Muscle fiber typology is associated with the incidence of overreaching in response to overload training

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Running title: Muscle fiber typology and overload training

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ABSTRACT

The aim of this study was to identify markers of training stress and characteristics of middle-distance runners related to the incidence of overreaching following overload training. Twenty-four highly-trained middle-distance runners (*n*=16 male; VO_{2peak}=73.3(4.3) $mL \cdot kg \cdot min^{-1}$; *n*=8 female, VO_{2peak}=63.2(3.4) $mL \cdot kg \cdot min^{-1}$) completed 3 weeks of normal training (NormTr), 3 weeks of high-volume training (HVTr; a 10, 20 and 30% increase in training volume each successive week from NormTr), and a 1-week taper (TapTr; 55% exponential reduction in training volume from HVTr week 3). Before, and immediately after each training period, an incremental treadmill-running test was performed, while resting metabolic rate (RMR), subjective fatigue responses and various resting blood biomarkers were assessed. Muscle fiber typology of the gastrocnemius was estimated by quantification of muscle carnosine using proton magnetic resonance spectroscopy and expressed as a z-score relative to a non-athlete control group. Twelve runners were classified as functionally overreached (FOR) following HVTr (decreased running time to exhaustion; TTE), whereas the other twelve were classified as acutely fatigued (AF; no decrease in running TTE). The FOR group did not demonstrate systematic alterations in RMR, resting blood biomarkers or submaximal exercise responses compared to the AF group. Gastrocnemius carnosine z-score was significantly higher in FOR (-0.44 ± 0.57) compared to AF (-1.25 ± 0.49, *p*=0.004, *d*=1.53) and was also negatively correlated with changes in running TTE from pre- to post-HVTr (r=-0.55, p=0.005) and pre-HVTr to post-TapTr (r=-0.64, p=0.008). Muscle fiber typology is related to the incidence of overreaching and performance super-compensation following increased training volume and a taper.

Keywords: OVERTRAINING; TRAINING LOAD; MUSCLE FIBER TYPE COMPOSITION; FATIGUE MARKERS; RECOVERY

New and noteworthy: Variability in the performance responses following an overload training period and subsequent taper were associated with the variation in the muscle fiber typology of the gastrocnemius. Runners with an estimated higher proportion of type I fibers (i.e., lower carnosine z-score) were able to maintain performance in response to an overload training period and subsequently achieve a superior performance super-compensation. These findings show that muscle fiber typology contributes to the variability in performance responses following training.

INTRODUCTION

A short-term (i.e., days to weeks) decrement in exercise performance induced by overload training has been termed functional overreaching (FOR), whereby performance restoration (2, 33), and sometimes super-compensation (24, 46), may occur after a recovery period (~1–3 wk) (41, 54). FOR is considered to be a *necessary* component of a training program to improve performance in highly-trained athletes (32, 34, 53, 66). Despite this, recent research suggests that FOR is associated with disturbed sleep (36, 39, 42, 53), higher incidence of upper respiratory tract infection (URTI) (36, 75), impaired metabolism (44, 76, 77) and blunted training and performance adaptations (2, 11, 18) compared to non-overreached athletes who completed the same relative increase in training load. Thus, clarification of markers of training stress that may be associated with the onset of FOR is essential to ensure that the risk of maladaptation to overload training can be mitigated.

Common physiological parameters associated with exercise stress in response to overload training include heart rate/rhythm derived indices (1, 43, 44, 46), subjective fatigue perceptions (7, 8, 24, 25, 29, 33, 62, 72), circulating hormone concentrations (24, 31, 47, 59), markers of inflammation (31), blood lactaemia (44, 73) and resting metabolic rate (RMR) (76, 77). Unfortunately, few of these parameters have been shown to consistently delineate between FOR and non-overreached athletes (9), with the possibility that changes simply reflect general responses to overload training rather than a state of overreaching. Furthermore, the categorization of overreached subjects in some of these studies was confounded (59, 72) and not in accordance with the consensus statement defining the classification of overreaching (53). Recent studies by Woods et al (76, 77) suggest that reductions in RMR (in both cyclists (77) and rowers (76)) may signal FOR in endurance athletes in response to increased training load. However, changes in RMR may also be reflective of a failure to increase energy availability (55) and/or maintain fat free mass (FFM)

(63), rather than a state of overreaching, but this needs further clarification. Recent research (44, 46) suggests that submaximal (44) and peak heart rate (44) may be reduced, while heart rate recovery (HRR) may be faster (1, 43) in FOR endurance athletes. However, others have questioned the discernibility of these heart rate measures to differentiate between FOR and non-overreached athletes (14, 71). As such, changes in the idiosyncratic physiological variables associated with FOR require further investigation.

Undertaking a period of overload training (e.g., increases of 30-40% of training volume for 3-4 weeks) does not always result in FOR. Indeed, studies report 33-69% (1, 2, 11, 17, 18, 36, 45, 46, 51) of athletes develop FOR following increases in training volume of this magnitude. FOR, in some cases (2, 11, 18), is associated with impaired training adaptations and attenuated performance super-compensation following an overload training period. However, it must be noted that a number of other studies (23, 24, 33) have also demonstrated a substantial performance super-compensation following an overload and taper period in athletes who were classified as being FOR. Why some athletes respond optimally to periods of overload training, while others do not is currently unknown. One potential explanation may be related to individual differences in skeletal muscle fiber type composition (i.e., ratio of type I and type II fibers; muscle fiber typology). Muscle fibers can be identified as pure (i.e., type I, IIa, IIx) or hybrid fibers that co-express two or more myosin heavy chain isoforms (i.e., I/IIa, I/IIa/IIx, IIa/IIx, I/IIx) (16). Although type IIa fibers can possess equally high or even higher mitochondrial volume as type I fibers in endurance trained athletes (19, 57), the cross-bridges (74) and sarcoplasmic reticulum Ca^{2+} pumps (58) of these fibers consume more ATP than type I fibers. This would result in a mismatch between the rate of energy supply and rate of energy use, likely resulting in more pronounced impairments in sarcoplasmic reticulum Ca^{2+} release and greater fatigability. In support of this premise, sarcoplasmic reticulum function is markedly depressed with fatigue in both control subjects

and trained athletes, and is dependent on fiber type, but appears to be minimally affected by chronic training status (either endurance or resistance training) (48). As such, trained individuals with a higher proportion of type II fibers may have greater fatigability (48, 49, 67), take longer time to recover (27, 49) and may adapt optimally to low-volume, high-frequency contractions (40). Conceivably, variation in muscle fiber typology between individuals may be related to the incidence of overreaching and performance super-compensation in response to increases in training volume, but this remains to be elucidated. Recently, Baguet et al (3) developed a non-invasive method to estimate muscle fiber typology, based on the proton magnetic resonance spectroscopy (^{1}H-MRS) derived measurement of muscle carnosine. This method provides a valid alternative to the invasive muscle biopsy based on the significant positive correlation ($P = 0.009$ and $r = 0.71$) between the percentage area occupied by type II fibers and muscle carnosine content (3) and the close level of agreement with the performance characteristics of various athletes (3, 12). More recent evidence from Lievens et al (49) showed that this non-invasive estimation of muscle fiber typology strongly influenced the extent of fatigue and time to recover in the acute period (5 h) following intermittent sprint exercise. However, it remains to be determined whether the variation in muscle fiber typology between individuals is related to the individual responses to a long-term period of overload training.

The aim of the present study was to investigate whether ^{1}H-MRS derived measurement of muscle fiber typology was associated with incidence of overreaching, training-induced fatigue and performance super-compensation following an overload training period and subsequent taper. In addition, we monitored various subjective and physiological variables to provide further clarification on whether these could differentiate between individuals who show no performance decrease despite high perceived fatigue (i.e., non-overreached) compared to those who are classified as FOR. We hypothesized that highly-trained middle-

distance runners who have higher muscle carnosine levels (i.e., higher estimated proportion of type II fibers) may display more severe symptoms of overreaching in response to an increase in training volume.

METHODOLOGY

Subjects

Twenty-four highly-trained middle-distance runners participated in this study; sixteen males (age 21.0 ± 3.6 yr, stature 181.3 ± 5.1 cm, body mass (BM) 70.6 ± 7.9 kg, VO_{2peak} 73.3 ± 4.3 mL·kg·min^{-1}) and eight females (mean ± SD: age 21.3 ± 3.2 yr, stature 171.2 ± 4.9 cm, BM 53.1 ± 6.0 kg, maximal oxygen uptake (VO_{2peak}) 63.2 ± 3.4 mL·kg·min^{-1}). Inclusion criteria specified that subjects were trained specifically for middle-distance races (800 m & 1500 m), had a consistent training history of at least 2 yr in these events, and were without major injury interruption for the previous 3 months. Male runners had personal best times for the 800 m and 1500 m of 119.4 ± 7.8 s (range: 108.3 – 133.4 s) and 238.0 ± 16.8 s (225.2 – 279.1 s), respectively, while female runners had times of 135.0 ± 8.6 s (124.1 – 153.4 s) and 284.1 ± 18.8 s (257.4 – 321.4 s), respectively. In the 3 weeks preceding the study, mean running training volume for male and female runners was 73.9 ± 19.2 km·week^{-1} and 53.9 ± 16.0 km·week^{-1}, respectively. Five females were taking oral contraception, while the other three reported regular menstrual cycles. All runners provided written informed consent prior to participating. Ethics approval was granted by the University's Human Research Ethics Committee (XXXXXX, removed for peer-review).

General design

The study period lasted 7 weeks, which was divided into three distinct training phases; (1): 3 weeks of normal training (NormTr) prescribed by the runners' coach, (2): 3 weeks of high-volume training (HVTr; weekly stepwise increase in training volume by 10, 20 and 30%

during each successive week from NormTr), and (3): a 1-week taper (TapTr; 55% exponential reduction in training volume from HVTr week 3 (4, 15, 56)). Before, and immediately after each training phase, runners performed a maximal incremental running test to determine the gas exchange threshold (GET), respiratory compensation threshold (RCT), time to exhaustion (TTE), peak heart rate (HR_{peak}) and VO_{2peak}. A venous blood sample was collected and body composition, RMR and energy intake were assessed at each time point. Subjects were scanned by ^{1}H-MRS according to Baguet et al (3) to estimate muscle fiber type composition of the right gastrocnemius medialis muscle. Subjects were classified as FOR when their performance in the maximal incremental running test decreased following HVTr by an amount greater than the smallest meaningful change (SMC) in performance determined from before and after the NormTr period.

Testing procedures and standardization

Subjects attended the laboratory on five separate occasions; twice before (familiarization and baseline visit) and once after NormTr, and again after HVTr and TapTr. Subjects were provided with a standardized dinner (~55 kJ·kg BM^{-1}, 2.0 g carbohydrate·kg BM^{-1}, 0.3 g fat·kg BM^{-1}, 0.6 g protein·kg BM^{-1}) to consume each evening prior to attending the laboratory. Subjects presented to the laboratory between 5:00 – 7:30 am after an overnight fast and refraining from strenuous exercise for at least 40 h. Laboratory conditions were controlled (22 - 23°C and 45-50% humidity) throughout all tests. During each testing session, subjects underwent an assessment of RMR and body composition by dual-energy x-ray absorptiometry (DXA). A fasted venous blood sample was taken from the antecubital vein following DXA. Subjects were then provided with a standardized breakfast (~40 kJ·kg BM^{-1}, 1.8 g carbohydrate·kg BM^{-1}, 0.2 g fat·kg BM^{-1}, 0.1 g protein·kg BM^{-1}) and rested quietly for 1 h before undertaking submaximal and maximal incremental running tests.

Resting metabolic rate (RMR)

Indirect measurement of RMR was conducted after 10 min of rest to allow time for familiarisation and complete relaxation. Subjects were advised to breathe normally and stay as rested as possible without falling asleep for the duration of the test. Pulmonary gas-exchange variables (ventilation, VO_2, and VCO_2) were measured breath-by-breath via an open-circuit metabolic system (Ultima CardiO_2; Medical Graphics Corporation, St. Paul, MN). To classify steady state, we adopted criteria from Schlein et al (21). In brief, steady-state conditions were established as 30 s mean VO_2 and VCO_2 coefficient of variation (CV) values of ≤10% for five consecutive minutes. RMR was reported in absolute (kcal·day^{-1}) and relative (kcal·kg of fat free mass (FFM)·day^{-1}) terms. Quality control and calibration procedures were undertaken prior to each test.

Dual-energy x-ray absorptiometry (DXA)

DXA was used to determine whole body bone mineral content, fat and FFM (Medix DR, Medilink, France). The DXA was calibrated with phantoms in accordance with manufacturer guidelines each day prior to measurement. All DXA scans were performed and analyzed by one trained technician, with emphasis on consistency of positioning subjects on the scanning bed. Scans were analysed automatically by the software, but regions of interest were subsequently confirmed by the technician. Short-term DXA measurement precision in our lab is 0.9%, 2.3% and 0.8% for whole body bone mineral content, fat and FFM, respectively.

Submaximal running test

Following a warm-up (5 min at 8 – 10 km·h^{-1}), subjects completed two, 4-min submaximal incremental stages on a motorised treadmill (HP cosmos Saturn, Traunstein, Germany), which was set at a speed equivalent to 100% of the GET which was determined in the familiarisation testing session. The treadmill belt was set at 1% gradient to reflect the

214 energetic cost of running overground at these speeds (38). Each of the two stages were
215 interrupted by a 60-s rest period to allow earlobe blood sampling for determination of blood
216 lactate concentration ([La]b) with a Lactate Pro 2 device (Arkray inc. Japan). HRR was
217 assessed during the recovery period following each 4 min stage and reported as the absolute
218 difference between HR at cessation of the submaximal stage and HR recorded after 60 s of
219 recovery standing on the treadmill. Pulmonary gas exchange was measured on a breath-by-
220 breath basis throughout each stage using a calibrated metabolic system (Cosmed Quark b^2,
221 Rome, Italy) and rating of perceived exertion (RPE) was measured in the last 30-s period of
222 each stage.

223 *Maximal incremental running test*

224 Following 5 min of rest after the submaximal running test, each subject performed an
225 incremental treadmill run to volitional exhaustion; starting at 10 $km \cdot h^{-1}$ and 1% gradient,
226 with speed increased by 1 $km \cdot h^{-1}$ each minute until a speed of 21 $km \cdot h^{-1}$. After 1 min at 21
227 $km \cdot h^{-1}$, the gradient was increased by 1% each minute until volitional exhaustion. RPE was
228 measured at the end of each stage and gas exchange variables (VO_2, VCO_2 and expired
229 ventilation (V_E)) were measured (as described for the submaximal exercise test) and
230 subsequently averaged into 30 s bins. GET was determined using the V-slope method and
231 RCT was determined using the V_E-versus-VCO_2 relationship described by Beaver et al, (6).
232 Two investigators performed threshold determinations independently, and a third investigator
233 was consulted if any disagreement occurred. HR was recorded each second (H10, Polar
234 Electro, Oy, Finland) to determine values corresponding to GET, RCT and HR_{peak}. HRR was
235 determined from the 60 s of recovery standing on the treadmill directly following the test.
236 VO_{2peak} was determined as the average of the two highest consecutive 30 s VO_2 values, while
237 TTE was used as a measure of running capacity. [La]b was measured from the earlobe at 1, 3,

238 5, and 7 min after completion of the test, with the highest [La]b value obtained at the end of
239 exercise considered $[La]b_{max}$.

240 *Energy intake*

241 To quantify changes in energy intake subjects were asked to keep diet records for three days
242 prior to each laboratory visit. Specifically, the three days of recording included the two days
243 immediately prior to the laboratory visit and either a weekend day within that week (to ensure
244 1 weekend day was included) or the third day prior to the lab visit (if at least one of the three
245 days fell on a weekend day). The principal investigator met each athlete to provide detailed
246 instructions on how to accurately record all food/fluid. Subjects were asked to record the time
247 of intake for all meals, the type of food/fluid (including brand names) and amounts
248 consumed. To improve validity, food records where EI < 1.39 × RMR were excluded from
249 the analysis [63]. One member of the research team (JC) analyzed all diet reports using a
250 dietary analysis software package (FoodWorks 7; Xyris, Queensland, Australia). To assess
251 inter-rater reliability, an experienced dietitian (CI) also analysed 10% of the diet reports (n =
252 36). The CV was 4.6% for energy intake, and 5.1% for carbohydrate, 7.1% for protein and
253 9.3% for fat intakes.

254 *^{1}H-MRS estimation of muscle fiber typology*

255 Muscle carnosine content was measured by ^{1}H-MRS in the soleus and gastrocnemius
256 medialis muscle of each participant's right limb in order to estimate muscle fiber typology.
257 We chose to estimate the muscle fiber typology of the gastrocnemius medialis and soleus
258 because; i) we can measure carnosine reliably in both of these muscles, ii) carnosine content
259 in the gastrocnemius medialis muscle has been positively correlated with the percentage area
260 occupied by type II muscle fibers [49], and; iii) the gastrocnemius medialis and soleus are
261 very active muscles during running. Indeed, relative to a maximal voluntary contraction, the

gastrocnemius medialis has the highest mean and maximal electromyographic activity, while the soleus has the second and third highest, respectively, compared to other prominent lower limb muscles (70). This suggests that the fiber composition of these muscles may be meaningful in the context of training induced fatigue and adaptations to running training. ^{1}H-MRS measurements were performed on a 3-T whole body magnetic resonance imaging (MRI) scanner (Philips Medical Systems, Best, The Netherlands). Subjects were lying in a supine position, while their lower leg was fixed in a spherical knee-coil. All the spectra were acquired using single voxel point-resolved spectroscopy (PRESS) with the following parameters; repetition time (TR) of 2000 ms, echo time (TE) of ~40 ms, number of excitations was 128 (carnosine) and 16 (water), spectral bandwidth was 2048 Hz, and an acquisition time of 4 min 16 s (carnosine) and 32 s (water). The voxel size was 40 mm x 15 mm x 20 mm. The voxel location was standardized in the center of the medial portion of both muscles. The same well-trained and experienced MRI technician (BK) was responsible for placing the voxel on all scans. The scan for each subject was completed within two weeks following the post-TapTr testing session. Each subject was scanned in the morning and had not completed any exercise prior to the scan. Spectral data analysis was carried out using jMRUI (version 6.0) with carnosine peaks fitted and expressed relative to the internal water signal

Carnosine content (mM) was calculated using following formula:

$$\mathrm{Cm} = \frac{(\mathrm{C_S})}{(\mathrm{H_2O_S})} \cdot \frac{(\mathrm{H_2O_{T1r}})}{(\mathrm{CT_{1r}})} \cdot \frac{(\mathrm{H_2O_{T2r}})}{(\mathrm{CT_{2r}})} \cdot \mathrm{H_2O_{muscle}} \cdot \mathrm{H_2O_{protons}} \qquad [1]$$

where Cm is the carnosine concentration, C_S is the carnosine signal, H_2O_S is the water signal, C_{T1r}, C_{T2r}, H_2O_{T1r}, H_2O_{T2r} are the relaxation correction factors for carnosine (earlier described by Baguet et al (3)) and water (earlier described by MacMillan et al (52)), H_2O_{muscle} is the

concentration of water in muscle, which was deducted from the molar concentration of water (55,000 mM) and the approximate water content of skeletal muscle tissue (0.7 L/kg wet weight of tissue) and H_2O_{proton} is the number of protons in water. The CV for test-retest inter-day carnosine measurements in our laboratory was 3.5% (soleus) and 4.3% (gastrocnemius; n = 15 subjects). The carnosine concentration was converted to a muscle- and sex-specific z-score relative to an age-matched control population of active, healthy non-athletes (males: n = 38; females: n = 30).

Training monitoring

To monitor training volume and intensity, each subject wore either an M430 GPS running watch (n=14; Polar, Kempele, Finland) or a Garmin Forerunner 235 (n = 8; Garmin, Canton of Schaffhausen, Switzerland) during every running session. Training intensity distribution was quantified from running speed using the total time-in-zone approach quantified by training analysis software (TrainingPeaks WEEKO+, Boulder, CO, USA). The percentage of training time spent with a running speed in each of the three training zones was quantified for each individual training session. The relative training time in each zone for all sessions was then determined. The three training zones according to the reference running speed values that corresponded to physiological thresholds obtained during the maximal running assessment were used: zone I (<GET), zone II (between GET and RCT) and zone III (>RCT). Subjects were also provided with a training diary instructing them to rate the global intensity (RPE; CR-10), volume and duration of all training sessions and races. The 10-point scale was divided into three training zones based on fixed RPE values with training zone 1 = RPE of 1–4, training zone 2 = RPE of 5–6; and training zone 3 = RPE of 7-10 (61). The total training time spent with an RPE within each one of these RPE derived training zones was determined. In addition, session RPE (sRPE) training load was determined by multiplying the intensity of

each training session by the duration of the session (28). Total weekly load was calculated for each week by summation of the daily loads.

Wellness and URTI questionnaires

Subjects rated their physical and mental well-being by means of a visual analogue scale (VAS) as 1–10, with 1 representing the most negative outcome. The questionnaire contained seven items and was completed at the end of each week throughout the duration of the study. Items included sleep quality, general mental well-being, general physical well-being, readiness to train, muscle soreness, fatigue and non-training stress. Subjects also completed the Wisconsin Upper Respiratory Symptom Survey (WURSS) to assess URTI severity and symptomatology (5) at the end of each week and were asked to provide their response relative to that week. The questionnaire included one global question, ten symptom-based questions and nine functional impairment questions. An overall URTI symptom score was calculated by summing the URTI severity score (0 = not sick, 1 = very mild URTI to 7 = severe) from the symptom-based and functional impairment questions (theoretical maximum score being 133) as proposed by Barrett et al (5). A single incidence of an URTI was defined as a period during which the weekly total symptom score was ≥21 and separated by 1 wk from another week with a total symptom score ≥21.

Blood sampling and analysis

Venous blood samples were collected during each of the laboratory visits (pre- and pre-HVTr, post-HVTr and post-TapTr) for determination of various blood biomarkers. Two 10 mL samples were collected into vacutainers containing either ethylenediaminetetraacetic acid (EDTA) or no anticoagulant (for serum). The EDTA tube was centrifuged immediately for 10 min at 1350 x g, while the serum tube was left to clot for 30 min before being centrifuged. The resultant samples were stored at -80°C for subsequent analysis. Serum ferritin, iron, total

iron binding capacity, direct and total bilirubin, total protein, urea, uric acid, lactate dehydrogenase (LDH), creatinine and C-reactive protein (CRP) were assessed using an automated clinical chemistry analyser (Au480, Beckman Coulter, Australia) according to the manufacturer's instructions for use (IFU). Total vitamin D (25-OH Vitamin D), total and free T4, total and free T3, thyroid stimulating hormone (TSH), thyroid uptake (i.e. the measure of the unbound thyroxine binding globulins in the blood), cortisol, testosterone, dehydroepiandrosterone sulphate (DHEA-S), human growth hormone (hGH) and interleukin 6 (IL-6) were assessed using an automated immunoassay analyser (Access 2, Beckman Coulter, Australia) according to the manufacturer's IFU. All parameters were calibrated and reported acceptable QC values prior to analysis. Testosterone:cortisol ratio and transferrin saturation were calculated following analytical quantification. A commercially available ELISA was performed to determine GDF-15.

Assessment of overreaching

In line with previous research (2, 46), the SMC was used as an overreaching threshold which was calculated as 0.5 × CV of TTE from the incremental running tests performed before and after NormTr. To be classified as FOR following HVTr, subjects had to report an elevated subjective fatigue rating following HVTr and show an individual performance decrement larger than the SMC. The remaining subjects who maintained or increased their performance, but also showed an elevated subjective fatigue rating following HVTr, were considered to be acutely fatigued (AF).

Statistical analysis

Results are expressed as mean ± SD unless stated otherwise. A two-way (group and training phase) analysis of variance (ANOVA) was used to identify differences in performance and physiological variables between FOR and AF groups. A three-way (group, training phase and

training zone) ANOVA was used to analyse the training intensity zone distribution data. If a significant main effect was found, pairwise comparisons were conducted using Tukey post-hoc analysis. Blood biomarker responses were also analysed using an analysis of co-variance (ANCOVA). The pre-NormTr values were entered into the model as a covariate in order to account for between-subject variations in blood biomarker levels that may arise from sex-based differences (65) as well as possible differences between naturally menstruating females and those using oral contraception (13). Regardless of group (FOR and AF), a two-way repeated measures analysis of variance (ANOVA) with Tukey post-hoc comparisons was used to compare blood biomarker responses between male and female subjects. The effect size (*d*) statistic was also calculated to assess the magnitude of difference between groups. The magnitude of difference was classified as small 0.2 to 0.59, moderate 0.6 to 1.19, large 1.2 to 1.99, very large 2.0 to 3.99, and extremely large >4.0 (37). All statistical analyses were performed using SPSS 25.0 (SPSS Inc, Chicago, IL, USA), with statistical significance accepted as $p < 0.05$. Test-retest reliability of running TTE, VO_{2peak} and RMR values were analysed using the CV.

RESULTS

Incidence of overreaching

The CV for initial incremental TTE tests was 6.3%, hence the SMC was considered 3.15%. Using this criteria, twelve subjects were classified as FOR following HVTr (decreased running TTE from pre- to post-HVTr), whereas the other twelve subjects were classified as AF (no decrease in running TTE).

Submaximal and maximal incremental running test

There were no between-group differences in the pre- to post-HVTr change in submaximal (running speed equivalent to 100% of GET) HR (AF: 2 ± 5 vs. FOR -1 ± 6 beats·min^{-1}),

381 [La]b (AF: -0.14 ± 0.58 vs. FOR -0.27 ± 0.61 mmol·L^{-1}), RPE (AF 0.0 ± 0.6 vs. FOR 0.1 ±
382 0.9 AU; p = 0.70), or HRR (AF -1 ± 5 vs. FOR 2 ± 6 beats·min^{-1}; all p > 0.05) when
383 measured at a running speed equivalent to 100% of GET.

384 There was a significant between-group difference for changes in HRR following exhaustive
385 running (AF = -1 ± 5 vs. FOR 5 ± 5 beats·min^{-1}; p = 0.01; figure 1), as well as the change in
386 RPE at RCT (AF -0.1 ± 1.4 vs. FOR 1.2 ± 1.6 AU; p = 0.02). Furthermore, HR_{peak} (-4 ± 3
387 beats·min^{-1}; p = 0.02) and $[La]b_{max}$ (-4.30 ± 1.80 mmol·L $^{-1}$; p = 0.002) were both reduced
388 from pre-HVTr to post-HVTr in the FOR group compared to the AF group, with both
389 parameters returning to pre-NormTr values following TapTr (figure 1). Running TTE and
390 VO_{2peak} did not change across the HVTr period in the AF group (+16 ± 17 s, +1.64 ± 1.80
391 mL·kg·min^{-1}), while there was a significant decrease in the FOR group (-49 ± 14 s, -2.33 ±
392 2.20 mL·kg·min^{-1}, p < 0.001). Compared to the FOR group, the AF group had a significantly
393 larger improvement in TTE from pre-HVTr to post-TapTr (absolute difference score: +37 ±
394 31 s; p = 0.04), while improvement in VO_{2peak} was similar between groups (AF: +3.52 ± 1.40
395 mL·kg·min^{-1}; FOR: +2.78 ± 1.80 mL·kg·min^{-1}; p = 0.45). There was no change in the GET
396 or RCT at any time point for either group.

397 *Muscle fiber typology*

398 The highly-trained middle-distance runners in the present study predominantly had negative
399 carnosine z-score values (20/24 runners), suggesting a higher proportion of type I fibers, but
400 the range was large in both soleus (z-score range: -2.51 to 1.00) and gastrocnemius (-2.02 to
401 0.46). Gastrocnemius carnosine z-score was significantly higher in FOR (-0.44 ± 0.57; range,
402 -1.32 – 0.46) compared to AF (-1.25 ± 0.49; -2.02 – -0.47, p = 0.004, d = 1.53; figure 2), but
403 not soleus carnosine z-score (FOR: -1.03 ± 0.92; -2.51 – 0.44, AF: -1.55 ± 0.93; -2.46 – 1.00,
404 p = 0.10, d = 0.56). Gastrocnemius carnosine z-score showed a significant negative

correlation with the change in running TTE from pre-HVTr to post-HVTr (r = -0.55, r^2 = -0.31, p = 0.005; figure 3) and pre-HVTr to post-TapTr (r = -0.64, r^2 = -0.41, p = 0.008; figure 3). Soleus carnosine z-score was not associated with the change in running TTE from pre-HVTr to post-HVTr (r = -0.21, r^2 = -0.04, p = 0.33), but was negatively correlated with the change in running TTE from pre-HVTr to post-TapTr (r = -0.45, r^2 = -0.20, p = 0.013).

RMR, body composition and macronutrient intake

The CV for absolute (MJ·day^{-1}) and relative RMR (kJ·kg·FFM·day^{-1}) was 5.1% and 4.8%, respectively. No significant time or time × group effect was evident for either absolute or relative RMR (table 1). Similarly, there was no significant change in BM, body fat percentage, or FFM in either group throughout the study period (table 1). There was a significant time effect for relative energy intake for the FOR group, whereby energy intake during the HVTr period (175 ± 71 kJ·kg·BM·day^{-1}) was greater than both pre-NormTr (148 ± 41 kJ·kg·BM·day^{-1}; p = 0.04) and NormTr (140 ± 36 kJ·kg·BM·day^{-1}; p = 0.004) periods. In the AF group, there was a non-significant 7 ± 18% increase in energy intake during the HVTr period (160 ± 39 kJ·kg·BM·day^{-1}) compared to the NormTr period (150 ± 17 kJ·kg·BM·day^{-1}; p = 0.62). There were no between group differences at any time point.

Blood parameters

There was no significant time or time x group effect for serum ferritin, iron, total iron binding capacity, direct and total bilirubin, total protein, urea, uric acid, LDH, creatinine, CRP, total vitamin D, total and free T4, total and free T3, TSH, thyroid uptake, cortisol, testosterone, DHEA-S, hGH, GDF-15, IL-6 or the testosterone:cortisol ratio (all p > 0.05; table 2). Regardless of group (FOR or AF), females had lower levels of ferritin, DHEA-S, LDH, testosterone and the testosterone:cortisol ratio at each time point compared to males (all p < 0.05).

Training monitoring

There were no between group differences in training volume at any time point. Training volume increased from NormTr (3 week mean; FOR 66.2 ± 21.2 km; AF 68.3 ± 19.5 km) throughout HVTr week 1 (FOR 77.3 ± 24.3 km; AF 75.9 ± 19.6 km), week 2 (FOR 85.9 ± 28.0 km; AF 84.5 ± 19.5 km), and week 3 (FOR 92.9 ± 30.1 km; AF 90.7 ± 19.9 km; all $p <$ 0.001), and was reduced during TapTr (FOR 43.2 ± 13.5 km; AF 42.1 ± 9.9 km; $p < 0.001$; table 3). There were no between group changes, nor was there a time effect on the running speed derived training intensity distribution (table 5). In contrast, the RPE derived training intensity distribution was altered during the third week of HVTr, whereby the FOR group spent a significantly greater time in zone 3 and reduced time in zone 1 (zone 1: 28.2 ± 4.8%, zone 2: 33.1 ± 6.2%, zone 3: 38.7 ± 3.8%) compared to each week of NormTr (all $p < 0.05$), the first week of HVTr (zone 1: 35.7 ± 6.5%; p = 0.01, zone 2: 35.9 ± 7.5%, zone 3: 28.4 ± 9.5%; $p = 0.004$) and TapTr (zone 1: 36.2 ± 8.1%; $p = 0.006$, zone 2: 34.7 ± 5.9%, zone 3: 29.1 ± 9.2%; $p = 0.009$). Conversely, there was no change in the RPE derived training intensity distribution in the AF group ($p > 0.05$).

Wellness questionnaires

There were no significant group × time interactions between AF and FOR for any items of the wellness questionnaire. Regardless of group, participants reported reductions in physical well-being, readiness to train and mood, as well as increased muscle soreness and fatigue following week two and three of HVTr (table 4). There was a significant effect of time for reductions in perceived sleep quality that were only evident in the FOR group after the second (5.6 ± 2.0 AU; $p = 0.009$) and third week of HVTr (5.4 ± 1.5 AU; $p = 0.004$), which returned to NormTr levels (7.7 ± 1.0 AU) after TapTr (7.8 ± 1.2 AU). During HVTr, seven subjects reported at least one episode of URTI, with five of these occurring from subjects in

453 the FOR group. There was a significant time effect in the FOR group for URTI symptom
454 score following the third week of HVTr (27.5 ± 36.8, $p = 0.002$).

455 **DISCUSSION**

456 In the present study, we have shown that ^{1}H-MRS derived measurement of muscle fiber
457 typology is associated with incidence of FOR following a period of overload training and
458 performance super-compensation following a taper. That is, trained middle-distance runners
459 who became FOR had a higher gastrocnemius carnosine z-score (estimated to have a higher
460 proportion of type II fibers) compared to those that were non-overreached following a period
461 of overload training, while FOR also had a reduced performance super-compensation
462 following a subsequent taper period. We also show that FOR is not associated with
463 systematic alterations in absolute or relative RMR, resting blood biomarkers, subjective
464 fatigue questionnaire ratings or submaximal exercise responses.

465 The incidence of FOR following HVTr observed in the present study (12/24 runners), is in
466 agreement with rates reported in other studies employing similar overload training periods
467 (i.e., increases of 30-40% of training volume for 3-4 weeks) (2, 36, 46, 51). The magnitude of
468 change in running capacity following HVTr (mean change: -16 ± 43 s, range: -120 to 65 s)
469 and TapTr (mean change: +38 ± 35 s, range: -20 to 120 s) was highly variable. Variation in
470 the individual muscle carnosine z-score values in the gastrocnemius did explain a significant
471 magnitude of the variability in the performance responses following HVTr and TapTr,
472 relative to pre-HVTr. Runners with an estimated higher proportion of type I fibers (i.e., lower
473 carnosine z-score) were able to maintain performance in response to overload training and
474 obtained a superior performance super-compensation following the taper. These findings
475 suggest that runners with an estimated higher proportion of type-I fibers are able to better
476 cope with increases in training volume and achieve superior performance adaptations. While

type II fibers can possess equally high or even higher mitochondrial volume as type I fibers in endurance trained athletes (19, 57), differences in cross-bridge (74) and sarcoplasmic reticulum Ca^{2+} pump ATP consumption (58) may result in greater fatigability (48, 49, 67), delayed recovery (27, 49) in type II fibers. Conversely, type I fibers are fatigue resistant (35), but may adapt optimally to low frequency, higher-volume contractions (60). Conceivably, individuals with a high proportion of type I fibers may therefore adapt more favourably to increases in training volume. On the other hand, individuals with a high proportion of type II fibers may develop greater residual fatigue from increased training volume and suboptimal adaptations in these fibers, resulting in impaired performance and a higher incidence of FOR. Indeed, short-term overload training can reduce type II fiber size (27), while improvements in maximal shortening velocity appears to be resigned to type I fibers, (27, 68). More recent work from Lievens et al (49) reported that the ^{1}H-MRS derived measurement of muscle fiber typology of the gastrocnemius was associated with the magnitude of fatigue within an intermittent sprint exercise session, as well as the recovery timeline in a well-controlled 5-h recovery period. Individuals classified as having fast-twitch typology had still not fully recovered maximal voluntary torque production of the knee extensors after 5 h of recovery, while those with slow-twitch typology had fully recovered after 20 min (49). Collectively, these findings (27, 49, 68) lead to the hypothesis that both acute and longer-term periods of overload training may result in residual fatigue possibly due to impairments in the contractile properties of type II fibers leading to impaired exercise performance, which may provide mechanistic evidence supporting the findings of the present study. Given that the runners in the present study classified as FOR had higher gastrocnemius carnosine z-score values (and presumably a higher proportion of type II fibers) compared to the AF group, it may be that the functional characteristics of these fibers were impaired by the HVTr period, leading to impaired running performance following the overload period. While soleus carnosine z-score

was significantly negatively correlated with the change in running TTE from pre-HVTr to post-TapTr, the associations between gastrocnemius carnosine z-score and the change in running TTE across both the HVTr and TapTr period were stronger compared to the soleus. While both the soleus and gastrocnemius have very high levels of relative muscle activation during running compared to other major lower limb muscles (70), it may be that the absolute contribution of the gastrocnemius muscle to ground reaction force during running is more influential than the soleus. Thus, the fiber type composition of the gastrocnemius may be more meaningful in the context of training induced fatigue and adaptations to running training compared to the soleus.

In the present study, each week of HVTr was completed with the same weekly distribution, type, and content of running training sessions as the corresponding week in NormTr but with the prescribed increased volume (i.e., +10-30%). While there were no between group differences in the total training volume (duration or distance covered), or the running speed derived training intensity distribution, subjects' perceptions of the training differed. During the third week of HVTr, the FOR group perceived more of the training sessions to have an RPE >6; thus, accumulating more time in training zone 3 using the RPE derived training intensity distribution. This also resulted in a significantly larger sRPE training load during the third week of HVTr compared to the AF group. While previous research indicates that the method of training-intensity quantification substantially affects computation of training intensity distribution (10), this is the first study to show clear delineation in training intensity distribution computed from two different measures of training intensity (external work rate and perceived intensity) in response to alterations in training volume. This is also supported by observations of significantly greater RPE at running speeds equivalent to the RCT in the FOR compared to AF group following HVTr. As such, runners in the FOR group perceived the intensity of running at speeds approximating the RCT to be substantially higher and

527 training sessions that incorporated similar running speeds were perceived to be more intense,
528 particularly during the third week of HVTr. While the running speed derived training
529 intensity distribution suggests that training intensity was not hampered during HVTr (i.e.,
530 similar time in zone 3 throughout the study), one limitation of the 3-zone training model is
531 that the third training zone includes all the training time accumulated with a running speed
532 greater than the speed equivalent to RCT. Given this spans a range of physiological and
533 mechanical characteristics (i.e., RCT to maximal sprinting speed), it may reduce the
534 sensitivity of detecting small decrements in running speed during training, where repetitions
535 may be completed at a running speed >RCT.

536 With the exception of a higher RPE at a running speed equivalent to RCT, other
537 physiological responses to submaximal exercise were unable to differentiate between FOR
538 and non-overreached participants in the present study. In contrast, reductions in VO_{2peak},
539 HR_{peak}, $[La]b_{max}$ and faster HRR during exhaustive running were greater in the FOR group
540 compared to the AF group. These findings are in agreement with previous literature reporting
541 reductions in VO_{2peak}, HR_{peak}, and $[La]b_{max}$ (14, 44) and faster HRR (1, 43) in FOR athletes.
542 However, these studies have also typically observed altered physiological responses during
543 submaximal exercise, but it should be noted that this is not a universal finding. Indeed,
544 Bellenger et al (8) suggests that HRR is only sensitive to changes in training status when
545 assessed after maximal exercise. Nonetheless, a key sentiment based on findings from the
546 present study and previous work (1, 8, 14, 43, 44), is that multiple physiological variables
547 should be measured to monitor fatigue associated with training; and changes in these
548 variables should be interpreted in the context of the specific training phase.

549 In the present study, both groups reported adverse effects based on changes to the majority of
550 subjective weekly wellness responses. However, only the FOR group reported impairments in
551 sleep quality (i.e., during the second and third week of HVTr) as well as higher URTI

552 symptom scores and URTI incidence in week 3 of HVTr. In addition, the FOR group had
553 moderately higher effect size differences (despite not being significant) in subjective fatigue
554 ratings after the first and second week of HVTr. These findings are consistent with previous
555 literature reporting impaired sleep and increased susceptibility to infection in overreached
556 endurance athletes (36) and exacerbated subjective fatigue ratings in athletes who become
557 FOR (1, 2, 36, 45). More recently, Ten Haaf (72) demonstrated that the combination of
558 changes in subjective fatigue and readiness to train after only 3 days of a cycling tour
559 correctly predicted 78% of the participants as either FOR or not using simple visual analogue
560 scales. However, despite not being significantly different, there was still a large reduction in
561 incremental cycling test peak power output in the FOR group approximately one month
562 following the cycling event which may indicate that at least some of these participants were
563 NFOR and not FOR (72). Nonetheless, while there is some evidence (1, 2, 36, 45) that
564 subjective questionnaires can differentiate between athletes who are FOR and not following
565 an overload training period, more research is needed to determine if these responses manifest
566 prior to a decrement in exercise performance.

567 Changes to a number of blood biomarkers have been associated with overload training
568 responses (31), but few have consistently been shown to differentiate between FOR and non-
569 overreached athletes. The present study involved quantifying the change to a range of blood
570 biomarkers, reflecting inflammation (IL-6 and CRP), metabolism (GDF15, thyroid
571 hormones), catabolic and anabolic biomarkers (DHEA-S, urea, total protein, testosterone,
572 cortisol and GH), muscle damage (lactate dehydrogenase), kidney function (creatinine) and
573 iron regulation (iron, ferritin and UIBC) relative to overload training responses. We failed to
574 observe any parameter (measured at rest) that was able to differentiate between FOR and
575 non-overreached athletes. These results are in agreement with Lehmann et al (47) who found
576 no significant changes in thyroid hormones in middle- and long-distance runners following a

two-fold increase in training volume. Previous research in cyclists and triathletes who were classified as FOR also indicated no changes in various hormone levels (testosterone, cortisol and growth hormone) (73). Likewise, changes in urea and markers of iron regulation do not appear to differentiate FOR and non-overreached athletes (22). Taken collectively, no resting blood biomarkers have been established as a sensitive predictor of FOR in endurance athletes.

Recently, GDF15 has been identified as a potential blood biomarker of overreaching (59). GDF15 is thought to be a stress-responsive biomarker related to the regulation of inflammatory processes (26), as well as appetite regulation (69) and bone metabolism (59). Poffe et al (59) substantially increased the training load of male subjects for 3 weeks and observed increased systemic levels of GDF15 in subjects who consumed a placebo drink (~292 ± 19 pg·ml^{-1} to 435 ± 29 pg·ml^{-1}). Results of the present study differ from those of Poffe et al (59), whereby we found no significant differences in systemic levels of GDF15 post-HVTr in both the FOR and AF groups. Several explanations for the contrasting findings may exist. For instance, the training status of subjects (healthy, non-specifically trained males vs highly-trained middle-distance runners), the nature of the overload training period (3-fold increase vs 10-30% increase) and the absolute levels of GDF15 (pre-training baseline: ~280 pg·mL^{-1} vs 541 pg·mL^{-1}), whereby the post-overload training GDF15 values reported in the male subjects of Poffe et al (59) (placebo group: 435 ± 29 pg·ml^{-1}) were still lower than the pre-NormTr levels of the highly-trained runners in the present study. It is possible that GDF15 concentrations may provide a general marker of training-induced physiological stress associated with the high training volume (67.1 ± 20.4 km·wk^{-1}) and frequency (6-8 running sessions·wk^{-1}) employed by the athletes in the present study. This suggests GDF15 may not be a sensitive marker to diagnose development of overreaching in trained athletes.

In the present study, we did not observe alterations in RMR in response to HVTr or TapTr. This finding contrasts that of two recent studies reporting a reduction in RMR with increased training load in well-trained endurance athletes (76, 77). The mechanism behind the reduced RMR in the previous studies (76, 77) is unclear. It is possible that the increased energetic demands of training, coupled with insufficient energy intake, are contributing factors. Indeed, despite a 21% increase in training load, participants in the study by Woods et al (76) did not increase their total energy or macronutrient intake, while trained cyclists in the study by Woods et al (77) had a significant reduction in FFM. Given that FFM (63) and energy availability (55) are major determinants of RMR, failure to increase energy intake and/or preserve FFM in response to increases in training load may be responsible for the reductions in RMR evident in these studies (76, 77). In the present study, the FOR group increased energy intake during the HVTr period (175 ± 71 $kJ \cdot kg \cdot BM \cdot day^{-1}$) compared to pre-NormTr (148 ± 41 $kJ \cdot kg \cdot BM \cdot day^{-1}$) and NormTr (140 ± 36 $kJ \cdot kg \cdot BM \cdot day^{-1}$). The AF group had a non-significant increase in energy intake during the HVTr period (160 ± 39 $kJ \cdot kg \cdot BM \cdot day^{-1}$) compared to pre-NormTr (149 ± 17 $kJ \cdot kg \cdot BM \cdot day^{-1}$) and NormTr (150 ± 17 $kJ \cdot kg \cdot BM \cdot day^{-1}$), while BM and FFM were preserved in both groups. As such, a reduction in RMR is not likely to be indicative of a given fatigue-induced training state per se (i.e., FOR), rather a reflection of an individual's inability to compensate for increases in training load by increasing energy intake.

A strength of the present study is the comprehensive attainment of data on energy availability before and after each training phase (i.e., energy intake, RMR and body composition) as well as pre-testing dietary standardization (evening meal and breakfast). Estimates of EI rely on the notoriously difficult task of gaining valid and reliable information about an athlete's habitual dietary intake by self-reporting which is prone to errors of underreporting (20). As such, we were only able to consider diet reports for 16 of the 24 subjects as eight subjects

(FOR = 4; AF = 4) were identified as having implausible food records (30). The taper characteristics in the present study are in line with recommendations based on a meta-analysis (15), modelling (4) and experimental studies (56) suggesting that a ~50% reduction in training volume in an exponential decay fashion over a period of 1 – 2 week can elicit peak performance improvements in endurance athletes. Despite this, it would have also been intriguing to extend our taper period beyond 1 wk, given the longer recovery time course of type II fibers (27, 50). It is likely that an optimal taper period should be individualised for each athlete (64), and it has been suggested that longer taper periods may be required following an overload training period due to greater stress and fatigue (66). Future research should investigate whether tapering strategies could be optimised by considering the muscle fiber typology of endurance athletes. Finally, the direct measurement of muscle fiber typology derived from a muscle biopsy may have provided further insight into the relationships between pure and hybrid fibers and the performance and physiological responses to alterations in training volume.

The main findings of the present study were that highly-trained middle-distance runners who became FOR following a period of overload training had a substantially higher gastrocnemius carnosine z-score (higher estimated proportion of type II fibers) and a reduced performance super-compensation following a subsequent taper period, compared to runners with a lower gastrocnemius carnosine z-score. We also showed that FOR was associated with altered perceptual responses to training but there were no systematic changes in RMR, resting blood biomarkers or submaximal exercise responses compared to runners who did not demonstrate impaired performance. These findings may have important applications in the development of individualized training advice and the monitoring of training load for endurance athletes undertaking overload training periods. More specifically, athletes with lower gastrocnemius carnosine Z-score values may have more favourable responses to periods of overload training

650 and a subsequent taper. This non-invasive estimation of muscle fiber typology could be used
651 a tool to a priori identify which athletes may be more likely to respond favourably to a
652 training volume overload period.

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882

883 **FIGURES:**

884 **Figure 1 –** Mean (95% confidence intervals) of time to exhaustion (A), VO_{2peak} (B) peak
885 blood lactate concentration (C), peak heart rate (D) and heart rate recovery (E) measured
886 before NormTr, before and after HVTr and after TapTr for the FOR group and AF group (n =
887 12 per group). A two-way (group and training phase) ANOVA with Tukey post-hoc analysis
888 was used.

889 [a]Significantly different compared to pre-NormTr, pre-HVTr and post-TapTr

890 [b]Significant difference between FOR and AF

891 [c]Significantly different compared to pre-NormTr and pre-HVTr

892

Figure 2 – Mean (95% confidence intervals) of the training intensity distribution, quantified as the percentage of total time spent in each of the three training zones based on running speed for the AF group (A) and FOR group (B) and based on rating of perceived exertion with AF group (C) and FOR group (D) during NormTr, HVTr (week 1, 2 and 3) and TapTr (n = 12 per group). A three-way (group, training phase and training zone) ANOVA with Tukey post-hoc analysis was used.

[a]Significantly different compared to NormTr, HVTr week 1 and TapTr

[b]Significantly different compared to NormTr, HVTr week 1 and TapTr

Figure 3 – Mean (95% confidence interval) of the gastrocnemius carnosine z-score of the AF and FOR group (n = 12 per group). A one-way ANOVA was used.

[a]Significant difference between FOR and AF

Figure 4 – Association between ^{1}H-MRS estimation of muscle fiber typology (gastrocnemius carnosine z-score) and the relative change in time to exhaustion from pre- to post-HVTr (A) and pre-HVTr to post-TapTr (B). Shaded area represents the smallest meaningful change (half the CV%). Linear regression was used and all subjects were included in the analysis regardless of group (i.e., FOR and AF; n = 24 in total).

TABLES:

Table 1 - Mean (SD) values for body composition, resting metabolic rate and macronutrient and energy intake measured before and after NormTr, and after the HVTr and TapTr period for the FOR group and AF group.

NormTr: normal training period, HVTr: high-volume training period, TapTr: taper training period, RMR: resting metabolic rate, FFM: fat-free mass, FOR: functional overreaching, AF: acutely fatigued

[a]Significantly different compared to pre-NormTr, pre-HVTr and post-TapTr

Table 2 - Mean (SD) values for subjective wellness questionnaire responses and upper respiratory tract infection symptom score and occurrences measured during each training phase for the FOR group and AF group.

NormTr: normal training period, HVTr: high-volume training period, TapTr: taper training period, URTI: upper respiratory tract infection, FOR: functional overreaching, AF: acutely fatigued

[a]Significantly different compared to each week of NormTr and TapTr. [b]Significant difference between groups

Table 3 - Mean (SD) values for weekly training duration, volume, load and distribution of training intensity during each training phase for the FOR group and AF group.

NormTr: normal training period, HVTr: high-volume training period, TapTr: taper training period, RPE: rating of perceived exertion, sRPE: session RPE training load, TID: training intensity distribution, FOR: functional overreaching, AF: acutely fatigued

[a]significantly different compared to each week of NormTr and TapTr

[b]significantly different compared to each week of NormTr, HVTr week 1 and TapTr

[c]significantly different compared to each week of NormTr, HVTr week 1 and 2 and TapTr

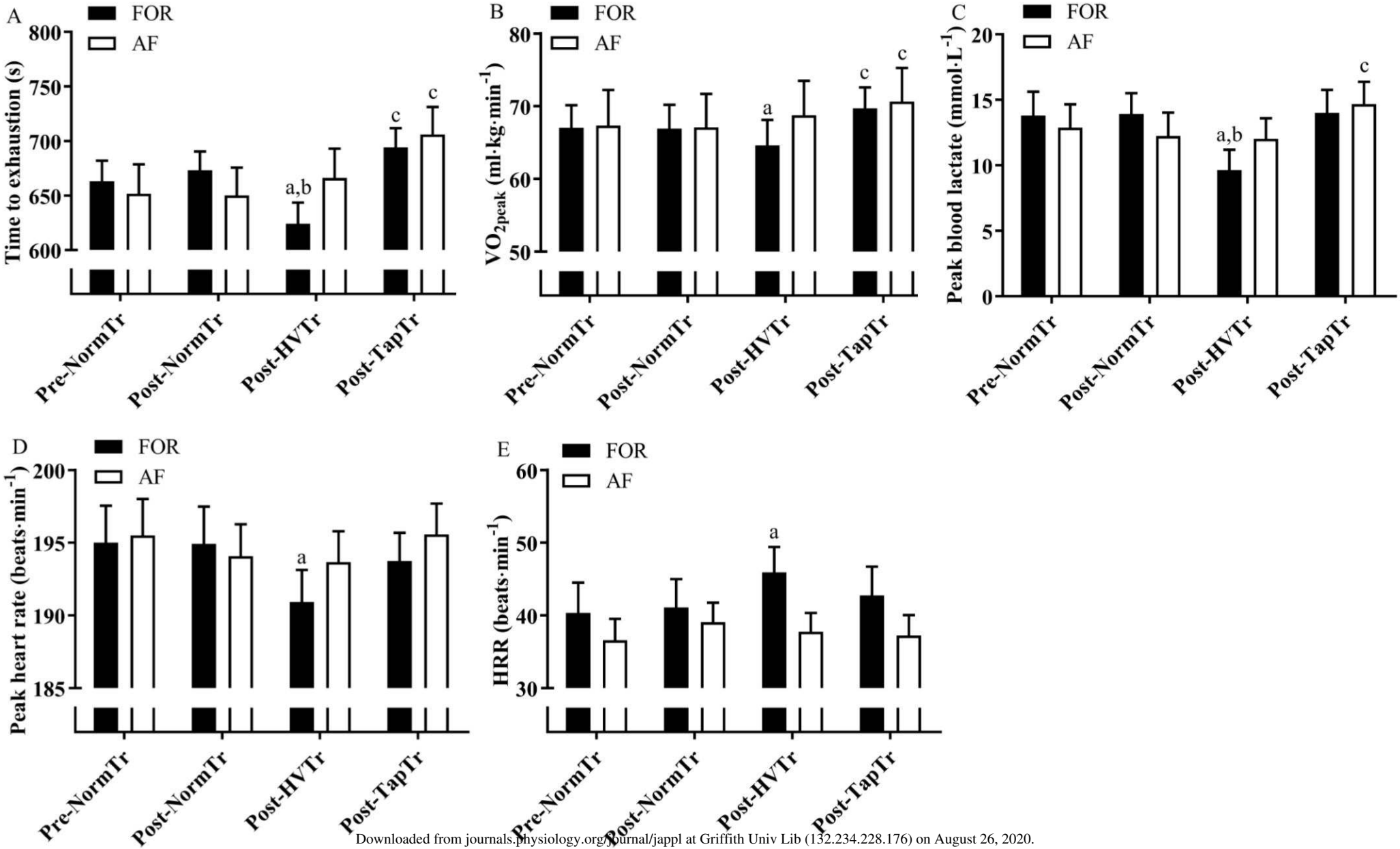
[d]significantly different compared to each week of NormTr and HVTr

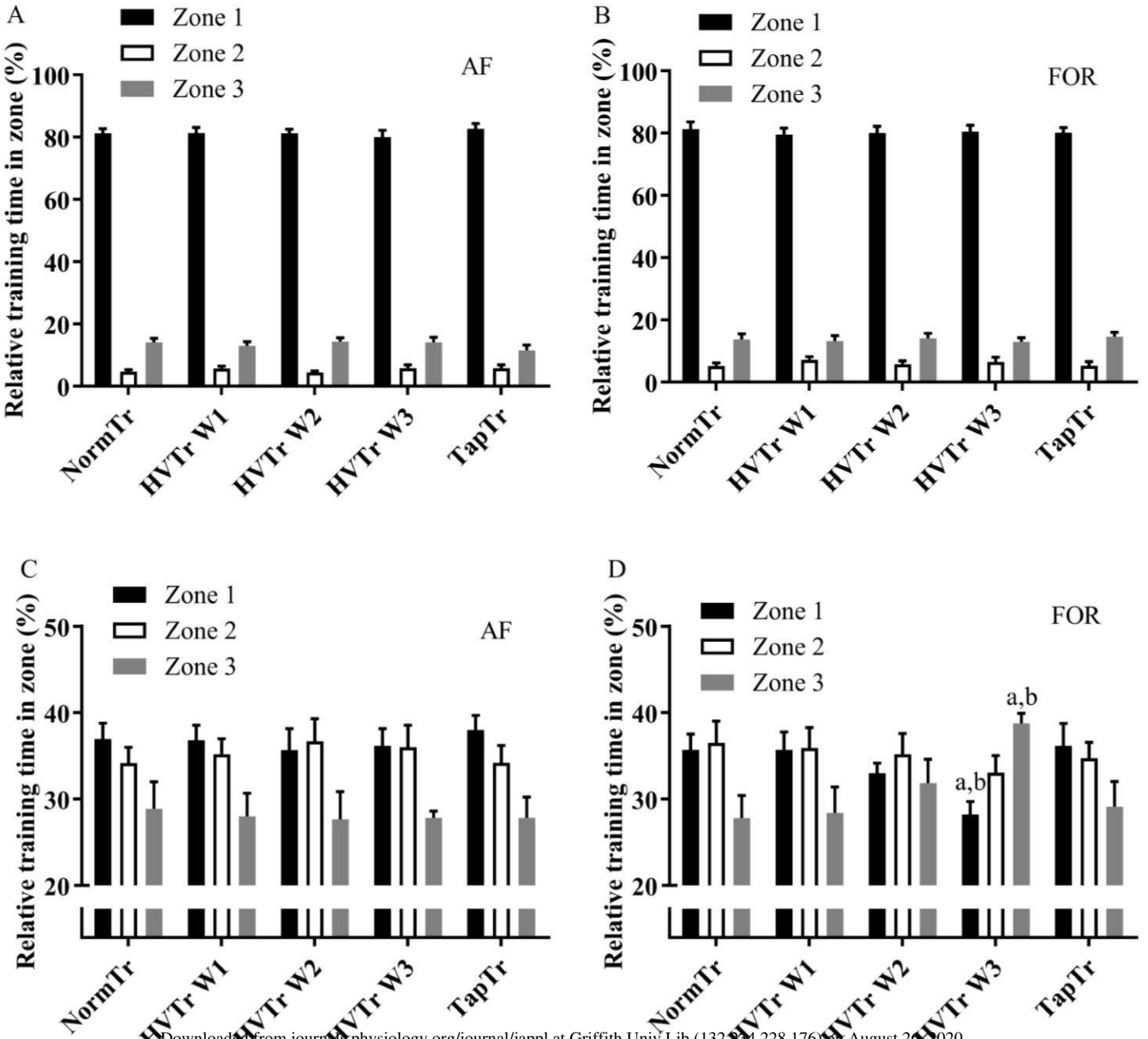
[d]relative training time in zone 3 significantly different compared to each week of NormTr, HVTr week 1 and TapTr

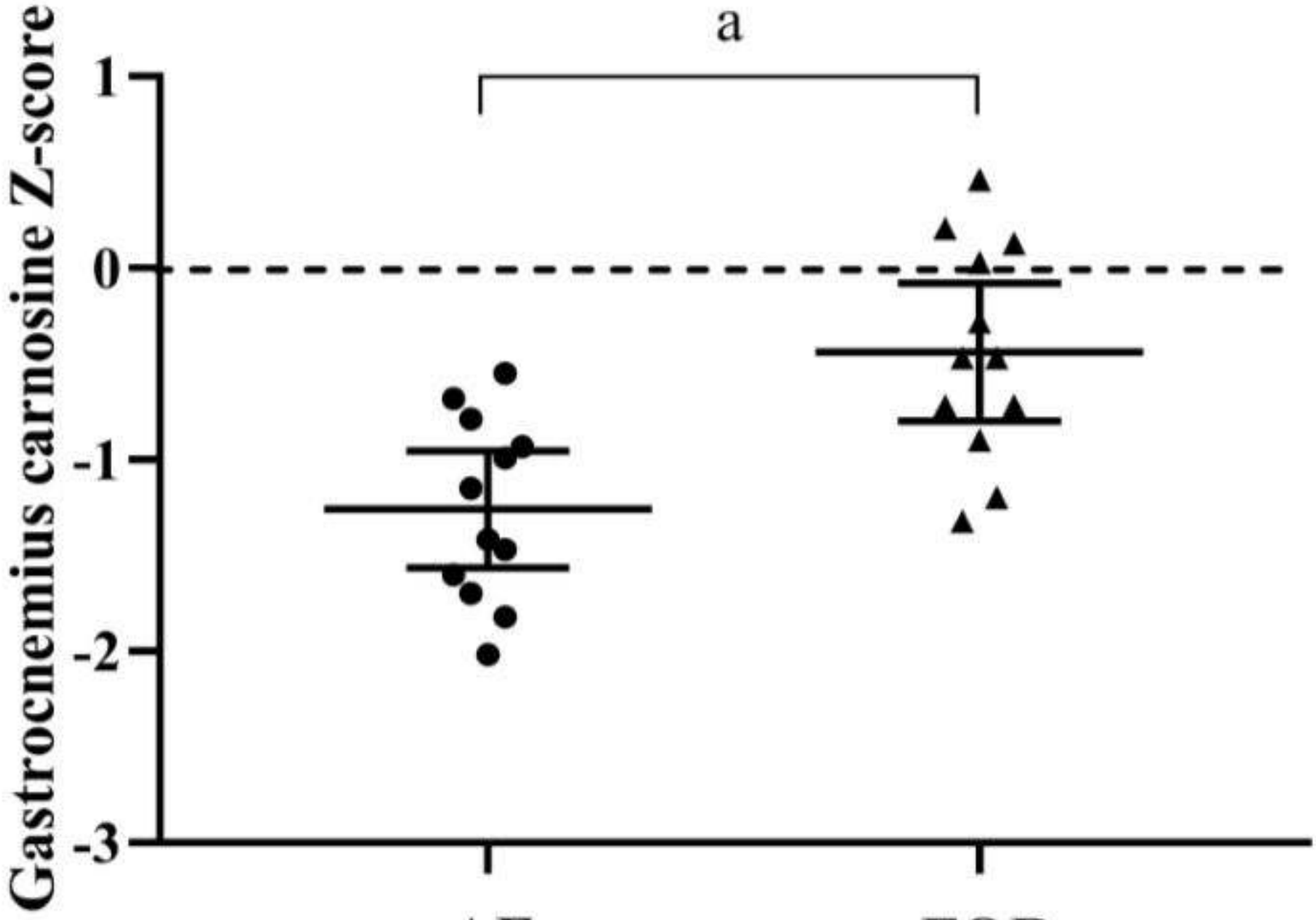
[d]relative training time in zone 3 significantly different compared to each week of NormTr, HVTr week 1 and TapTr

Table 4 - Mean (SD) values for the blood biomarkers measured before and after NormTr, and after the HVTr and TapTr period for the FOR group and AF group.

NormTr: normal training period, HVTr: high-volume training period, TapTr: taper training period, TIBC: total iron-binding capacity, UIBC: Unsaturated iron-binding capacity, VitdA: vitamin D (total 25(OH)D), GDF15: growth differentiation factor-15, FT3: free triiodothyronine, FT4: free thyroxine, TotT3: total triiodothyronine, TotT4: total thyroxine, TSH: thyroid stimulating hormone, TU: thyroid uptake, DHEA-S: dehydroepiandrosterone sulphate, hGH: human growth hormone, CRP: C-reactive protein, IL-6: interleukin-6, LDH: lactate dehydrogenase







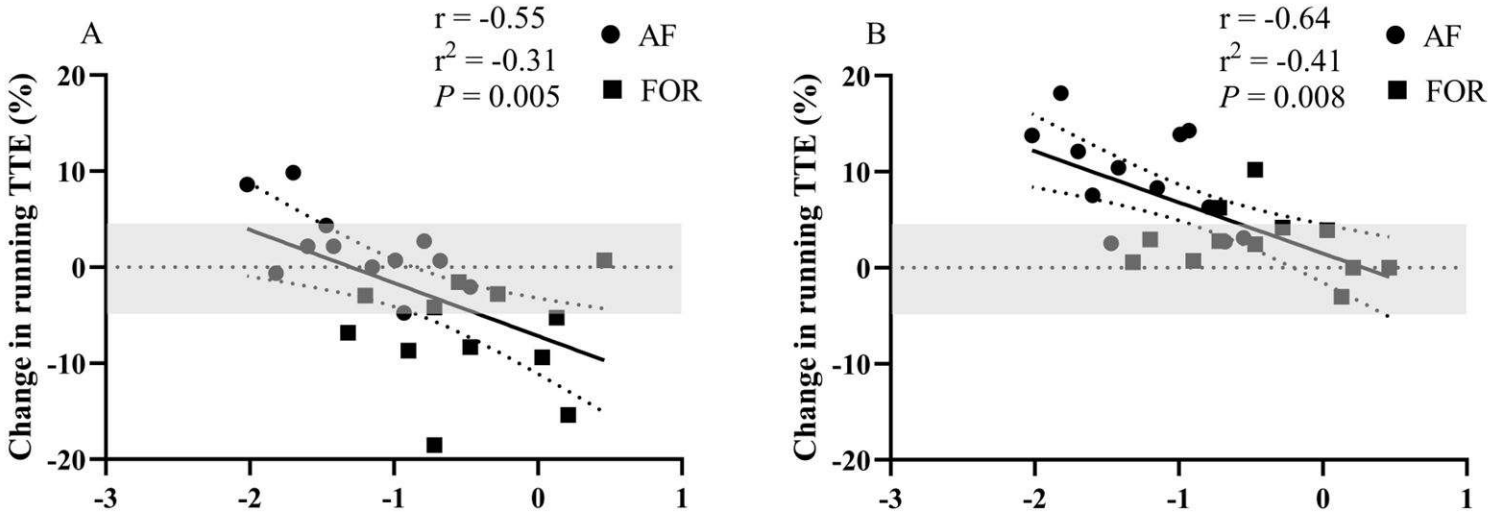


Table 1 - Mean (SD) values for body composition, resting metabolic rate and macronutrient and energy intake measured before and after NormTr, and after the HVTr and TapTr period for the FOR group and AF group.

Variable	Group	Pre-NormTr	Pre-HVTr	Post-HVTr	Post-TapTr
Body mass (kg)	AF	67.5 ± 10.4	68.0 ± 10.9	67.4 ± 11.1	67.6 ± 10.7
	FOR	62.9 ± 9.2	62.8 ± 9.3	62.7 ± 9.4	62.5 ± 9.7
Lean body mass (kg)	AF	54.5 ± 9.44	54.9 ± 9.78	54.5 ± 9.96	54.5 ± 9.48
	FOR	50.7 ± 8.14	50.6 ± 8.16	50.6 ± 8.10	50.5 ± 8.41
Bone mineral content (kg)	AF	3.25 ± 0.42	3.29 ± 0.44	3.35 ± 0.45	3.30 ± 0.45
	FOR	3.19 ± 0.43	3.22 ± 0.43	3.23 ± 0.44	3.22 ± 0.44
Fat mass (kg)	AF	9.75 ± 1.94	9.88 ± 1.96	9.51 ± 1.98	9.71 ± 1.97
	FOR	9.10 ± 0.96	8.97 ± 1.22	8.84 ± 1.24	8.72 ± 1.23
Body fat parentage (%)	AF	14.6 ± 1.61	14.7 ± 2.81	14.2 ± 2.83	14.6 ± 2.79
	FOR	14.6 ± 1.54	14.4 ± 1.70	14.2 ± 1.52	14.0 ± 1.45
Absolute RMR ($MJ \cdot day^{-1}$)	AF	6.86 ± 1.23	6.57 ± 1.07	6.81 ± 1.28	6.69 ± 1.32
	FOR	7.01 ± 0.91	7.15 ± 1.33	6.85 ± 1.08	6.58 ± 1.18
Relative RMR ($kJ \cdot kg\ FFM \cdot day^{-1}$)	AF	127.7 ± 11.6	121.9 ± 10.3	125.9 ± 14.4	123.4 ± 10.5
	FOR	122.9 ± 15.8	124.2 ± 13.7	119.5 ± 9.3	115.0 ± 13.9
Energy intake ($kJ \cdot kg \cdot day^{-1}$)	AF	148.6 ± 17.4	149.6 ± 16.7	160.0 ± 39.5	144.7 ± 20.7
	FOR	148.5 ± 41.3	140.5 ± 36.4	174.6 ± 71.2[a]	156.5 ± 58.2
Carbohydrate intake ($g \cdot kg\ BM \cdot day^{-1}$)	AF	3.96 ± 0.49	3.86 ± 0.53	4.33 ± 1.10	3.41 ± 1.17
	FOR	3.89 ± 1.37	3.77 ± 1.25	4.47 ± 2.47	4.16 ± 1.64
Protein intake ($g \cdot kg\ BM \cdot day^{-1}$)	AF	1.54 ± 0.27	1.62 ± 0.39	1.73 ± 0.44	1.44 ± 0.23
	FOR	1.41 ± 0.47	1.49 ± 0.26	1.92 ± 0.61[a]	1.52 ± 0.50
Fat intake ($g \cdot kg\ BM \cdot day^{-1}$)	AF	1.40 ± 0.23	1.45 ± 0.28	1.44 ± 0.47	1.40 ± 0.37
	FOR	1.51 ± 0.41	1.32 ± 0.37	1.63 ± 0.57	1.51 ± 0.65

NormTr: normal training period, HVTr: high-volume training period, TapTr: taper training period, RMR: resting metabolic rate, FFM: fat-free mass, FOR: functional overreaching, AF: acutely fatigued

[a]Significantly different compared to pre-NormTr, pre-HVTr and post-TapTr

Table 2 - Mean (SD) values for subjective wellness questionnaire responses and upper respiratory tract infection symptom score and occurrences measured during each training phase for the FOR group and AF group.

		NormTr			HVTr			TapTr
		1	2	3	1	2	3	1
Sleep quality	FOR	7.7 ± 1.0	7.6 ± 1.2	7.8 ± 1.1	6.2 ± 1.8	5.6 ± 2.0[a]	5.4 ± 1.5[a]	7.8 ± 1.2
	AF	7.0 ± 2.0	6.6 ± 1.8	6.8 ± 1.4	6.8 ± 1.6	6.1 ± 2.3	6.1 ± 2.1	6.8 ± 1.3
Physical well-being	FOR	7.4 ± 1.9	7.2 ± 1.8	7.3 ± 1.8	6.0 ± 1.4	5.8 ± 1.5[a]	5.3 ± 2.1[a]	7.9 ± 1.2
	AF	7.3 ± 1.4	7.2 ± 1.1	7.3 ± 1.4	5.9 ± 1.6	5.7 ± 1.9[a]	6.1 ± 1.3	7.5 ± 0.6
Readiness to train	FOR	7.9 ± 1.2	7.4 ± 1.7	7.3 ± 1.8	5.9 ± 2.1[a]	5.8 ± 1.9[a]	3.7 ± 1.8[a]	8.3 ± 1.2
	AF	7.6 ± 1.4	7.6 ± 1.5	7.8 ± 1.4	5.8 ± 2.7[a]	5.9 ± 2.4[a]	5.0 ± 2.3[a]	7.8 ± 1.0
Muscle soreness	FOR	2.5 ± 1.5	3.0 ± 2.1	3.1 ± 2.0	5.4 ± 1.7[a]	5.4 ± 1.6[a]	6.2 ± 2.0[a]	3.7 ± 2.4
	AF	3.0 ± 1.5	2.8 ± 1.8	2.7 ± 1.3	4.9 ± 1.1[a]	5.2 ± 1.6[a]	5.7 ± 1.4[a]	4.0 ± 1.5
Fatigue	FOR	3.2 ± 1.3	3.6 ± 1.9	4.2 ± 1.3	5.6 ± 1.4[a]	6.4 ± 1.3[a]	6.8 ± 1.5[a]	4.7 ± 2.3
	AF	3.7 ± 1.4	4.4 ± 1.6	4.3 ± 1.5	5.3 ± 2.3	5.8 ± 2.6[a]	5.7 ± 2.7[a]	3.9 ± 1.4
Non-training stress	FOR	2.8 ± 2.4	2.9 ± 2.5	2.8 ± 1.3	3.0 ± 1.8	2.7 ± 2.1	2.7 ± 1.5	2.4 ± 0.9
	AF	2.4 ± 1.9	2.7 ± 1.2	3.7 ± 1.9	3.8 ± 2.7	2.8 ± 1.8	2.7 ± 2.1	2.8 ± 1.8
Mood	FOR	7.9 ± 0.9	7.6 ± 1.1	7.3 ± 1.5	5.6 ± 1.3[a]	5.0 ± 1.6[a]	4.4 ± 1.1[a]	7.3 ± 1.4
	AF	7.7 ± 1.7	7.7 ± 1.6	6.9 ± 1.9	5.4 ± 1.5[b]	5.0 ± 2.1[a]	5.2 ± 2.2[a]	7.0 ± 0.9
URTI symptom score	FOR	2.9 ± 3.8	2.8 ± 4.9	4.3 ± 9.5	2.3 ± 2.9	17.1 ± 24.4	27.5 ± 36.8[ab]	3.8 ± 4.7
	AF	7.0 ± 13.4	3.0 ± 3.2	3.0 ± 3.2	7.7 ± 8.0	7.5 ± 11.2	8.5 ± 17.4	1.8 ± 3.7
URTI occurrence	FOR	0	0	0	0	2	3	0
	AF	1	0	0	1	1	0	0

NormTr: normal training period, HVTr: high-volume training period, TapTr: taper training period, URTI: upper respiratory tract infection

[a]Significantly different compared to each week of NormTr and TapTr

[b]Significant difference between groups

Table 3 - Mean (SD) values for weekly training duration, volume, load and distribution of training intensity during each training phase for the FOR group and AF group.

		NormTr			HVTr			TapTr
		1	2	3	4	5	6	7
Weekly running training volume (min)	FOR	359 ± 38	362 ± 40	367 ± 40	396 ± 43[a]	442 ± 56[b]	481 ± 62[c]	226 ± 42[d]
	AF	355 ± 60	365 ± 65	362 ± 69	389 ± 72[a]	437 ± 70b	473 ± 64[c]	225 ± 2[d]
Weekly running training volume (km)	FOR	68.3 ± 18.9	68.6 ± 21.1	67.8 ± 20.3	75.9 ± 19.6[a]	84.5 ± 19.4[b]	90.7 ± 19.9[c]	42.1 ± 9.9[d]
	AF	70.3 ± 23.3	71.3 ± 21.8	71.6 ± 23.2	77.3 ± 24.3[a]	85.9 ± 28.0[b]	92.9 ± 30.1[c]	43.2 ± 13.5[d]
sRPE (AU)	FOR	1779 ± 258	1804 ± 258	1800 ± 51	1967 ± 276[a]	2299 ± 295[b]	2613 ± 393[c]	1130 ± 274[d]
	AF	1731 ± 334	1816 ± 333	1784 ± 381	1917 ± 386[a]	2160 ± 383[b]	2337 ± 341[c]	1103 ± 122[d]
Running speed derived TID (% training time in zone 1/2/3)	FOR	80/5/15	81/6/13	80/6/14	79/7/14	80/6/14	80/7/13	80/5/15
	AF	82/5/13	80/6/14	81/5/14	81/6/13	82/5/13	80/6/14	83/6/11
RPE derived TID (% training time in zone 1/2/3)	FOR	36/36/28	35/37/28	36/37/27	36/36/28	33/35/32	28/33/39[e,f]	36/35/29
	AF	39/32/29	36/35/29	36/35/29	37/35/28	36/36/28	36/36/28	38/34/26

NormTr: normal training period, HVTr: high-volume training period, TapTr: taper training period, RPE: rating of perceived exertion, sRPE: session RPE training load, TID: training intensity distribution

[a]significantly different compared to each week of NormTr and TapTr

[b]significantly different compared to each week of NormTr, HVTr week 1 and TapTr

[c]significantly different compared to each week of NormTr, HVTr week 1 and 2 and TapTr

[d]significantly different compared to each week of NormTr and HVTr

[d]relative training time in zone 3 significantly different compared to each week of NormTr, HVTr week 1 and TapTr

[d]relative training time in zone 3 significantly different compared to each week of NormTr, HVTr week 1 and TapTr

Table 4 - Mean (SD) values for the blood biomarkers measured before and after NormTr, and after the HVTr and TapTr period for the FOR group and AF group.

Variable	Group	Pre-NormTr	Pre-HVTr	Post-HVTr	Post-TapTr
Ferritin (ug/L)	FOR	55.9 ± 32.9	54.7 ± 26.3	49.4 ± 31.2	46.7 ± 26.3
	AF	62.3 ± 37.4	65.7 ± 39.0	60.3 ± 35.4	60.2 ± 29.3
Iron (umol/L)	FOR	20.5 ± 7.8	16.6 ± 6.1	19.7 ± 10.2	16.0 ± 6.8
	AF	18.8 ± 5.6	17.9 ± 5.6	18.3 ± 7.5	22.6 ± 12.6
TIBC (umol/L)	FOR	56.0 ± 8.9	57.1 ± 10.9	59.6 ± 9.6	56.8 ± 5.9
	AF	56.4 ± 8.0	60.4 ± 7.3	60.3 ± 6.8	64.9 ± 12.3
Transferrin saturation (%)	FOR	36.6 ± 14.0	29.7 ± 10.5	32.7 ± 13.1	27.9 ± 9.7
	AF	33.6 ± 9.6	36.1 ± 21.6	30.5 ± 12.1	35.1 ± 20.6
Total protein (g/L)	FOR	68.2 ± 5.5	68.5 ± 5.3	69.8 ± 7.1	67.3 ± 4.97
	AF	64.8 ± 9.7	68.9 ± 4.33	68.0 ± 6.0	73.3 ± 12.7
UIBC (umol/L)	FOR	1.20 ± 0.28	1.18 ± 0.28	1.13 ± 0.22	1.09 ± 0.30
	AF	1.33 ± 0.30	1.36 ± 0.46	1.34 ± 0.21	1.33 ± 0.31
Urea (umol/L)	FOR	9.08 ± 2.07	9.08 ± 2.08	8.93 ± 1.87	9.97 ± 1.69
	AF	9.8 ± 1.79	10.5 ± 2.82	10.0 ± 1.43	10.0 ± 1.70
Uric Acid (umol/L)	FOR	2.62 ± 1.25	2.37 ± 0.88	2.17 ± 0.90	2.19 ± 0.85
	AF	1.92 ± 0.78	2.02 ± 1.57	1.97 ± 1.19	1.75 ± 0.97
VitdA (ng/mL)	FOR	42.2 ± 14.4	45.7 ± 16.2	48.6 ± 16.1	52.7 ± 20.3
	AF	48.6 ± 27.7	52.5 ± 21.4	52.6 ± 20.5	55.1 ± 26.4
GDF-15 (pg/mL)	FOR	571 ± 262	514 ± 266	457 ± 243	466 ± 220
	AF	510 ± 230	474 ± 255	490 ± 207	482 ± 212
FT3 (pg/dL)	FOR	3.62 ± 0.79	3.57 ± 0.79	3.45 ± 0.62	3.57 ± 0.61
	AF	4.49 ± 1.78	4.57 ± 1.91	4.13 ± 0.85	4.25 ± 1.11
FT4 (ng/mL)	FOR	0.92 ± 0.32	0.94 ± 0.31	0.89 ± 0.27	1.04 ± 0.48
	AF	1.46 ± 1.05	1.22 ± 0.80	1.00 ± 0.39	1.16 ± 0.46
TotT3 (ng/dL)	FOR	40.2 ± 3.21	40.4 ± 3.51	39.9 ± 4.24	40.9 ± 5.19
	AF	42.0 ± 5.06	41.1 ± 4.42	41.3 ± 4.70	44.6 ± 6.66
TotT4 (ug/dL)	FOR	35.5 ± 9.73	40.4 ± 11.8	39.9 ± 9.55	40.9 ± 6.25
	AF	37.6 ± 8.29	42.6 ± 7.41	42.0 ± 9.52	42.3 ± 16.2
TSH (ulU/mL)	FOR	5.50 ± 1.08	5.75 ± 0.88	6.20 ± 1.48	5.44 ± 0.97
	AF	4.81 ± 0.96	5.78 ± 1.45	5.46 ± 0.78	5.77 ± 1.29
TU (%)	FOR	314 ± 55	331 ± 61	359 ± 79	308 ± 73
	AF	307 ± 74	317 ± 44	327 ± 62	349 ± 86
Cortisol (ug/dL)	FOR	13.4 ± 3.71	13.7 ± 2.52	11.2 ± 2.56	13.1 ± 4.01
	AF	15.0 ± 4.80	13.0 ± 4.64	14.4 ± 5.2	13.3 ± 4.2
Testosterone (ng/dL)	FOR	5.98 ± 3.88	5.96 ± 3.94	4.95 ± 2.61	5.31 ± 2.97
	AF	5.00 ± 3.76	5.30 ± 3.89	5.11 ± 3.62	5.07 ± 3.75
Testosterone:cortisol ratio (ng/dL)	FOR	0.48 ± 0.35	0.46 ± 0.33	0.47 ± 0.26	0.45 ± 0.26
	AF	0.39 ± 0.30	0.45 ± 0.37	0.42 ± 0.32	0.44 ± 0.33
DHEA-S (ug/dL)	FOR	210 ± 59.3	221 ± 76.0	208 ± 57.6	207 ± 63.5
	AF	251 ± 86.4	275 ± 163	267 ± 109	261 ± 103.9

hGH (ng/dL)	FOR	2.87 ± 3.31	79 ± 1.04	3.18 ± 3.60	1.41 ± 2.19
	AF	0.72 ± 1.44	2.09 ± 5.33	2.45 ± 6.44	2.45 ± 6.59
Creatinine (umol/L)	FOR	84.6 ± 9.01	86.1 ± 7.09	87.5 ± 8.40	83.8 ± 8.86
	AF	76.3 ± 14.0	83.6 ± 12.0	83.6 ± 13.4	87.8 ± 13.9
CRP (mg/L)	FOR	0.98 ± 0.02	0.98 ± 0.01	0.98 ± 0.01	0.91 ± 0.25
	AF	0.95 ± 0.10	0.92 ± 0.17	0.94 ± 0.12	0.93 ± 0.16
IL-6 (pg/dL)	FOR	1.08 ± 0.45	0.93 ± 0.28	1.01 ± 0.30	0.88 ± 0.35
	AF	1.20 ± 0.82	1.39 ± 1.96	1.16 ± 1.06	1.02 ± 0.66
LDH (U/L)	FOR	179 ± 36.3	181.9 ± 38.0	171.6 ± 18.7	159.7 ± 12.2
	AF	171 ± 41.6	175 ± 24.9	179 ± 27.8	194 ± 33

NormTr: normal training period, HVTr: high-volume training period, TapTr: taper training period, TIBC: total iron-binding capacity, UIBC: Unsaturated iron-binding capacity, VitdA: vitamin D (total 25(OH)D), GDF15: growth differentiation factor-15, FT3: free triiodothyronine, FT4: free thyroxine, TotT3: total triiodothyronine, TotT4: total thyroxine, TSH: thyroid stimulating hormone, TU: thyroid uptake, DHEA-S: dehydroepiandrosterone sulphate, hGH: human growth hormone, CRP: C-reactive protein, IL-6: interleukin-6, LDH: lactate dehydrogenase