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BLOOD BIOCHEMICAL CHANGES AFTER DRAMATIC INCREASE IN RUNNING TRAINING VOLUME: EXPLORATORY STUDY IN 3 ELITE SOLDIERS

BIOKEMIČNE SPREMEMBE V KRVI PO DRAMATIČNEM POVEČANJU OBSEGA TEKAŠKE VADBE: RAZISKOVALNA ŠTUDIJA PRI TREH ELITNIH VOJAKIH

ABSTRACT

Ultra-Endurance running training is a powerful stressor for all biological systems and depends mainly on its volume and intensity. Although the high physical demands, soldiers are an unstudied group and information on exercise indicators are essential. This study aimed to observe the changes in serum biochemical indicators in previously endurance trained elite soldiers after a 17-week training program with a dramatic increase in running volume. Three subjects (#1: 26 years, 169,5cm; #2: 27 years, 167,9cm; #3: 27 years, 180,7cm) running daily between 10-12 km/day, increased their running volume to prepare the participation in a 100-km ultramarathon race. For 17 weeks the training program included 10-12 sessions per week, corresponding to 200-260 km. Average daily running volume was 35.8±6.2 km. Blood samples were taken for analysis of urea, creatinine, glucose, cholesterol and triglycerides, AST, ALT, CK, aldolase, Na, chloride, P, Ca, K, Fe, Mg and cortisol. Despite a marked drop in iron and a rise in phosphorus, the overall mineral status remained within laboratory reference values. ALT, AST, Aldolase showed slight changes while a marked increase was found in CK. Creatinine decreased and urea maintained the high starting values. Changes of glucose, cholesterol and triglycerides had no clinical significance. After the 17-week the cortisol increased to outside of the reference values in two participants. This study shows that a dramatic increase in running training volume experienced by previous trained runners is mainly reflected in basal blood chemistry through the reduction of iron and creatinine and increase of cortisol.

Keywords: endurance training, enzymes, cortisol, iron

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IZVLEČEK

Vadba teka za izjemno vzdržljivost je močan stresor za vse biološke sisteme in je odvisen predvsem od njegovega obsega in intenzivnosti. Kljub visokim fizičnim zahtevam so vojaki neraziskana skupina, zato so informacije o kazalnikih vadbe bistvenega pomena. Namen te študije je bil opazovati spremembe biokemičnih kazalnikov v serumu pri predhodno vzdržljivostno treniranih elitnih vojakih po 17-tedenskem programu vadbe z dramatičnim povečanjem obsega teka. Trije preiskovanci (#1: 26 let, 169,5 cm; #2: 27 let, 167,9 cm; #3: 27 let, 180,7 cm), ki so dnevno pretekli med 10 in 12 km/dan, so povečali obseg teka, da bi se pripravili na udeležbo na ultramaratonskem teku na 100 km. Program vadbe je 17 tednov vključeval 10-12 vadb na teden, kar ustreza 200-260 km. Povprečni dnevni obseg teka je bil 35.8 ± 6.2 km. Odvzeti so bili vzorci krvi za analizo sečnine, kreatinina, glukoze, holesterola in trigliceridov, AST, ALT, CK, aldolaze, Na, klorida, P, Ca, K, Fe, Mg in kortizola. Kljub izrazitemu padcu železa in porastu fosforja je splošno stanje mineralov ostalo znotraj laboratorijskih referenčnih vrednosti. ALT, AST in aldolaza so se rahlo spremenile, medtem ko se je CK izrazito povečala. Kreatinin se je zmanjšal, sečnina pa je ohranila visoke začetne vrednosti. Spremembe glukoze, holesterola in trigliceridov niso bile klinično pomembne. Po 17 tednih se je kortizol pri dveh udeležencih povečal in presegel referenčne vrednosti. Ta študija kaže, da se drastično povečanje obsega tekaške vadbe, ki so ga doživeli predhodno trenirani tekači, odraža predvsem v bazalni biokemiji krvi z zmanjšanjem železa in kreatinina ter povečanjem kortizola.

Ključne besede: vadba za vzdržljivost, encimi, kortizol, železo

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https://doi.org/10.52165/kinsi.29.2.220-231

INTRODUCTION

Changes induced by a single physical load are temporary and return to basal level within few days. Nevertheless, arduous training regimens imposing continuous loads can elicit changes that persist over time. Long-distance running training is a powerful stressor that induces specific adaptations according to the intensity, volume and frequency of training loads. Prolonged endurance running can lead to biochemical changes consistent with tissue destruction that provoke leakage of intracellular muscle components into the extracellular fluid (Bäcker, Richards, Kienzle, Cunningham, & Braun, 2023). Several enzymes can leak to plasma after long lasting exertion. Strenuous exercise leading to liver hypoxia can cause cell injury with subsequent release of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Pettersson et al., 2008). Despite AST enzyme reflects the integrity of hepatocytes the behavior of its plasmatic expression, in particular its post-exercise increase, in addition to the elevation of myoglobin, troponin I, fragments of the myosin heavy chain and total creatine kinase concentrations, are relevant indicators in the diagnosis of lesions of skeletal muscle tissue and cardiac muscle tissue (Clarkson, Kearns, Rouzier, Rubin, & Thompson, 2006). In order to show overload or liver damage AST/ALT ratio (De Ritis ratio) has been used (Ndrepepa, 2023) and when elevated it may indicate increased risk associated with hepatic and extrahepatic diseases indicating abnormalities at the level of basic metabolism. While AST and ALT are enzymes related with damaged hepatic cells, skeletal muscle injury produces high serum levels of Aldolase and creatine kinase (CK) (Brancaccio, Lippi, & Maffulli, 2010). The adaptations are mainly dependent on the subjects' training level although age, gender, race, muscle mass and climatic conditions can interfere with overall responses (Brancaccio, Maffulli, & Limongelli, 2007). Rodrigues dos Santos (2017) showed that after a 50-km running less trained runners, when compared to their more trained counterparts, showed a much higher rise in CK, AST, ALT, and Aldolase therefore, it seems that the training level determines the enzymatic response to effort. In addition, in the same study it was observed that the less trained runners had a delayed recovery. It seems that higher basal values of CK are associated with higher training volume and the reduction of training volume is reflected in the reduction of plasma CK (Houmard et al., 1990).

Studies on the behavior of blood lipids induced by exercise are inconsistent being difficult to establish a dose-response relationship (Leon & Sanchez, 2001). It seems that regular exercise provokes a 24% mean reduction in blood triglycerides (Trejo-Gutierrez & Fletcher, 2007). Immediately after exercise, plasma triglyceride concentration decreases with the concomitant increase of free fatty acids indicating a strong lipid mobilization. And it seems that 24 hours of rest are sufficient to recover baseline values (Barakat, Kerr, Tapscott, & Dohm, 1981).

Long-lasting running leads to changes in the hypothalamic-pituitary-adrenocortical axis, increasing plasma cortisol concentration that only decreases significantly 2 days after exertion (Bobbert et al., 2005). It is also known that there is a close relationship between the iron status and physical performance in humans (McClung & Murray-Kolb, 2013). Exercise-induced hemolysis has been implicated in the sub-optimal status of endurance-trained athletes (Weight, Byrne, & Jacobs, 1991). Sodium, potassium, calcium, magnesium, and chloride are the crucial electrolytes that regulate muscle contraction (Kuo & Ehrlich, 2015). Sodium and chloride are the major electrolytes for hydric balance but are easily restored with an adequate and normal salted diet (Maughan & Shirreffs, 1997). When athletes during workouts match their fluid losses with the available commercial sport drinks, a significant intake of minerals is achieved and fluid balance and training capability is restored (von Duvillard, Arciero, Tietjen-Smith, & Alford, 2008).

High training volume is crucial for 100-km race performance (Knechtle, Knechtle, Rosemann, & Lepers, 2010) what put a big challenge for body’s adaptation. This study sought to verify the biochemical changes induced by a severe increase in training volume, for 17 weeks, in previous moderate trained runners preparing for participation in a 100-km ultramarathon race.

METHODS

Participants

Three male soldiers from the Portuguese Army Elite Corps (Special Forces) participated in this study. Participant’s age and height were as follows: subject #1 (26 years; 169.5 cm), subject #2 (27 years; 167.9 cm), and subject #3. (27 years; 180.7 cm). These participants were not typical athletes but very active elite soldiers that run as a fundamental part of their physical military preparation and, have more than 5 years of running training. They regularly engage in military orienteering races and sporadically in civil road races. Their periodical medical screening showed no health constraints, besides participants were non-smokers and non-alcoholic drinkers. During the year prior to this study participants usually ran 10-12 km daily. The participants can be considered non-elite runners (average rate of 4 min/km for the half-marathon) very far from middle- and long-distance elite runners’ performance (average rate ≤

3 min/km at half-marathon). Prior to signing an informed consent form participants were informed about the benefits and risks of taking part in the current study, which was approved by the ethics board of the Faculty of Sport of the University of Porto. Experimental procedures were in accordance with Helsinki Declaration and ethical principles for medical research involving human subjects.

Training protocol

With the objective to compete in a military ultramarathon (100 km) participants executed 10-12 training sessions per week, totalizing 200-260 km. Average daily running volume was 35.8±6.2 km. Running intensity was controlled by Polar heart rate monitor with sensor chest strap. Low to moderate running pace was selected (130-160 beats per minute corresponding to 70-85% maximum heart rate) for continuous uniform running with 2 fartlek sessions per week (10 accelerations of 300m) inducing heart rates close to the maximum. They practiced twice a day on Tuesdays, Wednesdays, Thursdays, Fridays and Saturdays; and had only one workout on Sundays (the longest one) and on Mondays (the shortest one). Every four weeks during the first 3 months a performance test (30 km) was conducted which improved over time (week 4: 2h00; week 8: 1h57; week 12: 1h55). The performance test was preceded by a resting day. In the last week before testing, volume training was reduced in half while maintaining the usual intensity.

It should be noted that with the exception of fartlek training session, the continuous uniform running sessions were conducted at intensities below those which the participants were previously accustomed. The fundamental training goal was the completion of an ultramarathon (100-km) at an adequate pace to avoid overexertion while achieving the best performance possible.

Nutrition

Throughout the duration of the study, participants were requested to maintain their usual dietary diversity, increasing energy and carbohydrate intake *ad libitum*. During the study participants had no nutritional supplements. During training they usually drank a commercial isotonic drink.

Parameters evaluated

Blood Chemistry: Urea, creatinine, glucose, cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), aldolase, calcium and phosphorus were measured with an auto-analyser Hitachi 705. Sodium, potassium, and chloride were analysed with a flame photometer Korning 480. Iron and magnesium were assessed by manual technique. Cortisol was analysed by radioimmunoassay. All blood samples were collected after an overnight fast (12h) and a 24 hours compulsory rest period following the last workout, to attenuate the effects of hemodynamic variations and acute hemodilution induced by prior workout. The blood sample collection took place before and after the 17-week protocol.

RESULTS

Table 1 shows a marked decrease in body mass in all participants. Almost all biomarkers experimented slight variations and remained within clinical reference values with the exception of cortisol that exceeded the reference values in two participants with the other participant in the upper borderline. While serum iron and creatinine decreased, phosphorus increased in all participants. Initial chloride values are above the reference values, decreasing after training.

Table 1. Weight and Biochemical alterations induced by 17 weeks of training.

Variables (reference values)	Subject #1			Subject #2			Subject #3		
	M1	M2	M2-M1	M1	M2	M2-M1	M1	M2	M2-M1
Body mass (kg)	68.5	66.5	-2	69.3	65.5	-3.5	80.0	75.5	-4.5
Urea (2.9-8.9 mmol/L)	5.3	8	2.7	10.7	9.2	-1.5	8	7.5	-0.5
Creatinine (60-132 µmol/L)	129	93	-36	109	94	-15	111	85	-26
Glucose (3.6-6.1 mmol/L)	4.94	6.16	1.22	4.77	4.77	0	5.72	5.49	-0.23
Cholesterol (3.9-7.2 mmol/L)	4.1	4.3	0.2	4.4	3.9	-0.5	4.5	3.5	-1
Triglycerides (0.45-1.69 mmol/L)	0.6	0.8	0.2	0.7	1	0.3	1	1.1	0.1
AST (0-0.58 µkat/L)	0.31	0.23	-0.08	0.38	0.33	-0.05	0.41	0.28	-0.13
ALT (0-0.58 µkat/L)	0.33	0.23	-0.1	0.28	0.29	0.01	0.31	0.31	0
AST/ALT (<1)	0.94	1	-	1.36	1.14	-	1.32	0.90	-
CK (60-400 U/L)	78	124	46	112	138	26	98	116	18
Aldolase (0-6 U/L)	1.1	1	-0.1	1.5	1.3	-0.2	1.1	1.1	0
Cortisol (0-25 µg/dL)	18.8	**27.3**	8.5	18.9	**28.4**	9.5	18	24.4	6.4
Iron (9-31.3 µmol/L)	20.9	15	-5.9	18.8	12.9	-5.9	31.7	20.4	-11.3
Calcium (2.1-2.6 mmol/L)	2.32	2.39	0.07	2.27	2.19	-0.08	2.17	2.3	0.13
Phosphorus (1-1.5 mmol/L)	1	1.29	0.29	**0.84**	1.16	0.32	**0.84**	1.23	0.39
Magnesium (0.75-1.25 mmol/L)	0.8	0.87	0.07	0.95	0.94	-0.01	0.95	0.9	-0.05
Sodium (136-145 mEq/L)	138	139	1	137	138	1	139	138	-1
Potassium (3.8-5.5 mmol/L)	4.4	4.2	-0.2	4	4.4	0.4	4.8	4.9	0.1
Chloride (96-106 mmol/L)	**109**	105	-4	**109**	100	-9	**108**	100	-8

Reference values from Kratz et al. (2004)

Note: kg: kilograms; mmol/L: millimoles per litre; µmol/L: micromoles per litre; µkat/L:micro-katal per liter; U/L: units per litre; µg/dL micro-grams per deciliter; mEq/L: Milliequivalents per litre; M1: first assessment (week 0); M2: second assessment (week 17); M2-M1: differences between assessments. Bold means values are outside normal range.

DISCUSSION

A high volume of running training is essential to prepare and complete a 100-km ultramarathon with the best possible performance (Knechtle et al., 2010). This basic condition places enormous metabolic, hormonal, and enzymatic challenges with several disturbances in body homeostasis and the magnitude of alterations correlates well with the severity of exercise.

Findings of this longitudinal study showed that 17 weeks of high-volume training, as preparation for an ultra-marathon, resulted in a decreased BMI and iron levels and increased levels of CK, cortisol, phosphorus and chloride.

Long-term exercise acutely promotes plasma and serum increase in various biomarkers as glucose, calcium, phosphorus, urea, creatinine, ALT, AST, CK (Kratz et al., 2002), mainly derived from the increased protein catabolism.

Athletes are prone to display high resting urea concentrations when they practice daily (Warburton, Welsh, Haykowsky, Taylor, & Humen, 2002). This condition was verified in our study, with subject #1 sharply increasing his basal urea (50.9%) while the other two subjects maintained their high starting values after the 17-week training period. The elevation and maintenance of high plasma urea are according to the daily succession of intensive workloads that accentuate protein metabolism (Atherton & Smith, 2012). After a 100-km ultramarathon race, in 765 min, eight runners increased their serum urea from 5.69 ± 1.06 mmol/L to 9.61 ± 1.96 mmol/L. Three possible mechanisms may explain this rise: (i) reduced rate of elimination provoked by exercise, (ii) hypohydration, or (iii) increased urea production from the breakdown of amino acids during exercise. It seems that urea concentration after exercise depends on exercise duration (Haralambie & Berg, 1976). After three long-distance cross-country ski races Refsum & Strömme (1974) found production rates of urea about 60-80 per cent higher than in resting conditions. The return to the pre-race urea level lasted several days. For these authors, increase in serum urea was mainly due to decreased excretion and, to a lesser extent, to higher production what conflicts with other authors. On the other hand, Frank et al. (2009) suggest the high urea values may be a consequence of both training and diet. We sought that the prolonged duration of training sessions and the consequent protein catabolism may be the main reason for our values.

Prolonged physical exercise increases serum creatinine. The longer the duration of the effort, the greater the increase in plasma creatinine (Janssen, Degenaar, Menheere, Habets, & Geurten, 1989). Plasma levels of creatinine tend to normalize 24 hours after exertion (Warburton et al., 2002). The marked decrease in the plasma levels of creatine seen in our participants is probably due to either, an exercise-induced increased glomerular filtration rate or an increase in the secretory component of the renal handling of creatinine (Irving et al., 1990). It seems that prolonged exercise tends to increase some biomarkers of kidney injury due mainly to hypohydration (Bongers et al., 2018). However, changes are transitory and in healthy and trained subjects, it is supposed to have no clinical significance. The marked decrease in plasma concentration of creatinine (-21.7%) verified in this study seems to be related to the increase in the rate of glomerular clearance and express a good renal function. In healthy athletes, glomerular clearance rate can remain high 48 hours after endurance exercise (Irving et al., 1990) reducing plasma creatinine concentration. The reduced values of creatinine found after the training period may be also related to the reduction of body mass, as according to Banfi, Del Fabbro, & Lippi (2006) a correlation was found between creatinine concentration and Body Mass Index. Our results are corroborated by Rodrigues dos Santos et al. (2007) who showed similar results after an ultramarathon in kayak.

Plasma glucose values, within laboratory references, indicate that an overnight fasting was well matched by the glucose content of the last meal or by cortisol-induced increasing in gluconeogenesis (Goldstein et al., 1992). The present results suggest that in trained athletes, changes in plasma lipids are slight even when training volume is dramatically increased, which is partially corroborated by Nagel et al. (1989). The plasma increase in tissue enzymes depends on exercise intensity (Fry, Morton, & Keast, 1991), the training level (Rodrigues dos Santos, 2017), and the biomechanical expression of effort . After getting accustomed to longer running distances, the effect of training leads to a better muscular coordination resulting in less tissue damage and consequently a reduced release of intracellular enzymes into the blood stream. Our results show that the enzymes ALT and Aldolase changed slightly while AST decreased sharply (-23.6%). ALT enzyme is higher in the cytoplasm, compared to the mitochondrial values (Glinghammar et al., 2009), a fact that could, in part, justify a greater efflux into the blood circulation, with repercussions at the plasma level in case of post-exercise liver damage. This marked decrease points to a hepatic adaptation to training (Mikami, Sumida, Ishibashi, & Ohta, 2004). The AST/ALT ratio (>1 in 2 participants) which might point to overload or hepatic damage shouldn't be a point of concern as the high CK levels indicates that the muscle tissue is the most likely source of these enzymes as response to exercise (Pettersson et al., 2008). Moreover, we found a reduction in AST from M1 to M2 as regular exercise, according to

Margaritis et al. (1999) seems to attenuate the effects of exercise, reducing plasmatic concentrations of AST.

After intensive exercise, serum CK remains high more than 24 hours (Brancaccio et al., 2007; Rodrigues dos Santos, 2017). Usually basal values of CK are higher in trained subjects (Mougios, 2007) exceeding the normative references for sedentary individuals. The slight increase (18.6%) verified in this study expresses the adaptation to the long-lasting workouts. These results are in line with the values found after an ultramarathon in kayak (Rodrigues dos Santos et al., 2007). In fact, the plasma expression of CK by exercise can be induced by different processes: i) hypoxia and/or muscular ischemia reactions resulting from exhaustive and prolonged exercise and ii) loss of homeostasis of calcium ions with direct consequences on the sarcolemma, mitochondria and sarcoplasmic reticulum (Armstrong, Warren, & Warren, 1991).

Long endurance exercise promotes a marked increase in serum cortisol (Rudolph & McAuley, 1998). After an initial increase, cortisol levels seems to increase with exercise intensity (Duclos, Corcuff, Rashedi, Fougère, & Manier, 1997). The high response in cortisol levels observed in our sample might indicate a good adaptation to the high catabolic environment induced by the demands of the training volume with high stress and fuel needs (Urhausen & Kindermann, 2002). On the other hand, impaired cortisol adaptation could be indicative of overtraining (Urhausen & Kindermann, 2002). Moreover, our data was collected in the morning and cortisol have been shown to peaking by this time a and lowering its values at night (Rudolph & McAuley, 1998).

As expected in response to sustained physical activity (McClung et al., 2009) iron levels decreased in our sample. The significant decrease of serum iron in this study (-31.8%) might have been caused by several mechanisms. The most common described in the literature include losses in sweat and urine, activity of hepcidin and could be related to hemolysis due to mechanical forces and oxidative stress (Kapoor et al., 2023) or dilutional pseudoanemia (Bärtsch, Mairbäurl, & Friedmann, 1998). Despite the decline in iron levels in all participants, only subject #2 decreased to values near the reference limits for men (≥13 g/dL). Low iron levels have been linked to reduced performance and fatigue due to reduced oxygen transport to muscles (Kapoor et al., 2023). Nevertheless, it seems, after ten days of total rest, serum iron tends to recovery to normal values (Rodrigues dos Santos et al., 2007).

The sharp increase (25.9%) of plasma phosphorus seen in this study may be related to the dietary profile of the participants. It was shown that dairy products and cereals/grains having inorganic phosphate additives significantly increase serum phosphorus concentration (Moore, Nolte, Gaber, & Suki, 2015). The increase in serum phosphorus seen in this study does not seem to have any clinical significance and is matched with the body's requirements for ATP and phosphorylated metabolic intermediates (Baker, McCormick, & Robergs, 2010). Long-lasting exercise may be associated with hypomagnesaemia, hypokalaemia and hyponatraemia (Warburton et al., 2002) but return to basal values 3 days after exertion (Spiropoulos and Trakada, 2003). Our values, all within the clinical references, demonstrate electrolyte balance at rest despite the normal disturbances experimented during exercise.

Changes in Na, K, and Mg are slight and without clinical significance what was partially corroborated by Rodrigues dos Santos et al. (2007). Knechtle et al. (2011) found prevalence of exercise-associated hyponatremia in male ultraendurance athletes what conflicts with our results. At the start of the study, chloride concentration was high, beyond clinical references in all participants. As previous training level was moderate, the hypothesis of exercise-induced renal dysfunction should be ruled out. These high values point to a high ingestion of dietary salt what is common in Portuguese cuisine. Our results are in line with those found in a Portuguese ultramarathon kayaker (Rodrigues dos Santos et al., 2007). The reduction in plasma chloride after intervention may be related to the increase in sweating rate and subsequent fluid intake, mainly water, that did not matched chloride losses by sweating.

The main limitation of our study, despite the number of participants, that being higher could allow us to have more robust results, is the lack of diet control. Future studies must have diet in consideration since many changes in blood parameters might be due to diet.

Despite these limitations, according to our data it seems the biochemical eexpression in response to exercise is related to level of training (in this case athletes trained in prolonged efforts) makes it possible to carry out efforts of this magnitude, with greater tolerance to biochemical changes, mainly enzymatic alterations related to foci of muscle and liver injury.

CONCLUSION

Our data show previous endurance trained participants might adapt to the increase in running volume with a marked decrease on body mass accompanied by a greater tolerance for prolonged and intense efforts expressed by a slight variation in most of biochemical indicators. However, the present study shows a significant increase in biomarkers such as cortisol, creatinine and a relevant reduction in iron levels in the body.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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