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Relationship of long-term macronutrients intake on anabolic-catabolic hormones in female elite volleyball players

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ABSTRACT

Introduction: Specific macronutrient distribution and training can alter acute and chronic hormone behavior and, subsequently, sport performance.

Objective: The main aim was to examine relationships between dietary intake and anabolic/catabolic hormone response in elite female volleyball players during a 29-week season.

Methods: Twenty-two elite female volleyballers (26.4 ± 5.6 years; 178 ± 9 cm; 67.1 ± 7.5 kg) had dietary intake (seven-day dietary recall and food frequency questionnaire), blood concentration of anabolic/catabolic hormones concentration, physical performance, and body composition assessed at four time points: a) T1: baseline/pre-testing; b) T2: eleven weeks after T1; c) T3: ten weeks after T2; and d) T4: eight weeks after T3. Hormones evaluated were: total testosterone (TT), free testosterone (FT) adrenocorticotropic hormone (ACTH), and cortisol (C), along with hormone ratios.

Results: Positive correlations were observed between carbohydrate/protein ratio with ΔFT ($r = 0.955$; $p < 0.001$), ΔTT/C ratio ($r = 0.638$; $p = 0.047$), and ΔFT/C ratio ($r = 0.909$; $p < 0.001$). Significant and negative correlations were found between protein intake with ΔTT ($r = -0.670$; $p = 0.034$), and FT ($r = -0.743$; $p < 0.001$), carbohydrate intake and ΔACTH ($r = -0.658$; $p = 0.006$). No relationships were observed regarding Δcortisol. On the other hand, there was no change ($p > 0.05$) in body mass or body mass index at any time point, and the sum of six skinfolds improved ($p < 0.05$) from T1 (86.5 ± 6.9 mm) to T4 (75.2 ± 5.6 mm) as did muscle mass (T1: 28.9 ± 0.7 kg *vs* T4: 30.1 ± 0.8 kg). Vertical jump, spike-jump and speed improved ($p < 0.05$) from T1 to T4.

Conclusions: A high carbohydrate/protein ratio was associated with positive changes in anabolism, while high protein and low carbohydrates (CHO) were associated with an attenuated anabolic response.

Key words: Dietary intake. Testosterone. Cortisol. Macronutrients. Performance.

RESUMEN

Introducción: la distribución específica de macronutrientes y el entrenamiento pueden alterar el comportamiento agudo y crónico de las hormonas y, posteriormente, el rendimiento deportivo.

Objetivo: el objetivo principal del estudio fue examinar la relación entre la ingesta dietética y la respuesta anabólica/catabólica hormonal de jugadoras de élite de voleibol durante una temporada de 29 semanas.

Métodos: se evaluó en 22 jugadoras de élite (26,4 ± 5,6 años, 178 ± 9 cm, 67,1 ± 7,5 kg) la ingesta dietética (mediante un registro de siete días y un cuestionario de frecuencia de consumo de alimentos), la concentración de hormona anabólica/catabólica en sangre, el rendimiento físico y la composición corporal en cuatro puntos durante la temporada: a) T1: pre-pretemporada; b) T2: once semanas después de T1; c) T3: diez semanas después de T2; y d) T4: ocho semanas después de T3. Las hormonas evaluadas fueron: testosterona total (TT), testosterona libre (FT), hormona adrenocorticotropa (ACTH) y cortisol (C); se calcularon distintos ratios hormonales.

Resultados: se observaron correlaciones positivas entre la ratio carbohidratos/proteínas con ΔFT (r = 0,955; p < 0,001), ΔTT/C (r = 0,638; p = 0,047) y ΔFT/C (r = 0,909; p < 0,001). Se encontraron correlaciones significativas y negativas entre la ingesta proteica con ΔTT (r = -0,670; p = 0,034) y FT (r = -0,743; p < 0,001), la ingesta de carbohidratos y ΔACTH (r = -0,658; p = 0,006). No se observó ningún tipo de correlación con el Δcortisol. Por otro lado, no hubo cambios (p > 0,05) en la masa corporal ni en el índice de masa corporal en ningún momento, mientras que el sumatorio de seis pliegues mejoró (p < 0,05) de T1 (86,5 ± 6,9 mm) a T4 (75,2 ± 5,6 mm), así como la masa muscular (T1: 28,9 ± 0,7 kg frente a T4: 30,1 ± 0,8 kg). El salto vertical, el salto de remate y la velocidad mejoraron (p < 0,05) de T1 a T4.

Conclusión: en conclusión, una alta ratio de carbohidratos/proteínas se asoció con cambios positivos en el anabolismo, mientras que una ingesta alta de proteína y baja de CHO se asoció con una respuesta anabólica atenuada.

Palabras llave: Ingesta dietética. Testosterona. Cortisol. Macronutrientes. Rendimiento deportivo.

INTRODUCTION

Volleyball is intermittent, and often has ≤ 48 hours between training and matches, thus recovery-status must be monitored to avoid diminished performance (1) during match play. Specifically, inadequate recovery or overreaching can lead to elevated cortisol (C) and decreased serum testosterone (T) (2-4). Importantly, enhanced resting testosterone/cortisol (T/C) ratio has been associated with increased strength (5).

Therefore, monitoring T and C can provide insight into an athlete's recovery/readiness (6), and can be a tool to program daily volume/intensity of training (7).

Physiologically, T and C are secreted via the hypothalamic-pituitary-adrenal axis. Adrenocorticotropic releasing hormone (ACTH) facilitates C release, which is exercise volume-dependent (8,9). The steroid hormone, T, has both anabolic and anti-catabolic effects, contributing to the growth/remodelling of tissues (10), and when elevated enhances muscle glycogen synthesis (11). Moreover, nutritional intake (10,12) and specific macronutrient intake can effect acute hormone levels (13-17), however, chronic hormone concentrations (5), not acute (18), are most suggestive of long-term performance changes. Indeed, chronic high protein (8) and fat consumption (19) have been associated with elevated resting T. Data have shown decreased resting T when the carbohydrate/protein (CHO/P) ratio favors protein (8,12), but this is mitigated with sufficient CHO (8), suggesting T/C ratio is CHO-dependent (20).

Despite the data, which exists regarding macronutrient intake and changes in T/C ratio, the majority of this data is related to short-term protocols and male athletes (21,22). Therefore, the primary aim of this study was to examine relationships between total energy and macronutrient intake in conjunction with controlled training on chronic anabolic/catabolic hormone changes in elite female volleyball players during a 29-week season. We hypothesized that a low CHO/P ratio would be associated with decreased T and T/C ratio, and increased C and ACTH. Further, we anticipated the opposite hormone response if a high CHO/P ratio was observed.

MATERIALS AND METHODS

Ethics committee

All participants were informed of the protocol and risks/benefits and signed a written consent form prior to participation. The study was designed in compliance with the recommendations for clinical research of the Declaration of Helsinki of the World Medical Association (2008). The protocol was reviewed and approved by the Ethics Committee of the University of Leon.

Participants and daily training

Twenty-two elite female volleyball players (26.4 ± 5.6 years; 178 ± 9 cm; 67.1 ± 7.5 kg) participate from two teams of ten teams from the Spanish First National Professional League that the previous season, were the best two teams of the regular league, participated in this study. Initially, 24 players began the study; however, two participants left the study (one in each group) during the experimental protocol due to injury sustained during the season. Of the participants, 62% are currently or have previously represented a national team (Spain: ten players; Argentina: one player; Brazil: three players; and Serbia: one player). All participants performed the same training program and matches throughout the season supervised by the same physician (Fig. 1). The training program was in accordance with team sport periodization theory (23), and similar to previous programs in this population (24). A standard training day involved two sessions: a) a morning session with 30 minutes of jogging and 90 minutes of resistance training; and b) an afternoon session, with a 3-hour volleyball practice. The specifications of this training program were followed every day except on match days and days immediately following a match. On post-match days players engaged in recovery training (i.e., 20 minutes jogging and stretching).

All athletes completed a medical history questionnaire and electrocardiographic and cardiopulmonary examinations. No participants had any diseases, nor smoke, drank alcohol or took medications (including oral contraceptives), which would alter hormone response. All volleyballers had normal menstrual cycles of 28-31 interval days before the study.

Experimental protocol and assessment plan

This study was designed to examine relationships between macronutrient consumption and hormonal changes throughout a 29-week competitive season. The 22 participants were evaluated for dietary intake, physical performance, anthropometrics and hormone levels at four time points: a) baseline/pre-testing (T1-before preseason); b) eleven weeks later (T2); c) ten weeks following T2 (T3); and d) eight weeks following T3 (T4).

Blood collection and biochemical analysis

Blood samples (10 ml) were collected from the antecubital vein from all players, at each time point in basal conditions, which followed an overnight fast and 36 hours removed from exercise. Further, for blood collection, the players arrived at the laboratory at 8:30 am, and upon arrival sat comfortably for 30 minutes. Additionally, blood samples were obtained during the early follicular phase to avoid coinciding with the menstruation or ovulatory phases. The blood sample was left in room temperature for ten minutes before 15 minutes of centrifugation at 4 °C and 3,000 rpm. Next, serum was separated and stored in aliquots at -20 °C until analysis. All analyses were conducted in accordance with the manufacturer's instructions.

Serum total testosterone (TT)

Serum TT was measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (DRG testosterone ELISA kit®, DRG Instruments GmbH, Marburg/Lahn, Germany). Intra-assay coefficient of variation (CV) was 4.3% and inter-assay CV was 9.2%. FT was obtained by a validated formula (25).

Sex hormone binding globulin (SHBG)

SHBG concentrations were measured by a chemoluminescence based on immunometric method (Immulite, 2000), which was from the same supplier as TT. Intra-assay CV was 3% and interassay CV was 7%.

Cortisol (C)

Serum C levels were measured by an enzyme-linked fluorescent assay with the aid of a multiparametric analyser (Minividas®, Biomerieux, Marcy l'Etoile, France). The substrate, 4-methyl umbelipherone, was used and was capable of a fluorescent emission at 450 nm after stimulation at 370 nm. Intra-assay CV was 5.7% and interassay CV was 6.2%.

Adrenocorticotropic hormone (ACTH)

ACTH was determined with a radioimmunoassay (RIA). Intra-assay CV was 4.8% and inter-assay CV was 6.7%.

Hormone ratio calculations

Total testosterone/cortisol ratio TT/C and FT/C ratios were calculated from TT, FT and C concentrations. Similarly, ACTH/C ratio was calculated from ACTH and C concentrations, and the TT/SHBG ratio was calculated from TT and SHBG concentrations.

Dietary assessment

Dietitians taught participants to accurately track food intake. After blood collection, at each time point, participants completed a validated food frequency questionnaire (FFQ) (26), used in a similar population (27). This FFQ asked the athletes to recall average energy intake between each time point. The FFQ included 139 different foods and drinks, arranged by food type and meal pattern. The FFQ had frequency categories based upon the number of times items were consumed per day, week and month. Daily consumptions of total energy (kcal) and macronutrients (in grams) were determined by dividing the reported intake by frequency in terms of days (26). Additionally, the participants completed a seven-day dietary recall at all four assessment points and were asked to recall their average total energy and specific macronutrient intake over the previous seven days as a check on the FFQ. When participants weighed food, that data was used; however, when it was not possible to weigh food, an estimation of serving size consumption was determined from either product names, the place of food consumption, or via standard weights of food items or portion size, which participants indicated in a picture booklet containing 500 photographs of various foods. Food values were converted into total kcal intake and specific macronutrient distribution using the online validated food software package (Easy diet© online version) (28).

Macronutrient intake was compared to the established recommendations for this population: a) total energy: 50-80 kcal/kg/BM/day (29); b) protein: 1.6-1.8 g/kg/BM/day (30); c) fat: 20-35% of total calories (31); and d) CHO 5-8 g/kg/BM/day (29). Further, CHO/P ratio was calculated as CHO (g)/protein (g).

Body composition and anthropometric measures

Anthropometric measurements (height, body mass [BM], and the sum of six skinfolds-∑6SF) were taken following the protocol of the International Society for the Advancement of Kinanthropometry (32), and the same certified investigator (ISAK level 3) took all anthropometric measurements. The ∑6SF (triceps, subscapular, supraspinale, abdominal, front thigh, medial calf) was obtained using a Harpenden® skinfold caliper, with a precision of 0.2 mm. Two measurements were taken at each site, and if two measurements at any site were greater than 2.6% technical error of measurement (TEM), then a third measurement was taken. Body mass index (BMI) was calculated using the standard formula: BM/height^2 (kg/m^2).

Limb girths and muscle mass were also assessed. Limb girths (cm) (relaxed arm, chest, waist, mid-thigh and calf girth) were measured with a metallic Lufkin® measuring tape (W606PM), with a precision of 1 mm. All TEMs for girths assessed were less than 0.45%. Muscle mass (kg) was calculated using the Lee equation (33). All girths were corrected for the skinfold at the site using the following formula: [corrected girth = girth - (π x skinfold thickness at the site)].

Physical performance tests

To evaluate changes in physical performance, a battery of tests representing volleyball-specific fitness were performed at each time point in an indoor sports hall with standard conditions (temperature: 21 °C and humidity: 60%). The tests were as follows: jump tests (vertical jump [VJ] and spike-jump [SPJ]), a 2 x 18 m sprint test, and the overhead medicine ball throw. These tests have been previously validated as a measure of volleyball-specific fitness and used in a similar population (34).

Statistical analyses

Results are expressed as mean ± standard error. The Shapiro-Wilk test was used to determine normality of data. A one-way repeated measures analysis of variance (ANOVA) was carried out by Greenhouse-Geisser test to check for significant differences between percentage change of hormone concentrations and hormone ratios from T1-T2, T2-T3, and T3-T4, as well as for changes in physical performance, and body composition parameters at each time point. A Bonferroni post-hoc test was applied for pairwise comparisons. Further, to examine relationships between dietary

intake (total kcal and macronutrients), bivariate correlations between changes in hormones and nutritional intake during the season were tested using Pearson or Spearman's rank order correlation test. The hormonal percentage change (%Δ) in hormone concentrations was also calculated from T1-T4, T1-T2, T2-T3, and T3-T4. Mean dietary intake for the entire 29 weeks was calculated by summing the average intake of each period and then dividing by 3 ([T1-T2 mean + T2-T3 mean + T3-T4 mean] / 3). Statistical analyses were performed using the IBM Statistical Package (SPSS version 22) and Graphpad Prism (Graphpad Software version 6, San Diego, CA). Significance was set at $p \leq 0.05$.

RESULTS

Reliability of FFQ and seven-day dietary records

The results regarding total energy intake and specific macronutrient consumption obtained by the FFQ in comparison to the seven-day dietary records were not significantly different ($p > 0.05$) (data not shown).

Hormone: percentage change

Reporting percentage change can effectively illustrate the magnitude of alteration in an observational study. Therefore, table I displays hormone values at T1 and %Δ of each hormone and hormone ratio in the following periods: T1-T2, T2-T3, and T3-T4. From T1-T2 there were no significant ($p > 0.05$) changes in any hormonal parameter. In T2-T3 there were significant increases ($p < 0.05$) in TT (26.1 ± 11.0%), SHBG (24.8 ± 11.8%), and TT/C (19.0 ± 12.1%), however, no change ($p > 0.05$) was observed in any other hormone parameter during this period. From T3-T4 there were significant changes ($p < 0.05$) in TT (+10.4 ± 4.9%), ACTH (11.5 ± 5.4%), and TT/C (7.0 ± 3.8%). Finally, %Δ from T1-T4 is displayed in figure 2.

Total energy and macronutrient-specific consumption

Table II shows daily total energy intake and specific macronutrient distribution during each period along with average total kcal and macronutrient consumption for the entire study. This table also displays the dietary established recommendations (ER) for this population. Total kcal and specific macronutrient intake were not different ($p >$

0.05) between periods. Compared to ER, athletes consumed lower total energy (41.8 ± 1.9 kcal) and CHO (4.5 ± 0.2g/kg/d) in each period and over the 29 weeks. Contrastingly, we observed greater average protein (2.1 ± 0.1 *vs* 1.6-1.8 g/kg/d) and fat consumption (35.3 ± 1.0 *vs* 20-35% of total kcal) as compared with ER. Additionally, observed CHO/P ratio was 2.22 ± 0.10 g/day, which was lower than ER.

Relationship between dietary intake and hormone concentrations

Table III displays specific values of significant bivariate correlations between changes in hormone concentrations and dietary intake parameters from T1-T4. Significant ($p < 0.05$) and positive correlations were present between: total energy-kcal/kg/day and ΔSHBG; CHO-g/kg/day and ΔSHBG; and between CHO/P with ΔFT, ΔTT/C ratio, and ΔFT/C ratio. Significant ($p < 0.05$) and negative correlations were present between: protein intake-% of total kcal and ΔTT; protein-% of total kcal and FT; CHO-g/kg/day and ΔACTH; and between CHO/P and ΔSHBG. No relationship was discovered between ΔC and dietary intake or between fat intake and any hormone parameter (data not shown).

Body composition and performance assessments

Specific values for body composition changes can be seen in table IV. There was no significant difference ($p > 0.05$) in BM and BMI between periods. There was a significant change ($p = 0.011$) in ∑6S from T1-T4 (-12.9%). Similarly, muscle mass decreased ($p = 0.005$) from T1-T4 (+2.4%).

Regarding vertical jump power (VJP), values at T2, T3, and T4 were significantly greater ($p < 0.001$) than at T1; however, T2, T3, and T4 were not different ($p > 0.05$) from each other. Similar to VJP, SJP was greater ($p = 0.003$) at T1, T2, and T3 in comparison with T4, and T2, T3, and T4 were not different ($p > 0.05$) from each other. Absolute VJ height was significantly greater ($p < 0.05$) at T3 and T4 as compared with T1 (0.31 ± 0.01 cm), but not at T2 ($p > 0.05$) (data not shown). Absolute SPJ height did not increase ($p > 0.05$) from T1 to T2, but was significantly greater ($p < 0.05$) at T3 and T4 (0.54 ± 0.02 cm) *vs* T1 (0.48 ± 0.01 cm; +12.5%) (data not shown).

Speed (2 x 18 m sprint) significantly improved ($p < 0.001$; +3.2%) from T1-T2, then plateaued until T4. Throwing distance for the overhead medicine ball throw (OMBT) approached significance from T1-T4 ($p = 0.092$; +1.9%).

DISCUSSION

The main finding supports our hypothesis that lower protein intake was significantly and inversely correlated with ΔTT ($r = -0.670$) and ΔFT ($r = -0.743$). Additionally, in support of our hypothesis we observed strong and direct relationships between increased CHO/P ratio and increased anabolism. Specifically, CHO/P ratio was directly correlated with ΔFT ($r = 0.955$), ΔTT/C ($r = 0.638$), and ΔFT/C ($r = 0.909$) over the 29 weeks. Total energy ($r = 0.774$) and CHO ($r = 0.681$) intakes were positively associated with ΔSHBG, and an inverse relationship was found between CHO intake and ΔACTH ($r = -0.658$). However, no relationship was observed between C and any dietary intake parameter. Finally, significant improvements were observed from T1-T4 in body composition (i.e., ∑6S and muscle mass).

Even though it is well established that adequate total kcal must be consumed for positive performance outcomes (29), the present study has achieved novelty as the first investigation to examine the seasonal relationship between dietary intake and temporal hormone response in elite female athletes. Interestingly, the average total energy consumption observed over the 29 weeks (41.8 ± 1.9 kcal/kg/day) was substantially lower than the established recommendations: 50-80 kcal/kg/day. However, the athletes in this study still significantly improved power/speed and body composition, and experienced positive hormonal changes (i.e., TT: +23.6%, FT: +40.1%, TT/C: +6.8%, FT/C: +25.1%) over the season, thus it seems that proper balance between training/recovery was achieved and the dietary intake was sufficient to elicit positive training/endocrine adaptations. A possible explanation for the perceived low energy intake, yet beneficial endocrine response, is that the total kcal recommendations for high intensity athletes lack sex-specificity (27). Nevertheless, it does seem that a minimum threshold for total energy exists to optimize the endocrine response, as currently, total energy intake was strongly associated with ΔSHBG ($r = 0.774$). Therefore, in terms of total energy consumption the findings are two-fold: a) female athletes who engage in high-intensity exercise can achieve appropriate

recovery while consuming lower total kcal/day than the established recommendations; and b) due to the positive relationship between total energy consumption and anabolic hormone concentrations, a minimum energy intake threshold likely exists to elicit conditions for proper training/recovery balance.

Adequate protein intake in conjunction with training can increase rate of skeletal muscle repair (35). Similarly, T contributes to tissue remodelling (10) and muscle glycogen synthesis (11). However, previous data has demonstrated a negative relationship between protein intake and TT (8). Similarly, the present data revealed a strong inverse relationship between protein intake (% of total kcal) with ΔTT ($r = -0.670$) and ΔFT ($r = -0.743$), but demonstrated a positive relationship between CHO/P with ΔFT ($r = 0.955$), ΔTT/C ($r = 0.638$), and ΔTF/C ($r = 0.909$). Therefore, the ratio of CHO/P seems to be of paramount importance to speed muscle tissue recovery. Interestingly, in the present study, mean protein intake over the entire season comprised 19.8 ± 0.6% of total kcals (2.1 ± 0.1 g/kg/d), which was greater than the ER (1.6-1.8 g/kg/d). However, despite the elevated protein intake we observed positive changes in TT, FT, TT/C, and FT/C, which can be explained by the significant relationships between these changes and CHO/P. Thus, as long as an appropriate CHO/P ratio is maintained, T will induce muscle glycogen synthesis (11), independent of absolute protein consumption. Additionally, a high-fat diet has increased endogenous T production in males, however, even though elevated fat intake (i.e., 35.3 ± 1.0% of total energy) in comparison to ER (i.e., 20-35%) was observed, there was no relationship between fat consumption and anabolism.

Typically, nutritional strategies have been more important for directly enhancing anabolism rather than blunting the catabolism (35), as various studies have demonstrated protein intake to yield no change in resting C concentrations (8,21,36). Changes in C due to nutrition have mostly been acute (16,37,38), however, it is important to note that an acute C increase is simply a regulatory response to maintain homeostatic blood glucose (39), and not catabolic. In agreement, we did not observe an association between resting C and any dietary parameter. Therefore, alterations in resting C are likely due to training and may take long periods of time (i.e., two years) to manifest (5).

The significant limitation of this study is the absence of a control group who consumed kcals/macronutrients consistent with ER. The control group would provide a basis to examine a cause-effect relationship between dietary intake and hormone response, or if hormone fluctuations are primarily training-dependent. Therefore, it must be highlighted that our results are simply correlational and causation cannot be known; thus, it is necessary for future research to include a control group to determine if a causative relationship exists in this population between CHO/P and increased resting testosterone. Further, the present study lacked measures examining muscle protein turnover, which could directly speak to the anabolic response. However, novelty has been achieved, as no study has examined the relationship between nutritional intake and anabolic/catabolic hormones over an entire volleyball season in elite female players and, in general, little data exist in regards to chronic mechanistic adaptations in elite female athletes.

CONCLUSIONS

In conclusion, dietary tracking over an entire 29-week season in elite female volleyball players revealed dietary intake which varied from ER in that: lower total energy and CHO were consumed, while greater protein and fat were consumed. Importantly, this study is novel as it is the first to show that CHO/P ratio is positively related to ΔFT, ΔTT/C, and ΔFT/C over an entire season. Practically speaking, elite female volleyballers should aim to maintain a positive CHO/P ratio to aid in maintaining an appropriate balance of the training/recovery paradigm during the season. Finally, further research should be performed with various nutritional strategies in order to determine any cause-effect relationships between nutrient intake anabolic/catabolic hormone concentrations.

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Declaration: all authors declare that all experiments comply with the current laws of Spain (country in which the experiments were performed).

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Table I. Absolute hormone levels at T1 (baseline) and the percentage change during each period

	T1	*Δ (T1-T2)*	*Δ (T2-T3)*	*Δ (T3-T4)*
TT ($ng \cdot ml^{-1}$)	0.29 ± 0.04	-12.2 ± 5.4	*26.1 ± 11.0*[a]	*10.4 ± 4.9*[a]
FT ($pg \cdot ml^{-1}$)	3.11 ± 0.64	5.3 ± 6.1	8.4 ± 14.8	27.2 ± 11.5
SHBG ($nMol \cdot l^{-1}$)	85.4 ± 18.4	-14.5 ± 9.9	*24.8 ± 11.8*[a]	0.9 ± 6.1
ACTH ($pg \cdot ml^{-1}$)	17.0 ± 2.0	49.1 ± 16.4	30.9 ± 10.7	*11.5 ± 5.4*[a]
Cortisol ($\mu g \cdot dl^{-1}$)	20.9 ± 3.1	11.8 ± 7.8	11.0 ± 5.3	1.5 ± 1.2
TT/C	1.90 ± 0.37	-18.7 ± 5.0	*n*	*7.0 ± 3.8*[a]
FT/C	0.022 ± 0.005	-3.4 ± 6.9	3.4 ± 15.9	24.1 ± 9.8

Data expressed as mean ± standard error of the mean. % Δ calculated as ($[T_{final}-T_{initial}] \times 100/T_{inital}$). T1: baseline/pre-testing; T2: eleven weeks after T1; T3: ten weeks after T2; T4: eight weeks after T3. TT: Total testosterone: FT: Free testosterone; SHBG: Sex-hormone binding globulin; ACTH: Adrenocorticotropic hormone; TT/C: Total testosterone/cortisol ratio; FT/C: Free testosterone/cortisol ratio. [a]Significant magnitude of change in the specific period as determined by the Bonferroni post-hoc test ($p < 0.05$).

Table II. Mean total energy and specific macronutrient intake during each period. The 29-week (total season) mean total energy and mean specific macronutrient intake, and the previously established recommendations for this population

	T1-T2	*T2-T3*	*T3-T4*	*Mean*	*ER*
Energy (kcal/day)	2,890 ± 88	2,790 ± 50	2,810 ± 75	2,830 ± 50	
Energy (kcal/kg/day)	42.4 ± 2.0	40.5 ± 2.1	*41.8 ± 2.0*[b]	41.8 ± 1.9	50-80
Protein (g/day)	143 ± 6.8	135 ± 6	138 ± 7	139 ± 6.9	
Protein (g/kg/day)	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	1.6-1.8
Protein (%)	20.0 ± 0.7	19.7 ± 0.6	19.6 ± 0.6	19.8 ± 0.6	
Fat (g/day)	115 ± 6	106 ± 4	108 ± 3.7	109 ± 3.2	
Fat (g/kg/day)	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	
Fat (%)	35.5 ± 1.6	34.9 ± 1.2	35.5 ± 1.1	35.3 ± 1.0	20 - 35
CHO (g/day)	305 ± 11	297 ± 6.1	305 ± 9	302 ± 6.5	
CHO (g/kg/day)	4.5 ± 0.2	4.4 ± 0.2	4.5 ± 0.2	4.5 ± 0.2	5-8
CHO (%)	42.7 ± 1.2	43.9 ± 1.1	43.7 ± 1.0	43.5 ± 0.9	
CHO/P ratio	2.16 ± 0.09	2.25 ± 0.11	2.27 ± 0.11	2.22 ± 0.10	

Data expressed as mean ± standard error of the mean. T1: Baseline/pre-testing; T2: Eleven weeks after T1; T3: Ten weeks after T2; T4: Eight weeks after T3. ER: Established recommendations; CHO: Carbohydrates; CHO/P: Carbohydrate/protein ratio. kcal/day: Kilocalories per day. kcal/kg/day: Kilocalories per kilogram per day; g/day: Grams per day; g/kg/day: Grams per kilogram per day; %: Percentage of macronutrient consumed of total calories.

Table III. Correlations between changes in hormones from Δ (T1-T4) (29 weeks) and energy and macronutrient intake (mean during season)

	Δ TT	*Δ FT*	*Δ SHBG*	*Δ ACTH*	*Δ Cortisol*	*Δ TT/C*	*Δ FT/C*
Energy							
kcal/kg/day	ns	ns	0.774**	ns	ns	ns	ns
Protein							
%	-0.670*	-0.746**	ns	ns	ns	ns	ns
CHO							
g/kg/day	ns	ns	0.681*	-0.658*	ns	ns	ns
Ratios							
CHO/P	ns	0.995**	-0.644*	ns	ns	0.638*	0.909**

TT: Total testosterone; FT: Free testosterone; SHBG: Sex-hormone binding globulin; ACTH: Adrenocorticotropic hormone; TT/C: TT/cortisol ratio; FT/C: FT/cortisol ratio. CHO: Carbohydrates; CHO/P: Carbohydrate/protein ratio. *p < 0.05. **p < 0.001.

Table IV. Anthropometric, body composition and physical performance results at each assessment period

	T1	*T2*	*T3*	*T4*	*p-value*
Anthropometric and body composition					
Body mass (kg)	66.8 ± 2.4	67.3 ± 2.3	67.3 ± 2.4	67.1 ± 1.5	0.701
BMI	21.0 ± 0.4	21.1 ± 0.4	21.7 ± 0.4	21.3 ± 0.4	0.776
∑6S (mm)	86.5 ± 6.9	76.5 ± 6.3	77.5 ± 6.3	*75.2 ± 5.6*[a]	*0.011*
MM Lee (kg)	28.9 ± 0.7	29.4 ± 0.8	29.6 ± 0.8	*30.1 ± 0.8*[a]	*0.005*
Physical performance tests					
Vertical-jump power (W)	1,635 ± 56	*1,730 ± 55*[a]	*1,791 ± 76*[a]	*1,842 ± 75*[a]	*0.001*
Spike-jump power (W)	2,024 ± 73	*2,114 ± 68*[a]	*2,167 ± 89*[a]	*2,223 ± 74*[a]	*0.003*
Speed (sec)	7.48 ± 0.08	*7.39 ± 0.08*[a]	*7.25 ± 0.08*[a]	*7.23 ± 0.08*[a]	*< 0.001*
OMBT (m)	7.75 ± 0.25	7.73 ± 0.23	*7.88 ± 0.26*[a]	*7.90 ± 0.25*[b]	0.092

Data are expressed as mean ± standard error of the mean. T1: Baseline/pre-testing; T2: Eleven weeks after T1; T3: Ten weeks after T2; T4: Eight weeks after T3; BMI: Body mass index; ∑6S (mm): Sum of six skinfold sites (triceps, subscapular, supraspinale, abdominal, front thigh, medial calf) reported in millimeters; OMBT: Overhead medicine ball throw. MM Lee: Muscle mass according to the Lee equation; W: Watts. Speed (sec.): Speed results in seconds of the 2 x 18 m sprint test; m: Meters; p-value: Effects tests within subjects by Greenhouse-Geisser test. [a]Significantly different from T1 according to the Bonferroni post-hoc test ($p < 0.05$). [b]Change approached significance with respect to T1. Bolded text denotes statistical significance.

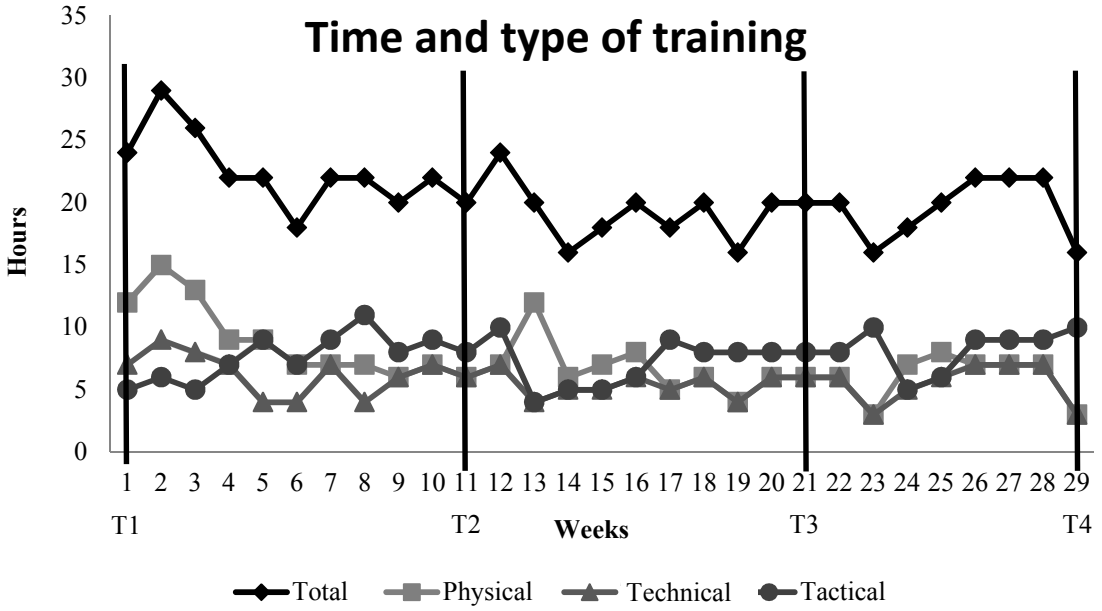

Fig. 1. Amount of time and type of training performed by players in each week of study.

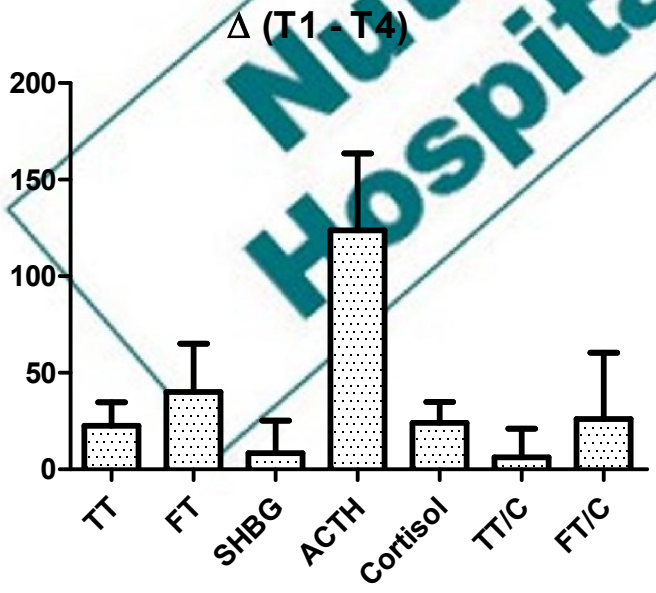

Fig. 2. Percentage change of each hormone parameter from T1-T4. Data expressed as mean ± standard error of the mean. Percentage change (%Δ) calculated as ($[T_{final}-T_{initial}] \times 100/T_{inital}$).