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SERUM ENZYMATIC CHANGES AFTER A 50-KM RUNNING IN SUBJECTS WITH DIFFERENT TRAINING LEVEL. A CASE STUDY

ALTERAÇÕES ENZIMÁTICAS INDUZIDAS POR UMA CORRIDA DE 50-KM EM SUJEITOS COM DIFERENTE NÍVEL DE TREINO. UM ESTUDO DE CASO

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Resumo: A corrida de longa duração normalmente provoca a emergência de sinais de lesão das fibras musculares e hepatócitos, como a libertação para o plasma de várias enzimas musculares e hepáticas. Contudo, os estudos bioquímicos relacionados com a corrida prolongada são escassos. O presente estudo teve como objetivo avaliar a concentração sérica de várias enzimas musculares e hepáticas após uma corrida de 50-km em sujeitos com diferente nível de treino. Com base no nível individual de treino, 4 participantes foram integrados em dois grupos: HT – 2 sujeitos com vários anos de treino e competição e LT – 2 sujeitos com poucos meses de treino sistemático de corrida. Amostras de sangue venoso foram obtidas para análise no sérum das enzimas creatina-quinase (CK), aldolase (ALD), aspartato aminotransferase (AST) e alanina aminotransferase (ALT), antes e imediatamente após a corrida e 24, 48, e 72 horas após a corrida. A análise estatística consistiu no cálculo da percentagem de variação entre os valores basais e após corrida. Todas as enzimas aumentaram imediatamente após a corrida para ambos os grupos com LT evidenciando maiores aumentos para a CK e AST. Um dia após a corrida, a CK atingiu o pico em ambos os grupos com expressivas diferenças entre grupos. ALD e ALT atingiram o pico de concentração um dia depois para HT. Para LT, ALD atingiu o pico dois dias após e ALT três dias após a corrida. Três dias após a corrida todos os valores mostraram tendência para reduzir, mas permaneciam marcadamente elevados para LT. O presente estudo demonstrou que enzimas séricas relacionadas com lesão muscular e hepática estavam aumentadas após corrida de 50-km. Enquanto a CK estava expressivamente elevada imediatamente após a corrida e atingiu o pico de concentração um dia depois, ALD, AST e ALT evidenciaram diferentes picos. O nível de treino dos sujeitos parece ser um fator importante relacionado com as alterações enzimáticas após uma corrida prolongada e durante o processo de recuperação.

Palavras-chave: corrida prolongada; CK; AST; ALT; ALD

Abstract: Running usually induces signs of muscle fibre damage expressed by several biomarkers. However, long lasting running biochemical related studies are scarce. The present case study aimed at evaluating muscle and

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hepatic enzymatic markers after a 50-km running event in a particular group of military participants. Based on individual training expertize, four participants were assigned in two groups: HT – 2 highly trained subjects with several years of running training and competition and LT – 2 low trained subjects with only few months of systematic running training. Venous blood samples were drawn for analysis of serum creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and Aldolase (ALD) before, and immediately after the race and 24, 48 and 72 hours after the race. Statistical analysis included the percentage of change between baseline and after trial. All the enzymes increased immediately after trail for both groups with LT showing greater increases for CK and AST. One day after running, CK peaked for both groups with expressive differences between groups. ALD and ALT peaked after one day for HT. For LT, ALD peaked after two days and ALT peaked after three days. AST peaked one day after for both groups but expressively higher for LT. Three days after exertion all values tended to decrease but remained marked higher for LT. The present study demonstrated that serum enzymes related to muscle and liver damage were increased after a 50-km run. While CK was expressively higher immediately after running and peak one day after, ALD, AST, and ALT showed different peaks. Participants' training level seems being a main determinant for enzymatic changes after prolonged running and during recovery.

Keywords: long lasting running; CK; AST; ALT; ALD.

Introduction

It is well documented that long-lasting exertion affects the activity of several intramuscular enzymes and its serum expression[1-3]. Serum enzyme activities such as creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and aldolase (ALD) which leak into circulation from damaged muscle have been used as indirect markers of exercise-induced muscle damage[4].

AST and ALT are found in significant quantities in liver and skeletal muscle and are related with damaged in both hepatic cells and muscle fibres [4,5]. ALD is probably present in all cells with particularly expression in liver, brain and skeletal muscle. Skeletal muscle damage produces high serum levels of ALD, particularly in the case of progressive muscular dystrophy[6]. CK (creatine kinase) is one of the most common serum biomarkers to assess myocardial injury[7] and skeletal muscle injury[3,8]. Total CK levels before and after exercise depend on age, gender, race, muscle mass, physical activity and climatic condition[9,10]. However, studies are conflictual because Jassal et al.[7] found that serum levels of CK post-race did not correlate with age, sex, BMI, level of training, or prior marathon experience.

It seems that serum CK and ALD activity depends more on exercise duration than on exercise intensity[11]. However, Noakes[12] has suggested that the increase in muscle enzymes, particularly CK, is related to both intensity and duration, with duration having the dominant

effect. Training level is also a major determinant for CK changes induced by exercise. After short-term supramaximal exercise trained subjects experiment lower CK concentration than untrained controls[11].

After exhaustive physical exercise some biomarkers are immediately measurable in serum while others have a delayed serum expression. Serum CK activity peaked 24 hr after exhaustive running, with a 15-fold rise, and returned to baseline after 1 week[13]. During a 200-km running race serum expression of AST was more pronounced than ALT in the middle and the end of the race[3].

The determination of serum CK, ALD, AST and ALT changes induced by exercise and their recovery can be important tools for training control. Few studies have analysed enzyme activity recovery after exercise related to runners' training level.

Then, the aim of this study was to evaluate the enzymatic changes induced by a 50-km running race in subjects with different training level, immediately after the race and during the recovery period.

Methods

Four participants, belonging to the Special Forces of the Portuguese Army, with different training level were divided in two groups: HT (High training status) - two subjects with several years of running practice and regular participation in road running, orienting, and cross-country races, and LT (Low training status) - two subjects which began systematic running training only 6 months before. The participants had regular medical screenings showing no health constraints. Prior to the race daily running volume varied between15-20 km. In the week previous to the running, distance of training was reduced for 8-10 km/day. The characteristics of the participants are shown in Table 1.

This study was conducted in accordance with the policy statement of the Declaration of Helsinki, adopted by the World Medical Association, regarding the ethical principles for medical research involving human subjects and approved by the Scientific Council of the Sport's Faculty of the University of Porto, Portugal. The participants were informed of the risks associated with their participation before giving voluntary written consent.

Table 1. Participants' characteristics

	HT (n=2)		LT (n=2)	
	Runner 1	Runner 2	Runner 3	Runner 4
Age (years)	24.3	25.4	22.8	23.1
Height (cm)	172.4	171.5	174.2	175.1
Weight (kg)	68.3	67.2	69.2	68.9
Fat mass (%)	9.0	9.3	10.8	10.6
VO_2max ($ml.kg^{-1}.min^{-1}$)	67.6	69.2	61.8	62.4

Maximal Oxygen Uptake

Expired respiratory gas fractions were measured using an open circuit breath-by-breath automated gas-analysis system (Cortex, Metalyzer, 3B, Leipzig, Germany). Before each test, flow, volume and gases were calibrated. HR was measured and recorded every 5 seconds using a HR monitor (Vantage NV, Polar Electro, Kempele, Finland) that was connected with the gas-analyzer system. The runners start a treadmill incremental test w8 km.h-1 with 2 km.h-1 increments every two minutes till exhaustion. The V̇O2max was considered when at least two of the following conditions were verified: a plateau in oxygen uptake against exercise intensity, a value of respiratory exchange ratio ≥ 1.10, a blood lactate ≥ 8 mmol.L-1 and 90% of theoretical maximal heart rate, were achieved.

Anthropometry

Anthropometrical measures included stature, mass and skinfold thickness at triceps, biceps, sub-scapular and supra-iliac sites. Body fat mass was estimated from the sum of these skinfolds using the Durnin & Womersley[14] formulae for body density calculation and Siri[15] equation for determination of body fat percentage.

Weather conditions during the race

The race was realized between 9 am and 13.30 pm, with the following weather conditions: partly sunny, 20-25 º C, 30-40% humidity, without wind.

Nutrition and hydration

Nutrition status was not controlled. However, participants were asked to maintain their nutritional habits. Throughout the run all participants have compulsorily drunk 1.500 ml of an

isotonic sport drink (6% carbohydrate, 25 mmol.L-1 sodium, and 5 mmol.L-1 potassium) with a rate of 500 ml per hour. In spite of fluid ingestion body weight loss was significant for all participants (1.5-2 kg).

Running results

The 50-km running times were for HT: 3h40' (runner 1) and 3h43’ (runner 2); for LT: 4h12’ (runner 3) and 4h17’ (runner 4).

Enzymatic assessment

With the subjects in the supine position 5 ml blood were taken from a cubital vein 2 hours after breakfast, 15’ before the race, and immediately after the race. For four subjects, blood samples were also taken in the first, second and third day of recovery. The blood samples were recoiled in heparinised tubes containing ethylenediaminetetracetate (EDTA) centrifuged for 10 min and analysed within 6 yours. Serum AST, ALT, ALD and CK were assessed with a Hitachi 705 device. All the subjects were familiarized with laboratory procedures because they were periodically assessed for health control.

Statistics

For each enzyme, percentage of change (%Δ) between baseline and after race was calculated; the difference between baseline and after the race was then divided by baseline value.

Results

Table 2 shows that, immediately after the race, CK increased for all subjects with LT showing the greatest increases and the highest peaks. Interestingly, ALD increased more expressively for HT immediately after the race but LT showed higher delayed peaks. AST peaked one day after exertion for all participants with LT peaking higher. ALT peaked one after exertion for HT while peaked higher and three days after the event for LT.

Table 2. Serum enzymatic changes after a 50-km running

	Baseline	After 50-km	%Δ	After 1 day	After 2 days	After 3 days
CK (U/L)						
Runner 1	79	205	159.5	386	211	128
Runner 2	72	337	368.1	486	236	209
Runner 3	113	2520	2130	2763	1541	1212
Runner 4	76	1131	1388	2444	1400	1322
ALD (U/L)						
Runner 1	1.0	3.0	200	3.9	3.2	2.4
Runner 2	1.1	4.4	300	4.5	3.8	2.6
Runner 3	1.3	1.9	72.7	18.5	18.6	16.0
Runner 4	0.8	1.9	137.5	16.7	17.1	15.8
AST (U/L)						
Runner 1	14	25	71.4	29	27	26
Runner 2	21	33	57.1	37	27	28
Runner 3	16	69	331.3	161	129	111
Runner 4	18	56	211.1	131	125	108
ALT (U/L)						
Runner 1	14	19	35.7	29	26	21
Runner 2	23	28	21.7	31	27	22
Runner 3	11	19	72.7	27	40	54
Runner 4	17	21	23.5	35	37	42

Note: Runners 1 and 2 were HT; runners 3 and 4 were LT

Discussion

Some enzymatic serum alterations during long lasting events can be attributed to the changes induced in the plasma volume [2]. However, in this study plasma volume changes must be neglected because the earliest serum enzymatic peaks were obtained 24 hours after exercise when plasma volume is fully recovered[16].

Serum enzymatic activity, after exertion, was not related to height, weight, %fat, free fat mass and absolute and relative VO_2max[17]. Age, sex, body mass index, training level and prior competitive experience did not correlate with serum levels of CK[7]. It seems that there is a genetic determination for the individual CK response to exertion[17]. However, the adaptation to a specific training load can modulate CK response. It was verified that CK values are consistently lower in runners than in strength/power athletes[8].

In this study, immediately after the run CK and AST changes were expressively higher from baseline for all subjects, ALD increasing was markedly higher for HT comparing with LT and ALT increase was similar for all subjects.

Athletes have basal values of CK higher than sedentary[10], however, in this study all basal values are within normal laboratory range what can be the outcome to the low intensity running training antecedent to the race. Daily training normally results in persistent serum elevation of CK[18]. The participants in this study have CK basal values varying from 76 to 113 U/L, all within normal laboratory range demonstrating a good adaptation to daily training loads. After the race our results are similar to those verified after marathon[7,19,20]. CK values show great variability among individuals[21]. Analysing the individual responses (Table 2) we can state that subjects 1 and 2 (subjects with several years of running practice) increased CK values to a maximum of 337 U/L, similar to those found by Kim et al.[16] after marathon. These subjects can be considered as low responders[9,17]. This lower responsiveness to exertion seems be more determined by training level than any special genetic trait. Higher CK values found in subjects 3 and 4 after the race and throughout recovery can be justified by the overall aggression induced by the race and accentuated by their lower training level[22]. According to the classification proposed by Brancaccio et al.[9] subjects 3 and 4 are high CK responders. This high responsiveness seems be related to the training level and eventually reduced by continuous running training. Studies about the modulation of systematic and continuous training on the acute biochemical response to physical exertion are lacking.

From our results, it seems that arduous physical stimuli induce more stressful responses in low trained subjects. As initial CK values were similar for both groups it can be speculated that higher CK values found in LT after the race resulted from a more pronounced mechanic and/or metabolic aggression. Higher CK levels in LT are on line with their comparatively lower capability to mobilize free fatty acids during exercise. It seems that higher CK responders are characterized by a reduced utilization of free fatty acids during exercise[17] what is directly related to the training level.

Although intensity is a major determinant for CK tissue release[23] it seems that prolonged exertion time is also correlated with CK increase[7] . Duration and intensity seem be independent factors for CK increase.

After 60-75 minutes of moderate intensity running, serum CK in collegiate athletes did not exceed 125 U/L [24] however, after a 246-km running race, CK was elevated for 43.763±6.764 U/L [25]; this value is an index of massive tissue destruction because a serum

elevation of CK by 100 U/L is associated with damage of approximately 2000 muscle fibres[8]. CK values of LT, one day after the race, are similar to those found by Apple et al.[26] one day after marathon.

Serum CK activity peaked 24 h after the run in all the 4 subjects corroborating other studies[9,12,13]. In our study, after 3 days of recovery, CK changes did not return to basal values what is corroborated by Kobayashi et al.[13]. LT showed a delayed recovery.

Few studies were focused on serum Aldolase (ALD) changes induced by exercise. In this study, all subjects had basal values of ALD within the normal laboratory range. Our results conflict with Karamizrak et al.[11] who stated that trained subjects display basal ALD values higher than sedentary. Immediately after the run, ALD increased markedly in HT, peaking one day after, while LT showed a slight increase peaking two days after. LT peaked expressively higher compared with HT. Our results are on line with those obtained in other studies[2,20]. The delayed ALD peak showed by LT is in accordance with Kanda et al.[4] who showed that after acute eccentric exercise ALD peaked only 96 h after exertion.

None of the subjects returned to their initial values after 3 days of recovery what is in accordance with other studies[4,27].

AST increased after the run peaking one day after for all subjects. Like CK, AST showed expressively higher increases and a delayed recovery in LT. ALT displayed similar increases for all subjects immediately after the race but while HT peaked one day after, LT need two days more to achieve their peak. The highest AST values found in LT are probably related to a more pronounced rhabdomyolysis[25] related to their lower training level.

Serum increase of ALT, enzyme of predominantly hepatic origin, could be related to plasma volume changes[2], while serum AST increase, which is widely distributed in all tissues, can be related to the systemic stress induced by exercise. Although, Kinoshita et al.[5] stated that increase on serum AST and ALT concentrations after exercise, can't be related with hepatocyte damage, ultra-endurance events can elicit a marked tissue destruction rising dramatically serum expression of AST and ALT. It seems that serum expression of these enzymes is clearly related to the duration of exercise; however our results highlighted the importance of training level as a major determinant for AST and ALT response to exercise.

After a 260-km running race, Skenderi et al.[25] found values of AST (1.182±165 U/L) and ALT (264±37 U/L) marked higher than those verified in this study and related to the duration of exertion what is confirmed by other studies[3,28].

Serum ALT values immediately after the race are similar for all the participants and showed slight increase. Contrarily to our findings, Burger-Mendonça et al.[29] showed no ALT differences after long distance triathlon. Post-exercise biochemical measurements can misinform about acute enzymatic adaptations provoked by exercise. For ALT, while experienced runners peaked 24 h after the race, novice runners peaked 3 days post-exertion with the highest values. This delayed response can be an index of prolonged hepatic damage conflicting with the statement of Kinoshita et al.[5].

Contrary to the position of Brancaccio et al.[6] who stated that aerobic exercise doesn't change membrane permeability our study, developed in aerobic conditions, demonstrated marked tissue enzymatic release, predominantly from skeletal muscle. These alterations were still visible 3 days after the race and their level seems be correlated with the degree of cellular damage[1]. This study clearly demonstrated that training level is a crucial factor for enzymatic response to long lasting exercise. The two subjects with the lowest training level exhibited a more pronounced muscular and hepatic aggression which is reflected not only in the higher increase of serum AST and CK immediately after the race but also by the highest Aldolase and ALT peaks obtained in the recovery period.

It can be concluded that a low-intensity pace 50-km running induce marked alterations on serum level of several enzymes. It seems that CK, ALD, AST and ALT increases are related to the runners' level of training. From this study it can be ascribed that runners with low training level show a higher enzyme peak after exertion and a delayed in the recovery time.

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