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Individual patterns in blood-borne indicators of fatigue – trait or chance

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Running title: Individual patterns in blood-borne indicators of fatigue

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ABSTRACT

Blood-borne markers of fatigue such as Creatine Kinase (CK) and Urea (U) are widely used to fine-tune training recommendations. However, predictive accuracy is low. A possible explanation for this dissatisfactory characteristic is the propensity of athletes to react with different patterns of fatigue indicators (e.g. predominantly muscular (CK) or metabolic (U)). The aim of the present trial was to explore this hypothesis by using repetitive fatigue-recovery cycles. 22 elite junior swimmers and triathletes (18 ±3 years) were monitored for nine weeks throughout two training phases (low-intensity, high-volume (LIHV) and high-intensity, low-volume (HILV)). Blood samples were collected each Monday (recovered) and Friday (fatigued) morning. From measured values of CK, U, free-testosterone (FT), and cortisol (C) as determined in the rested and fatigued state, respectively, Monday-to-Friday differences (Δ) were calculated and classified by magnitude before calculation of ratios (ΔCK/ΔU and ΔFT/ΔC). Coefficient of variation (CV) was calculated as group-based estimates of reproducibility. Linear mixed modelling was used to differentiate inter- and intra-individual variability. Consistency of patterns was analysed by comparison to threshold values (<0.9 or >1.1 for all weeks). Reproducibility was very low for fatigue-induced changes (CV ≥100%) with inter-individual variation accounting for 45-60% of overall variability. Case-wise analysis indicated consistent ΔCK/ΔU patterns for seven individuals in LIHV and seven in HILV; five responded consistently throughout. For ΔFT/ΔC the number of consistent patterns was two in LIHV and three in HILV. These findings highlight the potential value of an individualised and multivariate approach in the assessment of fatigue.

KEYWORDS: Exercise, Regeneration, Reproducibility, Surrogate markers, Training

INTRODUCTION:

The decisive difference in performance separating the winner from a challenger is generally tiny in today's competitive sports, in particular among elite athletes (1). As such, maximising training adaptation by fully utilising the limits of bearable training load is critical for success. However, such an approach is associated with the risk of accumulating fatigue, non-functional overreaching and ultimately the overtraining syndrome (21). Therefore, monitoring of fatigue and recovery is an important aspect in the regular fine-tuning of training recommendations in competitive sports.

A key feature of exercise-induced fatigue is a decline in discipline-specific performance capacity. However, repeated exhaustive performance tests are hard to integrate in the training regime and would contribute considerably to the overall fatigue burden of athletes. Therefore, various surrogate markers have been proposed including a wide range of blood-borne parameters (2, 16, 21, 25-26) as well as psychological (11) and autonomic (16, 24) measures (6, 21). Blood-borne parameters are particularly attractive surrogate markers of fatigue and recovery because of their obvious objectivity, their high accuracy and precision of measurements, the minimal interference with the training process, and, in most cases, a clear physiological concept concerning their connection with exercise and fatigue (18).

Ideally, a surrogate measure of fatigue and recovery is characterised by high reliability of their values, at any given level of fatigue and large fatigue-induced changes. Surprisingly, so far no parameter could be established which has adequate sensitivity and reproducibility for the monitoring of fatigue and recovery during athletic training cycles (6, 11, 15). In particular, gross variability is high and little is known about the proportion of between and

within-subject differences (3). This problem concerns measured values as well as fatigue-induced changes of virtually all blood-borne indicators presented in the literature thus far (16). The known mechanisms behind this variability include lifestyle dependent (e.g. nutrition (21), hydration, sleep (19) as well as subject inherent (e.g. age, sex, ethnicity (4)) and methodological factors (e.g. strict circadian and procedural standardization needed in particular for hormone and autonomic measures (16, 21, 24)).

Another possible explanation for this unsatisfactory characteristic could be a variable pattern of fatigue-induced changes between athletes. If some athletes responded predominantly with changes in parameter A and others with changes in parameter B, a group-based analysis of fatigue-induced changes will inevitably show high variability for changes in either measure. This explanatory approach originated from observations of experienced team physicians from endurance disciplines with the routine parameters Creatine Kinase (CK) and Urea (U). CK is commonly used as marker of muscular strain and is particularly elevated with exercise modes including high levels of eccentric work and peak force (4, 16). By contrast U, the excretal form of nitrogen in the human body, reflects protein catabolism occurring with high calorie turnover and metabolic strain (16, 21). While the majority of athletes are reported to have variable relationships between the two parameters, some athletes consistently show a marked, fatigue-dependent increase in CK with marginal changes in U and for some other individuals from the same discipline the observed relationship was reversed. Similar observations have been made for changes in Free-testosterone (FT) and Cortisol (C). FT is the biologically active form of testosterone, the most potent anabolic hormone. FT strongly promotes a multitude of anabolic pathways essential for recovery after physical exercise. These include protein synthesis, nutrient uptake into muscle cells and glycogen resynthesis. C is a catabolic hormone mediating e.g. protein breakdown for gluconeogenesis and glycogenolysis during

prolonged exposures to stress. The ratio FT/C reflects the anabolic/catabolic balance and has been shown to be reduced during non-functional overreaching and the overtraining syndrome (16, 25, 26).

Therefore, the three-fold purpose of this study was to determine a) the reproducibility of four routine (CK, U, FT and C) blood-borne parameters and their fatigue-induced changes, b) the proportion of between- and within-subject variability of the fatigued induced changes, and c) the main aim was to observe whether consistent individual patterns of fatigue-induced responses for different markers are existent.

METHODS:

Experimental approach to the problem

The general design of this study represents an observational approach. Elite junior athletes were monitored for a total of nine weeks during two distinct training phases as described by the team coaches (low intensity, high volume (LIHV) and high intensity, low volume (HILV), respectively). According to the repetitive structure of the weekly microcycles, blood samples were collected before the first training of the day on Mondays (recovered after resting on Sunday) and Fridays (fatigued after a week of training).

Subjects

A total of twelve (eight male, four female) athletes completed the study. Only junior elite swimmers and triathletes from a federal Olympic Training Centre were eligible for this trial. Further inclusion criteria included all athletes being required to complete three or more weeks of training throughout a training period to be included in the analysis and to be free from any form of illness or injury. All participants gave a written consent to take part in the study; for

101 those under the age of consent, parental permission was obtained. The institutional review
102 board in the spirit of the Helsinki declaration approved the study. The timeline of subjects
103 throughout the study is displayed in figure 1.
104
105 Figure 1 about here
106
107 **Procedures**
108 Four, well studied, routine parameters of fatigue and recovery were selected as outcome
109 measures for the study (26). These parameters were chosen due to their potential to form
110 logical and meaningful pairs (ratios) (26). These include, CK which represents the muscular
111 and U which represents the metabolic aspects of fatigue (17), as well as, FT and C of which
112 the ratio has been previously established as a marker of the anabolic / catabolic balance (25).
113
114 Venous blood samples were obtained from the anti-cubital vein in a supine position by
115 standard protocol, following 10-15 min of seated rest. Blood was collected during the
116 morning hours prior to the first training session of the day. Samples representing the
117 recovered state were collected on Mondays after a day of rest, whilst samples representing
118 fatigued status were collected on Fridays, in the morning before training, following a week of
119 continuous training (Monday to Thursday). Training was logged for every athlete by the
120 responsible coaches and checked by the research team to verify repetitive microcycles
121 (Figure 2). Athletes were asked to keep meals and eating patterns consistent throughout the
122 measuring period, no standardised food intake protocol or food diaries were upheld.
123
124 Figure 2 about here
125

Blood samples were transported immediately to the laboratory for appropriate procedures. Serum tubes were centrifuged at 2,500 revolutions per min for 10 min and aliquoted in 1 ml tubes. CK and U were measured immediately in singlicate assays, using a Unicel DxC600 synchron clinical system (Beckmann Coulter GmbH, Krefeld, Germany). The remaining aliquots were frozen within 1 h from sampling and stored frozen at -80°C until analysis. After completion of the respective training phase, FT (measured in duplicate, whereby the mean of the two values were used for analysis) and C (singularly) were measured using a commercial ELISA an Access 2 Immunoassay System (Beckman Coulter, California, USA) measured kit (Labor Diagnostika Nord, Nordhorn, Germany). Blood concentrations are expressed in 'commonly used' clinical units (CK, U/L; U, mg/dl; C, µg/dl; FT, ng/ml). For standardised units listed as follows are the conversion factors:

U- mg/dl to nmol/l = x0.357

C - µg/dl to nmol/l = x27.59

FT – ng/ml to nmol/l = x3.50

Prior to blood collection, each participant completed the Acute Recovery and Stress Scale (ARSS) (12) to confirm that indeed the weeks of training did cause a sensation of perceived fatigue.

Statistical analysis

Statistical analysis was conducted using SPSS v21.0 (SPSS inc., Chicago, USA). Normal distribution was checked using the Shapiro Wilks tests. Although for some outcome measures this test was slightly above the significance level for certain time points, the distributions from the respective histograms were not skewed. Therefore, parametric procedures were applied throughout. Descriptive statistics are presented as means and standard deviations

(SD). A mixed linear model was fit to the data for inferential testing and estimation of between and within-subject variability, respectively (random effect: subject ID; fixed effects: fatigue status (Monday vs. Friday) and training period (LIHV vs. HILV)). Mean coefficients of variation (CV) were calculated to analyse the between-week reproducibility of measured values (separately for fatigued and recovered states, respectively) and their fatigue induced changes. All CV analyses were conducted using a macro from a published Microsoft Excel Spreadsheet (10). CVs were calculated separately for LIHV and HILV respectively. Students paired *T*-tests were used to compare Monday and Friday questionnaire results for both LIHV and HILV, significance was set at an alpha (*P)* level ≤0.05.

The proceedings for the analysis of response patterns are illustrated in Figure 3. This novel approach was designed to allow for the transparent and reproducible operationalisation of the initial research question. Firstly Monday-to-Friday differences (Friday (fatigued) minus Monday (recovered)) were calculated for each individual parameter (ΔCK and ΔU, ΔFT and ΔC, respectively). The respective ratios (ΔCK/ΔU; ΔFT/ΔT) based on changes in the individual parameters categorised by their magnitude were then created. The upper / lower limits for the extreme categories were set at mean difference ± 2 SD. To characterise the pairwise response pattern, the ratios of categorised changes in CK and U, as well as for FT and C, were calculated. Overall, group-based reproducibility of ratio values (ΔCK/ΔU, ΔFT/ΔC) was assessed using CV as described above. Individual cases were then evaluated by whether the ratio consistently fell into the same range during all weeks of a training phase. The authors deemed any value ≥1.1 indicated a CK or FT response and a value of ≤0.9 indicates a U or C response from their respective pairs.

Figure 3 about here

For a qualitative evaluation of response patterns involving all four parameters (subjectively observed pattern shape and change in shape), categorised changes were illustrated using spider diagrams with a diamond representing each week, the shape of this diamond can be used for week-by-week comparison of response and further inform the practitioner of 'responder' type and alterations in athlete response. A single spider diagram was used for each of the training phases.

During analysis it became apparent that several Monday values of CK were considerably higher compared to the preceding Friday. Therefore, when Monday values were elevated by more than the estimated week-by-week random variability (CV) compared to the preceding Friday, the week was excluded from the analysis. In total nine CK values were excluded.

RESULTS:

The results of the ARSS indicated that during the LIHV phase each of the eight dimensions were significantly different between Monday (rested) and Friday (fatigued) $P = <0.05$. During the HILV phase dimensions one to six and eight were significantly different between Monday (rested) and Friday (fatigued) $P = <0.05$, moreover, there was a trend of significance for dimension seven $P = 0.06$

The characteristics of the subjects included in the analysis were; age 18 ± 3 y, height 177 ± 7 cm, mass 67 ± 9 kg. No significant differences were found between swimmers and triathletes for age, height, mass, years trained, any of the four blood-borne outcome measures or their changes ($P = <0.05$ in all cases) or relative number of existent patterns. For sexes, although

200 the number of participants does not yield statistical power, qualitatively, they were observed
201 to response in a similar manner.
202
203 CK, U, FT and C Monday and Friday measured values and the respective fatigue-induced
204 differences within each training phase are presented in Table 1. According to the fixed effects
205 results from the linear mixed model the difference between all Monday and Friday values
206 independent of training phase (fixed effect: fatigue status) was significant for CK and U *(P* =
207 <0.01 & *P* = 0.01 respectively), whereas for FT and C the numerical difference failed to
208 reach statistical significance (*P* = 0.46 & *P* = 0.74 respectively). The effect of training phase
209 on the week-by-week changes (LIHV vs. HILV) were significant for U (*P* = <0.01) but not
210 for CK (*P* = 0.31), C (*P* = 0.92) and FT (*P* = 0.09).
211
212 Table 1 about here
213
214 Usual group-based measures of reproducibility indicate a moderate-to-low reproducibility in
215 the measured values for Mondays and Fridays in all outcome measures (CV 12-51%). Very
216 poor reproducibility was seen for the fatigued-induced changes in all outcome measures with
217 a mean CV ≥100 % in all cases (Table 2). Exemplary figures of the individual courses
218 indicating the fatigue-induced changes in the associated blood-borne indicators are displayed
219 in Figure 4. The respective graphs for Monday and Friday measured values for all parameters
220 are provided as supplementary material (http://links.lww.com/JSCR/A14). According to the
221 random effects results from the linear mixed model the proportion of between-subject
222 variability from total variability is 45% for CK, 57% for U, 51% for FT and 57% in C.
223
224 Table 2 about here

225

226 Figure 4 about here

227

228 Ratios of categorised responses (bivariate response patterns) are displayed in Table 3.
229 Athletes with a consistent pattern within a training phase are highlighted in degrees of grey.
230 Case-wise analysis indicated consistent ΔCK/ΔU patterns for seven individuals in LIHV and
231 seven in HILV; five responded consistently throughout. For ΔFT/ΔC the number of
232 consistent patterns was two in the LIHV and three in the HILV phase. Selected exemplary
233 spider diagrams conveying patterns including all four parameters, using their categorised
234 values are displayed in figures 5a, 5b, 5c and 5d. These indicate a visual interpretation over
235 an array of blood-borne parameters.

236

237 Table 3 about here

238

239 Figure 5 about here

240

241 **DISCUSSION:**

242 In competitive and elite sports there is a high awareness of the athletes' individuality (9).
243 Despite this awareness, formalised, objective standards (like normal ranges for individual
244 fatigue markers) are still mostly based on group means and main effects leaving the
245 individualisation to the experience and subjective valuation of the coach. This proof-of-
246 concept trial was designed to offer a simple, cost efficient and understandable approach to
247 assess and handle the athletes' individuality in a more objective way. Moreover, the current
248 study aimed to address the premise that the pattern of response remains consistent in various
249 athletes during training micro cycles. The commonly used, group-based measurements of

reproducibility, demonstrated a high degree of random variability in fatigue-induced responses. This was seen in all parameters examined and within the response patterns. However, when data were analysed on the individual level, consistent relationships between the magnitudes of fatigue-induced changes in the selected parameters were apparent in a proportion of athletes. This finding supported the study aims and seminal practical observation that individualised patterns of fatigue indicators is present in certain athletes.

The comparison between the two differing training phases (LIHV and HILV, respectively) extends this main finding by contributing multiple aspects. On the one hand side it corroborates the description of consistent, individual patterns, as seen in the current study, as a variety of athletes elicited the same blood-borne response, despite differences in training characteristics between the two phases. On the other hand side, this points to the need of taking current training characteristics into account when interpreting fatigue indicators, in particular when the consistency of response patterns between training phases has not been confirmed before for the concerned individual.

At present, the high variability of surrogate markers for fatigue and recovery leads to wide reference ranges and thereby severely limits their diagnostic value (19). The individual patterns of fatigue-associated responses, apparent in our data, may partly explain this variability. This is supported by the important contribution of inter-individual variation to overall variability in measured values and responses. Beyond the multivariate approach associated with the assessment of response patterns, this insight may translate to individualise ranges of normality for individual markers and thereby to an improvement in their diagnostic accuracy for the assessment of fatigue. Such personalised normal ranges, which, for other parameters, are already successfully implemented in the athlete's biological passport (ABP)

(20). The concept of the ABP is a means of monitoring an individual's long-term haematological or steroid profile, whereby, when large discrepancies are discovered between the history of an athlete's values and values obtained in a recent test implies that there is something that has altered the physiological condition of the athlete, be it from an act of doping or a medical condition which would warrant further investigation (20). This concept exemplifies the paradigm of personalised medicine while avoiding additional cost and effort. However, the practical applicability of this approach for the assessment of fatigue and recovery in competitive sports remains to be demonstrated, i.e. by establishing a better long-term outcome compared to another approach.

Previous research that investigates blood-borne indicators of fatigue is predominately based on a two-dimensional concept of fatigue. In other words, changes in fatigue status were mainly quantified as "more" or "less" fatigue with little attention to qualitative differences in fatigue states (25). However, mere quantification may not be sufficient to fully characterise the fatigue status of athletes who may not only be "more" or "less" but also "differently" fatigued. An explanatory example is the relationship between the muscular aspect of fatigue, reflected by an increase in CK (as a result from accumulated membrane damage; as compared to metabolic fatigue reflected e.g. by an increase in urea (as a result of limited carbohydrate availability and protein turnover) (17). The $\Delta CK/\Delta U$ ratio makes this qualitative aspect of fatigue measurable. By contrast the components of the $\Delta FT/\Delta C$ ratio reflect the same aspect of fatigue (anabolic-catabolic balance) and the ratio is established in order to increase contrast and facilitate detection. However, it remains to be seen whether and to which extent the analysis of these ratios can be established across varying other training stimuli and sports.

As previously stated, the two-dimensional approach to fatigue could prove to be insufficient in the overall quality of an athletes fatigue level. Furthermore, the use of few parameters to monitor this fatigue could also prove to be insensitive in said fatigue determination. The plots (Figure 5) were created to visualise 'responder type', for several dimensions. This tool can potentially serve as independently as its own fatigue marker (Figure 5a & 5b), this could act, whereby if the shape dramatically changes can indicate an extreme or different stressor placed upon the athlete. Furthermore, this concept could lead to a progression in the future when the similarities between the multivariate responses are valuated objectively by bioinformatics approaches (e.g. neural networks).

Exercise mode or the characteristics of certain disciplines are well known factors which can influence changes in fatigue indicators that occur during normal training cycles (5, 8). Prominent examples of relevant discipline characteristics affecting fatigue indicators are eccentric force production and calorie turnover (4). Training status and adaptation to the specific training load are important subject-inherent factors. To exclude such obvious sources of variability, a homogeneous sample of junior elite athletes from two related disciplines was included. As such, our results did not reveal any difference between disciplines in the measured values of the blood-borne markers, in the extent of the fatigue-induced changes or in the number of response patterns.

It is beyond the scope of this study to uncover the causes for the observed inter-individual differences in patters of fatigue markers. However, it seems plausible that determinants include subject-inherent factors such as muscle fibre distribution. Totsuka et al., (23) previously showed that those athletes with a lower cross-sectional area of the quadriceps femoris muscle were "high responders" in CK production. Other inter-individual differences

could include consistent lifestyle characteristics e.g. nutritional habits. An example may be caloric restriction or protein supplementation, which would both favour increases in urea concentration (14). Research in the individualisation of an athlete's response is clearly warranted to further our current understanding of the fatigue and recovery spectrum in regards to the specific nature not only of certain disciplines but also of each individual athlete (9).

Given the novelty of the approach, this study bears some of the limitations typical for a field-based proof-of-concept trial. Due to the observational character of the study, the opportunities for standardisation and control were limited to the training and blood sampling. The behaviour of subjects outside the normal training routines could not be controlled, comparable to circumstances during routine training periods. The lack of standardisation outside the training bouts became apparent with some CK values on Mondays being clearly higher in comparison to the preceding Friday. This is most probably due to unaccustomed spare time activities during the weekend. To alleviate this issue and avoid skewed results, Monday CK values were excluded from analysis when the value compared to the preceding Friday was higher than the expected random variability indicated by the CV (nine cases). While this added to the complicacy of the analyses and led to a loss in analysable data, non-standardised spare time activity is commonplace even in elite sports. Therefore, this study design contributes to the external validity of the obtained results.

In sport science the "gold standard" for evaluating fatigue is testing the maximal, discipline specific ability of an athlete and noting differences in occasion (26). Less physically demanding exercise based measures such as exercise heart rate at submaximal workloads or jump height have also been published (13, 22). However, as any exercise tests interferes with

the training routine this was not acceptable for the recruited elite athletes and their coaches. Therefore, the main effects of established blood-borne fatigue markers, validated questionnaires and the training load from daily training logs were used to ascertain changes in fatigue status. These included an individualised observation of each athletes training schedule, the overall significant differences in CK and U and the significant differences in the vast majority of the questionnaire results.

While the athletes were informed to keep their meals as similar as possible throughout the days prior to and of the morning of blood collection, no food diaries were kept. This may potentially contribute to within-subject variation, in particular for urea. In addition, outcome measures for this study were limited to four classical fatigue indicators. In future research, a higher number of indicators should be included the selection of which may be either hypothesis-driven or exploratory.

Aiming at a balanced and applicable definition of what is a “consistent response” (and in the absence of previous published work) a narrow and symmetrical “neutral zone” for the respective ratio was combined with a strict notion of “consistent” (above (≥1.1) or below (≤0.9) neutral for all weeks studied) this had been fixed a priory by the research team. The aim was to ensure contrast between response types while avoiding to be overly restrictive in the classification of individual weeks. A systematic evaluation of different cut-off values may be warranted in the future but requires follow-up studies with a higher number of subjects. Assessing consistency of larger patterns by visual inspection of the respective spider diagrams bears a preliminary character due to subjective component. However, in some cases there was an undisputable similarity of patters within a training phase. In larger follow-up trials, quantification of this similarity may be attempted using e.g. neural networks.

375

376 **PRACTICAL APPLICATION:**

377 The use of longitudinal observations of several micro cycles in the present study confirmed
378 that: considerable contribution of inter-individual differences to the large overall variation in
379 blood born markers of fatigue, their changes with training and recovery as well as in the
380 relative magnitude of changes in different parameters (patterns). Therefore:

381

382 • Individualised interpretation of observed values will probably help to overcome the
383 longstanding problem of large variability in surrogate markers of fatigue in all
384 different forms of athletes.

385 At present coaches and team physicians should be encouraged to consider previous
386 observations in the individual athlete in addition to fixed reference ranges. Future
387 research is warranted to develop objective algorithms for the individualization of
388 normal ranges in fatigue assessment. Starting points may be the statistical approaches
389 used in the athlete biological passport (20) or in the field of "personalized medicine"
390 (7).

391 • Patterns of changes in fatigue indicators may provide additional information as
392 compared to individual parameters, at least in athletes with consistent responses.
393 However, the possible increase in diagnostic accuracy remains to be determined in
394 experimental follow-up trials.

395

396 **CONCLUSION:**

397 The present observational study is the first to systematically distinguish consistent individual
398 patterns of response in blood-borne parameters of fatigue in a proportion of athletes.
399 Together with the considerable between-subject variability in individual markers and their

changes, this clearly points to the potential value of individualised diagnostic approaches as compared to group-based 'normal ranges' of individual markers when optimal accuracy is intended, as it is usually the case in high-performance competitive sports.

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FIGURE CAPTIONS:

Figure 1: Details of the participants' timeline throughout the study.

Figure 2: Details of mean training loads for swimmers and triathletes during both low intensity high volume (LIHV) and high intensity low volume (HILV) training phases.

Figure 3: Schematic representation of data preparation for analysis of response patterns.

CK: Creatine Kinase; U: urea; FT: Free-Testosterone; C Cortisol; Δ: Monday to Friday changes.

Figure 4:

Individual courses of mean fatigue-associated differences (Δ) of response in the measured blood-borne parameters. Each line pertains to a single subject, whereby; line type and marker symbol remains constant per individual.

CK: Creatine Kinase; U: urea; FT: Free-Testosterone; C Cortisol; LIHV: low intensity high volume; HILV high intensity low volume.

Figure 5: Selected spider diagrams of categorised responses in blood-borne markers:

a and b: Examples of consistent response pattern.

c and d Examples without consistent response pattern.

Supplementary Figure 1: Individual courses of the mean fatigue-associated response in blood-borne parameters, Mondays (Mon) and Fridays (Fri). Each line pertains to a single subject, whereby; line type and marker symbol remains constant per individual.

CK: Creatine Kinase; U: urea; FT: Free-Testosterone; C Cortisol; LIHV: low intensity high volume; HILV high intensity low volume.

Table 1: Mean ± standard deviation of raw values and mean differences of Monday and Friday values over both training phases.

	LIHV						HILV				
Time point	Week 1	Week 2	Week 3	Week 4	Week 5	Mean	Week 1	Week 2	Week 3	Week 4	Mean
CK (U/L)											
Mon	190.7±74.7	187.3±90.5	209.1±95.1	178.2±57.2	173.2±52.0	191.3±70.5	224.7±83.9	207.5±92.0	194.9±82.8	194.6±101.1	208.4±77.1
Fri	262.1±150.3	286.3±126.9	256.4±141.3	280.5±133.5	271.9±164.4	271.4±119.4	275.4±144.8	264.7±137.5	261.1±105.5	240.8±117.7	263.7±110.2
Δ	71.5±98.9*	99.0±99.2*	47.9±104.9*	110.6±115.5*	115.2 ±158.1*	76.3±73.9*	35.5±69.8*	19.2±37.8*	66.3±37.8*	22.5±65.1*	35.9±54.1*
U (mg/dl)											
Mon	32.9±8.4	31.1±5.3	33.4±8.5	31.2±4.6	28.8±7.0	32.2±7.0	35.1±7.5	36.6±7.4	37.6±7.4	32.3±5.7	35.4±7.1
Fri	36.1±5.3	34.4±7.6	33.6±8.8	35.5±10.4	33.5±6.8	34.9±8.3	37.5±8.9	36.7±6.0	35.6±5.4	35.6±5.7	36.3±6.7
Δ	2.7±5.0*	3.1±4.3*	-0.6±3.8	9.2±7.5*	4.1±8.1*	3.8±5.1*	2.4±3.1*	0.1±2.9	-2.1±2.8	3.3±4.0*	0.9±3.2
FT (ng/ml)											
Mon	13.0±10.4	13.7±11.2	10.8±10.0	11.5±9.6	10.6±9.8	12.2±10.3	9.2±10.6	9.6±13.3	9.4±11.5	10.3±16.8	9.6±13.3
Fri	15.8±13.9	10.9±10.0	10.1±7.5	9.6±7.3	10.4±8.7	11.5±9.9	10.8±16.2	9.6±12.9	9.3±12.1	12.7±17.5	10.6±14.9
Δ	1.6±2.6	-2.1±2.4	-1.6±2.8	-1.2±2.0	0.4±4.0	-0.9±2.5	1.5±3.2	0.0±0.4	-0.1±0.8	1.8±2.5	0.8±1.7
C (µg/dl)											
Mon	13.1±8.4	12.6±6.3	11.5±7.1	13.1±8.5	14.0±8.6	12.6±7.6	15.1±10.1	11.1±7.4	17.7±12.3	9.7±3.4	13.4±8.9
Fri	15.1±10.4	10.8±7.8	13.7±6.8	11.4±7.3	14.8±9.8	12.7±8.1	14.1±9.0	11.4±4.8	10.1±3.6	12.6±7.1	12.1±6.5
Δ	3.1±4.0	-1.0±2.3	2.3±4.5	-0.9±4.1	4.2±10.3	0.9±3.7	-0.9±2.0	0.3±3.2	-7.5±5.9	2.9±4.0	-1.3±3.7

Note: Creatine kinase (CK), Urea (U), Cortisol (C) and Free-testosterone (FT); Monday (Mon), Friday (Fri); Low intensity high volume training phase (LIHV), High intensity low volume training phase (HILV).
*****P = <0.05.

Table 2: Mean coefficients of variation (CV).

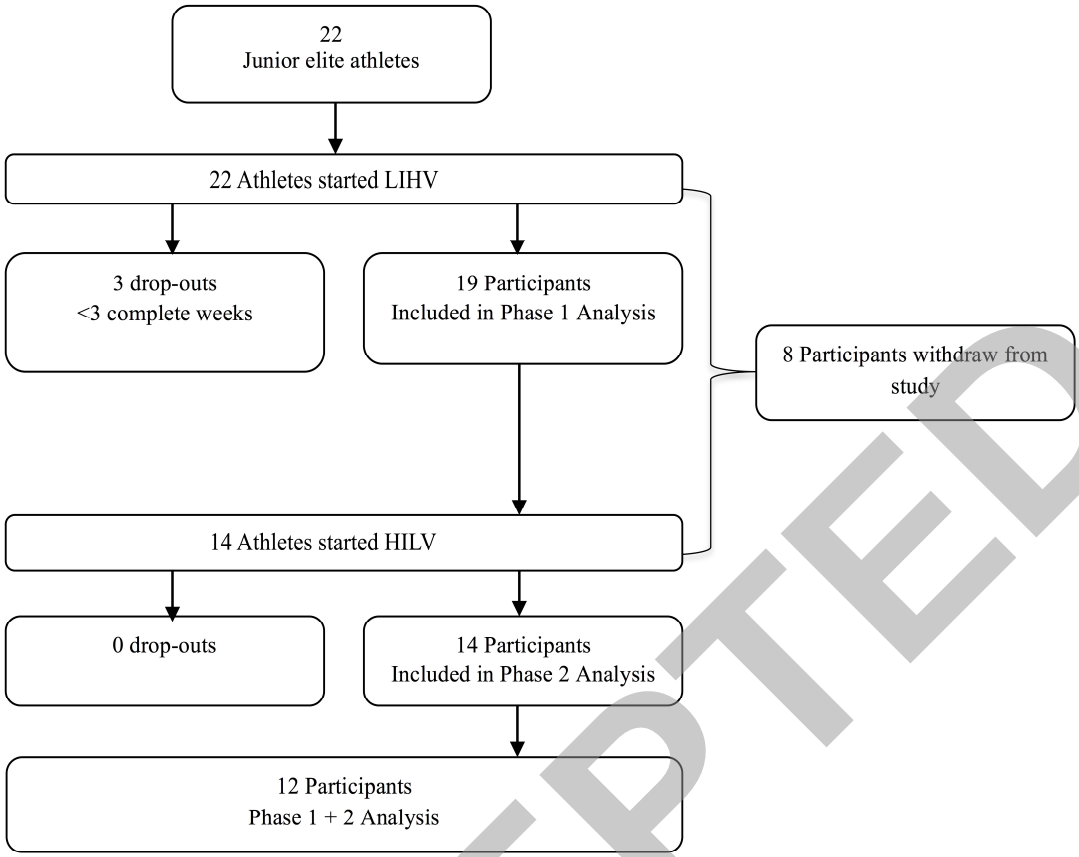
Blood parameter	CK			U			C			FT		
Time point	Mon	Fri	Δ	Mon	Fri	Δ	Mon	Fri	Δ	Mon	Fri	Δ
LIHV	27.3	34.7	164.7	19.0	14.2	224.7	32.5	38.8	568.4	22.2	19.8	444.3
HILV	22.4	28.2	109.5	14.2	12.1	1334.6	50.6	37.1	152.9	22.5	26.3	383.3

Note: CV expressed as %.
Creatine kinase (CK), Urea (U), Cortisol (C) and Free-testosterone (FT). Mondays (Mon), Fridays (Fri) and differences (Δ). Low intensity high volume training phase (LIHV), High intensity low volume training phase (HILV).

Table 3: Bivariate ratios of fatigue induced changes in outcome measures.

ΔCK/ΔU	Week 1	Week 2	Week 3	Week 4	Week 5		Week 1	Week 2	Week 3	Week 4
Subject 1	0.7	3.3	1.0	1.8	1.3		1.0	0.8	8.0	1.0
Subject 2	0.8	2.0		1.4	1.3		**1.2**		**1.7**	**1.2**
Subject 3		**2.7**	**5.5**	**3.0**	**3.3**		**3.7**	**1.4**	**3.3**	**7.0**
Subject 4	**0.5**		**0.9**	**0.6**	**0.8**		**0.6**	**0.8**	**0.7**	**0.6**
Subject 5	0.6	8.0	2.3	1.7	1.1		0.6	1.2	7.0	1.0
Subject 6		**2.2**	**5.0**	**1.8**	**1.2**		**2.3**	**1.5**	**1.2**	**7.0**
Subject 7	**0.5**	**0.8**	**0.4**		**0.6**		0.8	1.4	1.3	1.0
Subject 8	2.8	0.5	2.8	1.1			**2.7**	**4.3**	**3.3**	**11.0**
Subject 9	**2.2**	**7.0**	**6.0**		**1.7**		4.0	1.5	4.0	1.0
Subject 10	**10.0**	**2.3**	**7.0**		**13.0**		**2.0**	**1.7**	**1.7**	**7.0**
Subject 11	**7.0**		**2.0**	**2.0**	**2.0**		**1.7**	**8.0**	**3.5**	
Subject 12	1.0		1.2	0.5	0.5		0.7	3.0	0.9	2.3
ΔFT/ΔC	Week 1	Week 2	Week 3	Week 4	Week 5		Week 1	Week 2	Week 3	Week 4
Subject 1	1.3	0.6	0.8	0.8	0.8		0.6	0.5	5.0	0.5
Subject 2	**1.4**	**1.7**		**1.5**	**1.2**		0.5	0.5	4.0	1.5
Subject 3		0.6	0.9	1.3	1.0		0.8	5.0	5.0	0.6
Subject 4	0.7	1.7	0.9	1.7	4.0		1.2	1.3	4.0	0.6
Subject 5	0.7	1.0	9.0	2.0	0.2		1.0	0.4	4.0	0.9
Subject 6		0.1	1.0	1.0	1.7		3.0	1.7	5.0	0.9
Subject 7	0.3	1.3	2.0		1.8		0.9	0.5	1.6	1.0
Subject 8	4.0	1.0	1.0	0.3			**0.4**	**0.3**	**0.3**	**0.1**
Subject 9	0.8	3.5	0.3		1.0		**1.5**	**1.5**	**2.0**	**2.5**
Subject 10	2.0	0.7	0.1	1.0	1.2		0.4	1.0	0.6	1.8
Subject 11	1.0		0.4	0.4	0.4		1.1	0.7	0.8	0.8
Subject 12	**1.2**		**1.2**	**1.3**	**1.3**		**0.6**	**0.5**	**0.4**	**0.4**

Note: Categorized Monday – Friday differences (Δ) for creatine kinase divided by urea (ΔCK/ΔU) and free-testosterone divided by cortisol (ΔFT/ΔC). Values of 1.1 or greater indicate a CK or FT response respectively, which are highlighted in darker grey; values of 0.9 or less indicate a U or C response respectively, which are highlighted in lighter grey.



Swimmers

LIHV	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
S (km)	Rest day	15.0 ± 0.2 L	14.7 ± 1.6 L	8.7 ± 1.8 H	14.3 ± 2.7 L	13.5 ± 2.8 L	5.7 ± 2.6 H
ST (min)		30 ± 0	30 ± 0	90 ± 0	30 ± 0	30 ± 0	
HILV	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
S (km)	Rest day	14.5 ± 9.5 H	15.3 ± 9.5 H	7.9 ± 0.5 L+S	14.5 ± 8.1 H	14.8 ± 0.1 H	Rest
ST (min)		30 ± 0	30 ± 0	60 ± 0	30 ± 0	30 ± 0	Day

Triathletes

LIHV	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
S (km)	Rest day	3.9 ± 0.5 L	3.6 + 0.7 L	3.9 ± 0.5 L	4 ± 0	3.4 ± 0.5 L	4.6 ± 0.9 L
R (km)			9.8 ± 1.3 L	10.7 ± 2.3 L	11.0 ± 2.4 L	7.0 ± 4.2 L	12.3 ± 7 L
C (km)				30 ± 0 L	45 ± 0 L+P	45 ± 0 L+P	42.0 ± 9.9 L
ST (min)		45 ± 0		45 ± 0		30 ± 0	
HILV	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
S (km)	Rest day	4.0 ± 0.7 L	3.8 ± 1.1 L	4.1 ± 0.3 L		3.9 ± 1.0 L	1.5 ± 0 L
R (km)			8.0 ± 1.6 H	8.0 ± 2.8 H+P	7.8 ± 1.4 H	7.0 ± 1.4 H	4.5 ± 0.7 H+S
C (km)				40.0 ± 8.7 L+C	32.5 ± 9.6 L+C		20.0 ± 0 H
ST (min)		41 ± 8		45 ± 0	45 ± 0	30 ± 0	

LIHV: Low intensity high volume. HILV: High intensity low volume
Mean times and/or distances for all days throughout the training period.
Activity type described (S = Swimming, R = Running, C = Cycling, ST = Strength training).
Intensity description: L = Endurance training low intensity
H = Endurance training high intensity
P = Force development training (Power)
S = Speed development training
C = Competition specific training