***
NEAT (kcal·day^{-1})	632	1097	903	1122	929	937	507	**855 ± 289**
EEE (kcal·day^{-1})	1120	409	421	467	474	0	337	**423 ± 407**
EPOC (kcal·day^{-1})	98	33	34	37	38	0	27	**34 ± 33**
24-hr WDEB (kcal·day^{-1})	-2729	-2280	-2108	-2340	-2622	809	2394	**-2165 ± 476**
EA (kcal·kgFFM·day^{-1})	-4	9	8	7	-3	64	89	**3 ± 7***
PHASE 3 +1 D to +1 WK								ǂ
EI (kcal·day^{-1})	3877	3203	4637	2978	3717	3902	5490	**5211 ± 2672#**
DIT (kcal·day^{-1})	395	352	432	332	325	451	885	**567 ± 398#**
NEAT (kcal·day^{-1})	1042	851	390	723	599	494	136	**767 ± 260**
EEE (kcal·day^{-1})	0	0	0	0	0	0	0	**48 ± 127#**
EPOC (kcal·day^{-1})	0	0	0	0	0	0	0	**4 ± 10#**
24-hr WDEB (kcal·day^{-1})	879	332	2038	39	801	855	2369	**1387 ± 1180#**
EA (kcal·kgFFM·day^{-1})	69	57	83	53	66	70	98	**73 ± 17#**

Daily data for combined time phases are presented as mean ± *SD* with singular values representing individual daily time points within specified time phases. *significantly different to all other equitable measurements at phases 1 and 3 ($p < 0.05$). #significantly different to all other equitable measurements at phases 1 and 2 ($p < 0.05$). [OCWI]Official competition weigh in day. [COMP]Competition day. †Calculated with values prior to OCWI. ⸸Calculated with values post OCWI. (WK = Week; D = Day; EI = Energy Intake; DIT = Diet Induced Thermogenesis; NEAT = Non Exercise Activity Thermogenesis; EEE = Exercise Energy Expenditure; EPOC = Excess Post Oxygen Consumption; 24-hr WDEB = 24 hour Within Day Energy Balance; EA = Energy Availability)

TABLE 2. Within day energy balance calculations for a training day 5 during the Phase 1 -8 WK time period of the intervention.

	INTAKE		EXPENDITURE					HOURLY CALCULATIONS		
TIME	**EI**	**DIT**	**EEE**	**EPOC**	**NEAT**	**SMR**	**RMR**	**TEE hr to hr**	**EB hr by hr**	**WDEB**
00.00-01.00	0	2	0	0	0	71	0	74	-74	-8465
01.00-02.00	0	0	0	0	0	71	0	145	-71	-8536
02.00-03.00	0	0	0	0	0	71	0	216	-71	-8607
03.00-04.00	0	0	0	0	0	71	0	287	-71	-8679
04.00-05.00	0	0	0	0	0	71	0	359	-71	-8750
05.00-06.00	0	0	0	0	0	71	0	430	-71	-8821
06.00-07.00	0	0	0	0	0	71	0	501	-71	-8892
07.00-08.00	0	0	0	0	46	0	71	618	-117	-9009
08.00-09.00	0	0	0	0	46	0	71	735	-117	-9126
09.00-10.00	0	0	0	0	46	0	71	852	-117	-9243
10.00-11.00	0	0	246*	0	46	0	71	1215	-363	-9606
11.00-12.00	379	11	0	12	46	0	71	1355	238	-9368
12.00-13.00	0	11	0	7	46	0	71	1490	-135	-9502
13.00-14.00	0	7	187^	0	46	0	71	1801	-311	-9813
14.00-15.00	595	22	0	9	46	0	71	1098	446	-9367
15.00-16.00	0	19	0	6	46	0	71	2091	-142	-9509
16.00-17.00	0	13	0	0	46	0	71	2221	-130	-9638
17.00-18.00	595	25	0	0	46	0	71	873	453	-9185
18.00-19.00	0	21	0	0	46	0	71	2501	-138	-9323
19.00-20.00	0	14	364#	0	46	0	71	2995	-495	-9818
20.00-21.00	0	7	364#	18	46	0	71	3502	-506	-10324
21.00-22.00	131	8	0	29	46	0	71	3656	-23	-10347
22.00-23.00	0	6	0	11	0	71	0	3744	-88	-10435
23.00-00.00	0	2	0	0	0	71	0	3818	-74	-10509
24 hr TOTAL	**1700**	**169**	**1162**	**93**	**684**	**641**	**1069**	**3818**	**-2118**	**-10509**

*Fasted Aerobic Continuous Running ^Resistance Training #Sport Specific Training (EI = Energy Intake; DIT = Diet Induced Thermogenesis; NEAT = Non Exercise Activity Thermogenesis; EEE = Exercise Energy Expenditure; EPOC = Excess Post Oxygen Consumption; SMR = Sleeping Metabolic Rate; RMR = Resting Metabolic Rate; TEE hr to hr = Total Energy Expenditure Hour to Hour; EB hr by hr = Energy Balance hour by hour; WDEB = Within Day Energy Balance) n.b. Measures have been rounded to whole numbers owing to 24 hr totals which may not equate

TABLE 3. Changes in blood clinical chemistry and DXA bone mineral density and content measurement throughout the intervention and post competitive recovery phases.

	PHASE 1			PHASE 2		PHASE 3	
	-8 WK	**-4 WK**	**-1 WK**	**-1 D**	**WI**	**+1 D**	**+1 WK**
ENDOCRINE RESPONSES							
Cortisol ($nmol \cdot L^{-1}$)	501	465	498	482	571	407	407
Testosterone ($nmol \cdot L^{-1}$)	21.5	18.7	11.2	9.4	4.0 ↓	9.7	19.8
IGF-1 ($nmol \cdot L^{-1}$)	32	30	25	22	21	21	30
Luteinizing Hormone ($U \cdot L^{-1}$)	3.4	3.2	2.1	1.8	1.0 ↓	3.1	4.0
Follicle Stimulating Hormone ($U \cdot L^{-1}$)	1.9	1.9	1.9	1.7	1.4 ↓	2.0	2.1
SHBG ($nmol \cdot L^{-1}$)	43	57	57	72	82 ↑	72	46
Insulin ($pmol \cdot L^{-1}$)	26	34	28	26	21	142 ↑	77 ↑
BONE TURNOVER & DXA BMD/BMC							
P1NP ($ug \cdot L^{-1}$)	112 ↑	159 ↑	131 ↑	124 ↑	112 ↑	132 ↑	139 ↑
β-Ctx ($ug \cdot L^{-1}$)	0.7 ↑	0.9 ↑	1.0 ↑	0.9 ↑	0.9 ↑	0.6 ↑	1.0 ↑
Total Body (minus head) BMD/BMC ($Z\text{-score}/g \cdot cm^2$)	0.4 (1.123)	0.5 (1.135)	0.4 (1.130)	0.7 (1.155)	-	-	0.4 (1.122)
Lumbar Spine BMD/BMC ($Z\text{-score}/g \cdot cm^2$)	0.8 (1.144)	0.8 (1.154)	1.0 (1.175)	1.0 (1.169)	-	-	0.9 (1.166)
ELECTROLYTE, RENAL & LIVER FUNCTION							
P_{osm} ($mOsmols \cdot kgH2O^{-1}$)	288	294	294	299	307 ↑	297	289
Sodium ($mmol \cdot L^{-1}$)	144	143	143	142	147 ↑	143	142
Urea ($mmol \cdot L^{-1}$)	4.2	5.8	5.6	6.6	8.3 ↑	4.8	4.2
Creatinine ($\mu mol \cdot L^{-1}$)	93	94	99	109	119 ↑	92	93
Albumin ($g \cdot L^{-1}$)	56 ↑	57 ↑	52 ↑	55 ↑	59 ↑	53 ↑	52 ↑
Globulin ($g \cdot L^{-1}$)	25	23	22	22	25	21	22
Total Protein ($g \cdot L^{-1}$)	78	80	74	77	84 ↑	72	71
LIPID PROFILES							
Total Cholesterol ($mmol \cdot L^{-1}$)	3.6	3.8	4.3	4.6	5.2 ↑	3.9	5.1 ↑
HDL ($mmol \cdot L^{-1}$)	1.3	1.2	1.3	1.4	1.6	1.3	1.7
LDL ($mmol \cdot L^{-1}$)	2.1	2.3	2.7	2.9	3.3 ↑	2.0	2.9
Triglyceride ($mmol \cdot L^{-1}$)	0.5	0.7	0.6	0.6	0.6	1.5	1.5

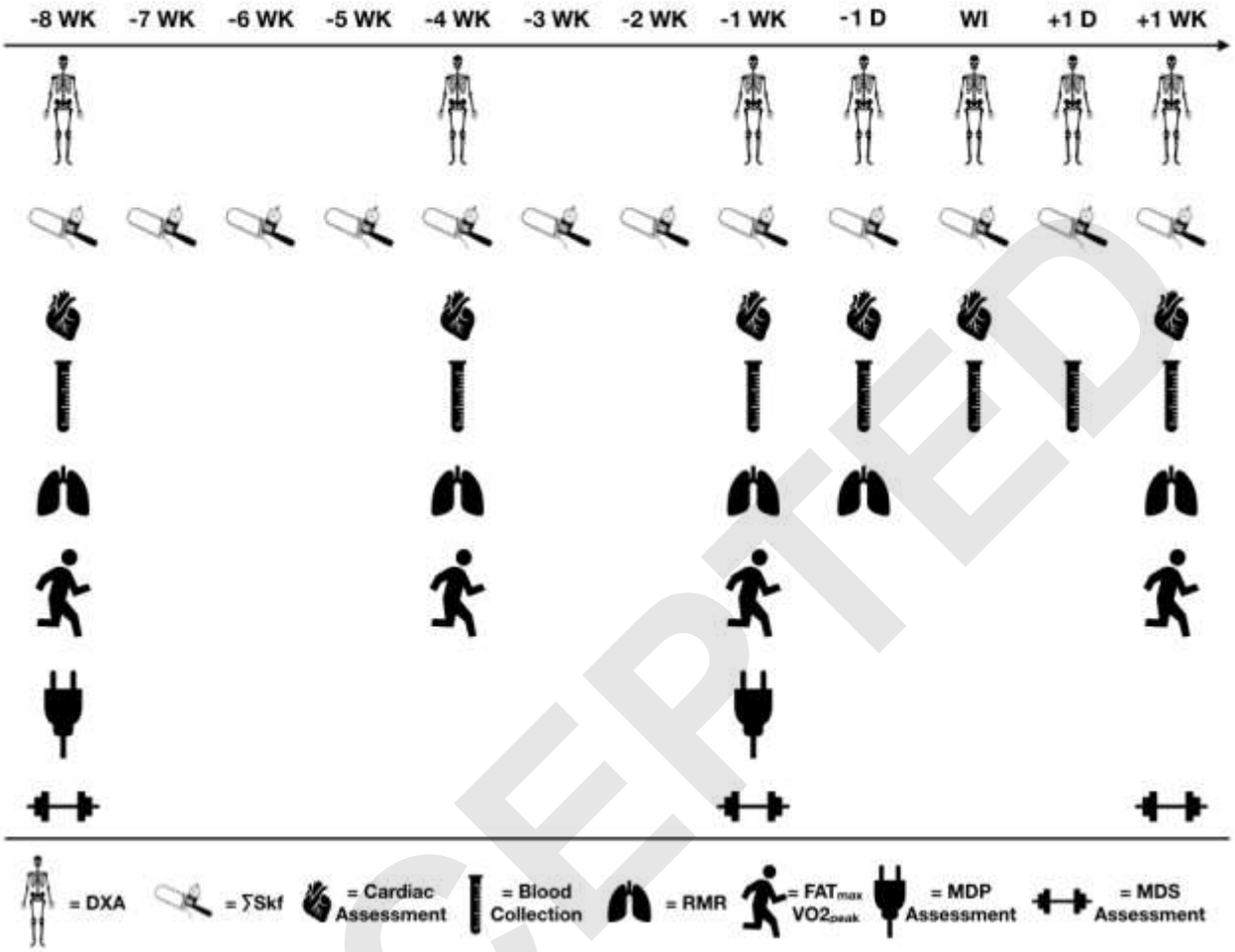
↓represents decrease and ↑ represents increase outside of normative clinical reference ranges (IGF-1 = Insulin-like Growth Factor 1; SHBG = Sex Hormone Binding Globulin; P1NP = Procollagen type 1 N-terminal propeptide; β-CTX = β-carboxy-terminal cross-linked telopeptide; BMD = Bone Mineral Density; BMC = Bone Mineral Content (values in parentheses); P_{osm} = Plasma Osmolality; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; WK = Week; D = Day; WI = Official competition weigh in

TABLE 4. Changes in profile of mood states and total mood disturbance scores throughout the intervention and post competitive recovery phases.

	PHASE 1			PHASE 2		PHASE 3	
	-8 WK	**-4 WK**	**-1 WK**	**-1 D**	**WI**	**+1 D**	**+1 WK**
Tension (AU)	5	7	9	5	8	13	1
Depression (AU)	3	6	8	4	4	2	0
Anger (AU)	3	6	9	10	9	7	1
Vigour (AU)	22	24	21	22	11	19	24
Fatigue (AU)	3	6	1	3	12	0	7
Confusion (AU)	6	6	6	5	6	3	1
Total Mood Disturbance Score (AU)	**-2**	**7**	**12**	**5**	**28**	**6**	**-14**

WK = Week; D = Day; WI = Official competition weigh in

Supplemental Digital Content 1. Diagram of measurements taken throughout phases 1 & 2 intervention and phase 3 post competitive recovery periods.



Supplemental Digital Content 2. CV%, range and sensitivity of measurement for all assessed blood clinical chemistry biomarkers.

Biomarker	CV%	Range*	Sensitivity**
Insulin ($pmol \cdot L^{-1}$)	<4.9	18.2 – 987.5	1.39
Insulin-Like Growth Factor-1 ($nmol \cdot L^{-1}$)	<7.2	2.9 – 61.4	0.58
Testosterone ($nmol \cdot L^{-1}$)	<4.4	1.4 – 38.3	0.09
Cortisol ($nmol \cdot L^{-1}$)	<3.8	140.1 – 1592.0	1.5
Luteinizing Hormone ($U \cdot L^{-1}$)	<2.2	1.0 – 95.6	0.10
Follicle Stimulating Hormone ($U \cdot L^{-1}$)	<3.7	0.5 – 128.7	0.10
Sex Hormone Binding Globulin ($nmol \cdot L^{-1}$)	<4.0	14.9 – 119.4	0.35
Procollagen type 1 N-terminal Propeptide ($ug \cdot L^{-1}$)	<4.1	12.8 – 1140.4	5.0
β-Carboxy-Terminal Cross-linked Telopeptide ($ug \cdot L^{-1}$)	<5.7	0.06 – 1.74	0.01
Plasma Osmolality ($mOsmols \cdot kgH2O^{-1}$)	<1.9	275.8 – 322.5	2.0
Sodium ($mmol \cdot L^{-1}$)	<1.5	132.8 – 148.2	20.0
Urea ($mmol \cdot L^{-1}$)	<1.3	1.8 – 12.4	0.5
Creatinine ($\mu mol \cdot L^{-1}$)	<1.1	61.9 – 177.3	15.0
Albumin ($g \cdot L^{-1}$)	<1.5	32.9 – 59.1	2.0
Globulin ($g \cdot L^{-1}$)	<2.1	17.5 – 35.2	2.0
Total Protein ($g \cdot L^{-1}$)	<2.5	60.7 – 89.3	2.0
Total Cholesterol ($mmol \cdot L^{-1}$)	<1.6	2.3 – 7.9	0.1
HDL ($mmol \cdot L^{-1}$)	<1.5	0.8 – 2.7	0.1
LDL ($mmol \cdot L^{-1}$)	<2.1	1.5 – 5.1	0.1
Triglyceride ($mmol \cdot L^{-1}$)	<1.9	0.3 – 5.8	0.1

CV% = Intra/inter coefficient of variation percentages; *Values are indicative of internal laboratory references inclusive of male and female cohorts of various age ranges; **Values are indicative of lowest detection limit as stated by assay manufacturer.