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ARTIGO ORIGINAL

Long distance training associated to HIIT protocol does not induce changes in blood biochemical markers in adult marathoners

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Keywords:
Liver damage;
Muscle damage;
Blood biomarkers;
Sports Science.

ABSTRACT

Objective: to verify blood markers during a 12-week training protocol and after Sao Paulo Marathon. **Methods:** Blood samples of 9 male marathoners were collected before (C1) and after (C2) 12-week training protocol, before (C3) and after (C4) marathon. Muscle and liver damage markers (creatine kinase [CK-MM], aspartate aminotransferase [AST], alanine aminotransferase [ALT]), oxidative stress levels (thiobarbituric acid reactive substances [TBARS]) and serum iron concentration were measured. **Results:** changes were identified comparing moment C4 to other moments for CK-MM and iron. For AST, ALT, and TBARS no differences were identified. **Conclusion:** strenuous exercises might elicit changes on blood markers, needing follow up strategies to avoid impairments to athletes' performance and health.

Palavras Chave:
Dano hepático;
Dano muscular;
Biomarcadores sanguíneos;
Ciências do Esporte.

RESUMO

Objetivo: verificar marcadores sanguíneos durante 12 semanas de treinamento e após a Maratona de São Paulo. **Metodologia:** amostras de sangue de 9 maratonistas foram coletadas antes (C1) e após (C2) o treinamento de 12 semanas, antes (C3) e após (C4) a maratona. Marcadores sanguíneos e hepáticos (creatina quinase [CK-MM], aspartato aminotransf. [AST], alanina aminotransf. [ALT]), níveis de estresse oxidativo (subst. reativas ácido tiobarbitúrico [TBARS]) e ferro sérico foram analisados. **Resultados:** Verificaram-se alterações de CK-MM e ferro entre C4 e os outros momentos. Para AST, ALT e TBARS não se encontrou diferenças. **Conclusão:** exercícios exaustivos podem causar alterações em marcadores sanguíneos, requerendo estratégias de monitoramento para evitar danos ao desempenho e saúde do atleta.

Palavras Chave:
Daño muscular;
Daño hepático;
Biomarcadores sanguíneos;
Ciencia deportiva.

RESUMEN

Objetivo: evaluar marcadores sanguíneos durante 12 semanas de entrenamiento y después del Maratón de São Paulo. **Metodología:** muestras de sangre de 9 maratonistas fueron recogidas antes (C1) y después (C2) el entrenamiento, y antes (C3) y después (C4) a maratón. Se analizaron marcadores sanguíneos y hepáticos (creatina quinasa [CK-MM], aspartato aminotransf. [AST], alanina aminotransf. [ALT]), estrés oxidativo (sustancias reactivas del ácido tiobarbitúrico [TBARS]) y de hierro. **Resultados:** Se encontraron cambios comparando C4 y otros momentos para CK-MM y hierro. Para AST, ALT y TBARS no se encontraron diferencias. **Conclusión:** ejercicios extenuantes pueden causar cambios en marcadores sanguíneos, requiriendo estrategias de monitoreo para evitar daños al desempeño y salud del atleta.

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INTRODUCTION

In the past decades, the number of marathoners has hugely increased. Nowadays it is usual to meet someone who has already crossed the finish line of a 42km running competition. Most of those "finishers" started exercising for health benefits, to avoid aging effects or inactivity-related diseases (Jastrzębski et al. 2015). The search for healthy habits and active lifestyle has driven people to practice high intensity and/or prolonged duration exercises, which are accompanied by extreme exertion efforts. It is possible to find data of hospitalization and even death during the course or after the completion of marathons, but each year a crescent number of recreational runners goes for long-distance competitions (Mathews et al. 2012).

The populations increased interest in long distance running has aroused sport scientists attention to factors concerning performance-related effects of endurance exercises (Waśkiewicz et al. 2012; Gatterer et al. 2013; Rowe et al. 2014; Gonçalves et al. 2015; Jastrzębski et al. 2015). Parameters related to body tissue damage, cardiac outcomes, vascular disease, physiological disturbances and dehydration were widely explored in endurance athletes, to verify the impact of their practice on the human organism (Patil et al. 2012; Gonçalves et al. 2015; Jastrzębski et al. 2015).

According to Bird, Linden and Hawley (2014), performing a bout of strenuous exercise may elicit physiological changes related to injuries or chronic diseases prevalence. Those changes commonly occur on concentration and/or activity of muscle, liver and heart damage biomarkers (i.e. muscular isoform of creatine kinase [CK-MM], aspartate aminotransferase [AST], alanine aminotransferase [ALT], cardiac troponins). Participating in endurance events provokes increase to several of these biomarkers (Kratz et al. 2002; Hoffman et al. 2012; Del Coso et al. 2013; Kłapcińska et al. 2013; Žákovská et al. 2017; Knechtle and Nikolaidis 2018), AST and ALT (Kratz et al. 2002; Del Coso et al. 2013; Kłapcińska et al. 2013; Shin et al. 2016; Knechtle and Nikolaidis 2018). Increased serum values of these parameters suggests muscle and liver damage during the effort (Bird et al. 2013).

It is commonly explored the association between exercise practice and oxidative stress rates (Siqueira et al. 2009; Nishida et al. 2010; Kabasakalis et al. 2011; Kłapcińska et al. 2013), being well established that moderate exercise training acts as an antioxidant factor, while exhaustive exercise plays the oxidant role (Gomez-Cabrera et al. 2008). The thiobarbituric acid-reactive substances (TBARS), a marker for lipid-peroxidation, have been recently studied to associate oxidative stress and prolonged efforts. Some studies found increased concentrations of TBARS after endurance and ultra-endurance competitions (Machefer et al. 2004; Kabasakalis et al. 2011; Kłapcińska et al. 2013; Stagos et al. 2015; Oliveira et al. 2017), reinforcing the hypothesis that strenuous efforts contributes to the augmentation of blood oxidative stress.

Studies have shown increased concentrations of serum iron after a half-marathon and a 48h-ultra marathon (Siqueira et al. 2009; Kłapcińska et al. 2013), respectively, potentially related to the occurrence of hemolysis (rupture of blood red cells), when associated with increment of other tissue damage biomarkers, as CK-MM, AST and ALT. In this study, besides the analysis of blood biomarkers before and after the competition, e.g. marathon, the behavior of these variables was observed throughout the training.

It is evident the amount of transversal studies investigating the effects of strenuous endurance single bouts on blood biomarkers, while it is also noted an apparent lack of longitudinal studies examining the effects of endurance training protocols on the same parameters and their modulations between different moments of the intervention, which justifies the execution of researches in such matter. Therefore, the aim of the present study was to verify the activity of tissue damage biomarkers on the skeletal muscle (CK-MM), liver (AST and ALT), as well as concentrations for oxidative stress markers (TBARS) and serum iron, before and after a twelve-week training protocol, before and after a Marathon. We hypothesized that the increase in training load over the twelve-week training will result in negative changes on damage biomarkers of the skeletal muscle, liver and for oxidative stress markers.

METHODS

Participants

Nine healthy marathon runners (sample size calculated by - software G*Power - Dusseldorf, Germany - statistical power of .80 and alfa of 5%) participated in this study (age 34.78 ± 10.62 years; height 170.15 ± 8.06 cm; body mass 70.54 ± 7.88 kg; fat % 13.83 ± 5.51). The inclusion criteria were: i) at least two years of training for long distance competition (running experience 5,2 ± 1,3 years); ii) they must have completed, at least, a marathon in less than four hours in the past six months; and iii) no history of musculoskeletal injuries nor surgical procedures in upper or lower limbs. Athletes completed a sheet for medications and supplements and there were no substances that could impair the results listed. The study was approved by the Ethics Committee of the Ribeirao Preto School of Physical Education and Sport (protocol 006641/2015, CAAE 41397214.8.0000.5659)

and was conducted in accordance to the Declaration of Helsinki ethical principles.

Training protocol

The training was conducted 4 times a week for 12 weeks (48 sessions) by the team's trainers. In general, the protocol consisted of 80% aerobic continuous training at the lactate threshold intensity (LTI), and 20% of high intensity interval training (HIIT), focusing on participating in Sao Paulo Marathon. Aerobic continuous training at the LTI consisted of stable and long runs, i.e. cadenced running (88-92% HR_{max}). HIIT was conducted by four sets of 3-5 minutes (98-100% HR_{max}) of run with 120 seconds active recovery between each set. The twelve-week intervention was divided into three periods: week 1 to week 5 (1st period- extensive), week 6 to week 10 (2nd period- intensive), and week 11 and week 12 (3rd period- tapering). The Sao Paulo Marathon was performed at the end of week twelve. In the first period, the training volume was 86.52 ± 6.68 kilometers. In the second period, it was 106.73 ± 8.26 kilometers and during the third period, it was 61.23 ± 4.38 kilometers. During the whole protocol, the sessions intensity was monitored using heart rate with a Polar Accurex Plus (Polar Electro Oy, Kempele, Finland).

Blood samples

Venous blood was collected at the same time and place, always early in the week (72 hours after the previous training session). Athletes were instructed not to perform physical exercises between the previous workout and the blood collection. Samples of 10ml were collected from antecubital vein before (C1) and after (C2) the training protocol, as well as before (C3) and right after (C4) Sao Paulo Marathon. The blood was immediately allocated in a container with dry ice and subsequently centrifuged at 2000g for 15 minutes for serum and plasma obtainment. After this procedure, plasma was separated into aliquots and immediately stored at - 80 °C (- 112 °F) for later analysis.

Blood analysis

Analysis for muscle damage, liver damage and oxidative stress markers were performed. To verify CK-MM activity, CK-MM Bioliquid Assay Kit was utilized (Laborclin, Pinhais, Brazil). To determine ALT and AST concentrations and activity, Alanine GPT (ALT) Bioliquid Assay Kit and GOT-AST Bioliquid Assay Kit (Laborclin, Pinhais, Brazil) were used, respectively. For TBARS determination, it was used MDA Assay Kit (BD Biosciences, San Jose, USA). For serum iron concentrations, the Iron Serum K017 Assay Kit (Bioclin, Sao Jose dos Campos, Brazil) was utilized. All procedures were performed according to the Kits specifications.

Statistical analysis

The obtained data were treated using the IBM SPSS Statistics software for Windows, version 22.0 (IBM Corporation©). Data were presented and analysed using descriptive statistics (mean ± SD). The normal distribution of the results through Shapiro-Wilk test and repeated measures Analysis of Variance (ANOVA) were further performed to identify possible differences. Whenever necessary, Bonferroni "post-hoc" was applied. Effect size (ES) of the differences between moments was calculated using Cohen "*d*" (Cohen, 1998). The "*d*" values were considered as follows: $d < 0.1$, trivial; $0.1 \leq d < 0.2$, small; $0.2 \leq d < 0.5$, moderate; $0.5 \leq d < 0.8$, large; $d > 0.8$, very large. The significance level adopted was 5 % ($p < 0.05$).

RESULTS

The average time to complete the marathon was 4,06 ± 0,74 hours, giving a mean running speed of 10,3km/h (pace of 5'50"/km). Regarding blood biomarkers, the Figure 1 shows CK-MM activity. It was found a significant increase in CK-MM activity at C4 (186.58 ± 118.54 UI/L) compared to C1 (45.26 ± 22.58 UI/L, $p < 0.001$, ES = 1.65 [very large]), C2 (63.58 ± 26.24, $p < 0.01$, ES = 1.43 [very large]) and C3 (69.12 ± 36.35, $p < 0.05$, ES = 1.34 [very large]).

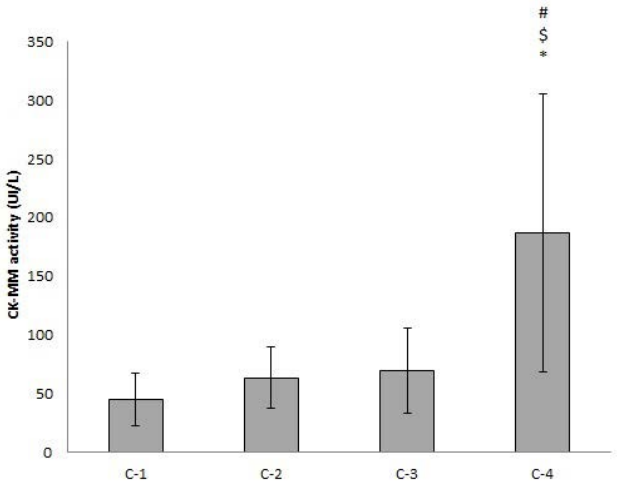

Figure 1. Values for muscular isoform of creatine kinase (CK-MM) activity, in arbitrary units, before (C1) and after (C2) training protocol, before (C3) and after (C4) Sao Paulo Marathon. (# = C1 vs. C4, $p < 0.001$; $ = C2 vs. C4, $p < 0.01$; * = C3 vs. C4, $p < 0.05$).

In Figure 2 are expressed the activities of liver tissue damage enzymes: AST (2A) ALT (2B). No significant differences were found for both variables at any moment ($p > 0.05$, ES = 0.08 to 1.32 [trivial to very large]).

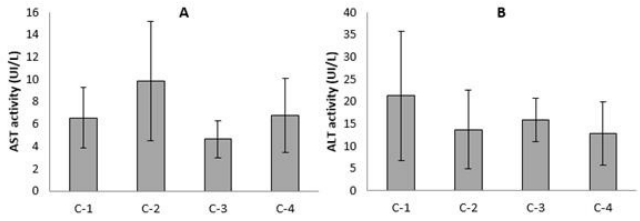

Figure 2. Values for aspartate aminotransferase (AST [2A]) and alanine aminotransferase (ALT [2B]) activity, in arbitrary units, before (C1) and after (C2) the training protocol, before (C3) and after (C4) Sao Paulo Marathon.

Results for TBARS (A) and serum iron (B) concentrations are plotted in Figure 3. No statistic difference was observed between any moments for TBARS ($p > 0.05$, ES = 0.02 to 0.31 [trivial to moderate]), but, similarly to CK-MM, serum iron increased significantly on C4 (392.92 ± 90.85 µg/dL) when compared to C1 (146.38 ± 52.95 µg/dL, $p < 0.001$, ES = 3.31 [very large]), C2 (209.01 ± 98.7 µg/dL, $p < 0.001$, ES = 1.93 [very large]) and C3 (152.38 ± 22.35 µg/dL, $p < 0.001$, ES = 3.63 [very large]).

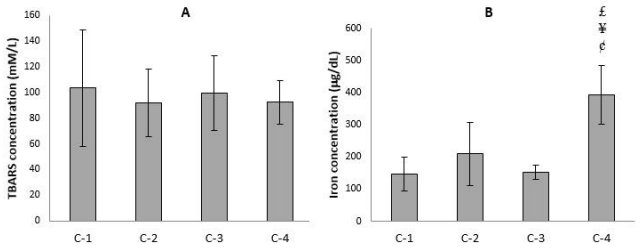

Figure 3. Values for thiobarbituric acid-reactive substances (TBARS [3A]), expressed as mMols of malondialdehyde, and free serum iron (3B) concentrations before (C1) and after (C2) the training protocol, before (C3) and after (C4) Sao Paulo Marathon. (£ = C1 vs. C4, $p < 0.001$; ¥ = C2 vs. C4, $p < 0.01$; ¢ = C3 vs. C4, $p < 0.05$).

DISCUSSION

The main findings of our study were the significant increase of CK-MM activity and free serum iron concentration after the competition (C4) compared to the other three moments (C1, C2, C3). These results show the onset of muscle damage and the possible occurrence of hemolysis during the marathon. In addition, the absence of substantial changes in other biochemical markers along the training protocol (C1 vs. C2) demonstrated the efficiency and security of the training protocol concerning muscle damage, liver damage and oxidative stress. Therefore, we used the load structure proposed in this study to ensure overcompensation throughout training season.

The increased activity of plasmatic CK-MM (~170%) after the marathon may be considered a typical response due to the practice of long distance running (Echegaray and Rivera 2001; Siqueira et al. 2009), showing that acute assessments of biologic samples may provide relevant evidences regarding biochemical alterations induced by endurance efforts. Siqueira et al. (2009) performed a study with 20 professional marathoners during a half-marathon, verifying increments of muscle damage markers activity (i.e. CK-MM), assenting the present research results.

It is also observed a maintenance of CK-MM activity during training protocol (C1 vs. C2) and, despite the progression of loads, no increment of this parameter was noticed, probably due to exercise-induced muscle adaptations. Fact possibly explained by apparently unusual exercises at the beginning (C1) turning into usual over the training course, and becoming less intense to the organism at the end of the twelve weeks (C2) (McHugh 2003). Another mechanism associated to this muscle adaptation may be related to myogenic satellite cells excitation, acting onto muscle fibers repairing (Snijders et al. 2014; Imaoka et al. 2015).

The inconclusive results for liver damage markers AST and ALT maybe occurred because peak activity of these biomarkers show up between 12 to 24 hours after effort is completed (Kratz et al. 2002; Waśkiewicz et al. 2012; Jastrzębski et al. 2015). However, other studies found higher levels of these parameters right after the completion of a prolonged exercise (Chevion et al. 2003; Wu et al. 2004; Shin et al. 2016). Our results corroborate with the findings of Del Coso (2013), with no significant changes (~112%) between moments before (C3) and after marathon (C4), once again confirming the security of the 12-week training protocol implemented.

Oxidative stress levels measured by TBARS did not present significant changes for any moments. In contrast, Machefer et al (2004) found expressive modulations in oxidative stress markers (e.g. TBARS) after an ultramarathon, assessing blood samples of six participants before and after competition. Similarly, Oliveira et al (2017) identified significant changes in TBARS and other Redox Status variables in athletes who completed a marathon in two different conditions, when confronted to basal levels. Nevertheless, strengthening our results, Kabasakalis et al. (2011) found no differences on TBARS levels when comparing before and after a 24hours simulated swimming competition. Additionally, Kłapcińska et al. (2013) did not encounter any differences in their study with amateur ultramarathoners. This lack of consistence in literature may be attributed to multiple factors as sample characteristics, type of exercise, intensity and duration of the effort and an extensive number of methods to evaluate oxidative stress (Kabasakalis et al. 2011).

As mentioned, our study found increased free serum iron concentration (~158%) when compared before and after marathon (C3 vs. C4, respectively), suggesting the occurrence of hemolysis. During an exhaustive endurance event, this factor possibly occurs due to repeated ground-feet impacts, decreasing levels of nitric oxide, increasing shear stress and oxidative stress rates (Chevion et al. 2003; Mastaloudis et al. 2004; Siqueira et al. 2009). In addition, the hemoglobinuria (high concentration of hemoglobin-protein in urine) may be associated with hemolytic anemia, impairing athletes' performance (Siqueira et al. 2009).

This study is not without limitations: a) the study was carried out with only one group (experimental). However, the athletes' follow-up in real conditions of training and competition hinders the division of the participants in two groups. In this sense, it is usual to observe in sport science studies that athletes are their own control subjects. In our research, the values obtained before training (C1) were considered baselines for comparisons with other moments (C2, C3 and C4); b) when considering antioxidant markers, it is advisable to monitor nutritional parameters to prevent any possible changes in redox status due to food/supplement intake. Unfortunately, such procedure could not be performed, as we did not have access to the athletes' diet information or nutrition record. Otherwise, this study also has relevant points: i) the protocol training applied demonstrated efficiency and security when it comes to muscle damage, liver damage and oxidative stress, ii) the evaluation procedures are inexpensive and ease to be performed, which increases their practical application, and iii) besides the amateur level, the recruited sample has great experience with running training and competition (at least one marathon completed per year in the past 2 years and 5,2 ± 1,3 years of long distance running experience), valuing the results achieved.

Moreover, this study stands as an evidence that it is possible to implement and perform accessible follow up strategies to accurately monitor training status of endurance athletes before, during and after target competitions without impairing training/rest schedule or individual performance. Nonetheless, more studies are recquired to determine response patterns for blood biochemical markers in different moments during athletes' preparation, with the correct approach of all variables concerning training periodization.

CONCLUSIONS

The present study shows significant changes in biochemical variables when comparing moments before and after marathon (C3 vs. C4), especially for CK-MM, suggesting muscle damage, and increased free serum iron concentration, which may indicate hemolysis. However, when verified moments before and after training protocol (C1 vs. C2) no significant changes were found for any variables.

Athletes' follow up, through laboratorial assessments, during their habitual training protocols and competitions contributes to diagnose silent disturbances, which may compromise health and performance of these individuals. Thereby, such evaluations provide information regarding athletes' training status, aiming to avoid under and overtraining.

DISCLOSURE STATEMENT

The authors have no relevant conflicts of interest to disclose.

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