Journal of Clinical Chemistry and Laboratory Medicine

Martins et al., J Clin Chem Lab Med 2019, 2:2

Research Article Open Access

The Effect of an Exhaustion Exercise in Male Athletes on Routine Clinical Chemistry Parameters

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Received date: October 17, 2019; **Accepted date:** October 30, 2019; **Published date:** November 06, 2019

Abstract

Introduction: Physical activity as a preanalytical variable can influence several biomarkers. The level of training, type, intensity and duration of exercise may influence a broad array of laboratory variables. The aim of this study was to describe the changes of some common clinical chemistry in response to an exhaustion exercise protocol.

Materials and methods: Thirteen adult male athletes participated in the study. A blood sample was collected with the athletes fasting and before exercise (M1). Then the participants completed an eccentric/concentric contraction knee extension/flexion exercise protocol until exhaustion. At this moment, a second blood sample was collected (M2). Protocol was repeated 2 weeks later and the mean of both measurements was considered for comparisons. Routine clinical chemistry parameters were analyzed in automated routine equipment's. Wilcoxon test were used to compare mean differences between two moments.

Results: As expected, there was an increase in muscle markers between M1 and M2 moments with significant differences for creatine kinase (P=0.023), C-reactive protein (P=0.033) and myoglobin (P=0.002). Also, were observed significant differences in GGT, Total-, HDL- and LDL-cholesterol (P=0.006, 0.015, 0.009 and 0.033, respectively) and with an acceptable biological variation bias. Beside the significant differences in both Moments, total protein (P=0.003), glucose (P=0.012), albumin (P=0.003), uric acid (P=0.001), magnesium (P=0.039) and phosphorus (P=0.001) exceed acceptable bias range.

Conclusion: Our results show that even after an intense exhaustion physical exercise only a small group of parameters showed changes that exceed the acceptable biological variation bias. In conclusion, we expect that this study contributes to a better understating of the effects of an intense exhaustion physical exercise on common analytical biomarkers.

Keywords: Clinical chemistry parameters; Exercise; Biological variation

Introduction

Physical activity as a preanalytical variable may influence several clinical chemistry parameters. It may produce very different responses and changes in a wide range of biomarkers depending on the biological characteristics of the analyte, the level and type of the training, the intensity and duration of the exercise and the time of recovery after training [1-4]. Individual lifestyle and biological rhythms should always be taken into consideration before sample collection to avoid clinical misinterpretations. It is known that reference values in athletes are different from healthy sedentary individuals and may not reflect a disease, but often an adaptation to regular training or changes occurred during and/or after exercise [5]. It is also known that the intensity and duration of training may influence the severity of muscle damage after an intense exercise [6-8], and that severity is more pronounced when the exercise involves eccentric/concentric contraction (ECC) [9]. When the muscle lengthens during contraction, which occurs during ECC type of exercise, greater stress and strain are placed upon the involved structures, inducing micro-injury at a greater severity compared to concentric and isometric muscle actions.

Actually, physical activity is a very important public healthcare issue, since regular practice of physical exercise provides important health benefits, preventing or delaying the onset of chronic disorders, improving fitness and even rehabilitation from injury [10]. Also, all the various aspects of Sport Medicine are, thereby, receiving growing focus from almost each and every clinical discipline, including laboratory medicine [11].

In this study was our aim describes the changes of some common analytical biomarkers in response to an exhaustion exercise protocol.

Materials and Methods

Subjects

Thirteen adult male athletes [22 (19-25) years; 72.4 (65.9-78.9) kg; 177 (172-182) cm; Median (Min-Máx)] from national-levels (8 jumpers, 2 throwers and 3 sprinters) volunteered to participate in this study. The study approved by the Ethics Commission of the University of Porto, was conducted in compliance with the World Medical Association's Declaration of Helsinki (2008). All participants were informed verbally and in writing regarding the experimental procedures before giving their written informed consent. They were instructed to fulfil a food record and a physical activity record for the 2 days prior the study. They were also asked not to make drastic changes in their diet and avoid strenuous exercise in order to ensure similar metabolic conditions. Exclusion criteria were previous acute knee/ankle injuries.

Experimental design

The athletes, fasting for more than 10 hours arrived at the laboratory and rested in a seated position while a blood sample was taken (M1). Then, they completed a 5 min warm-up on a cycle ergometer, with intensity ranging 70-100 rpm. After that, participants were correctly positioned and strapped in the test chair and performed a protocol comprising a minimum of 300 ECC knee extension/flexion repetitions until exhaustion. This protocol was consisting of 3 bouts of ECC knee extension/flexion exercise at 60s-1 with 200 sec rest time between the 3 sets composed by 100 repetitions each one. Immediately after exhaustion, a second blood sample was collected (M2). The same protocol was repeated 2 weeks later and the mean of both measurements was considered for comparisons.

Parameters (Unit)	Before exercise (M1)	Immediately after exercise (M2)	p-value*	Bias (%)	Acceptable bias† (%)
CRP (mg/L)	0.53 (0.83)	0.62 (0.93)	0.033*	3	21.8
Myoglobin (ng/mL)	56.3 (46.9)	111.0 (82.0)	0.002*	66	8.2
CK (U/L)	372.0 (693.0)	299.0 (736.8)	0.023*	5.6	11.5
ALP (U/L)	65.5 (24.5)	64.5 (26.5)	0.207	2.3	6.7
LDH (U/L)	181.5 (41.8)	194.0 (54.0)	0.152	17.4	4.3
AST (U/L)	20.5 (12.0)	20.0 (13.8)	0.162	6.2	6.5
ALT (U/L)	10.5 (6.0)	10.5 (7.0)	0.418	3	11.5
GGT (U/L)	18.0 (11.0)	19.5 (10.8)	0.006*	6.2	11.1
Aldolase (U/L)	8.5 (6.7)	7.8 (6.8)	0.727	-4.6	-
Total Protein (g/L)	75.4 (4.1)	77.8 (4.2)	0.003*	4.5	1.4
Glucose (mmol/L)	4.4 (0.4)	4.9 (0.5)	0.012*	7.7	2.3
Triglycerides (mmol/L)	0.8 (0.5)	0.7 (0.4)	0.533	-1.5	9.6
Total-cholesterol (mmol/L)	4.4 (1.6)	4.6 (1.4)	0.01^{9*}	2.7	4
HDL-cholesterol (mmol/L)	1.2 (0.1)	1.3 (0.2)	0.009*	4.2	5.6
LDL-cholesterol (mmol/L)	3.0 (1.0)	3.1 (1.0)	0.033*	2.4	5.5
Albumin (g/L)	46.7 (4.3)	48.3 (3.3)	0.003*	4.6	1.4
Uric Acid (µmol/L)	333.1 (77.3)	362.9 (83.3)	0.001*	7.5	4.9
Urea (mmol/L)	5.7 (1.7)	5.7 (2.0)	0.304	0.7	5.5

Table 1: Differences between moments M1 and M2, Descriptive data are reported as medians (interquartile range).

Biochemical analysis

Two venous blood samples were collected from each athlete at the two moments with them in seated position. One sample was collected to a tube containing tripotassium ethylenediaminetetraacetic acid (EDTA) from Venosafe® (Terumo Europe, Leuven, Belgium) and the other was collected to a serum separator Venosafe® tube containing an additive gel. Blood was them centrifuged at 4500 rpm during 15 min and in this serum fraction were analyzed C-reactive protein (CRP), creatine kinase (CK), alkaline phosphate (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamyltranspeptidase (GGT), aldolase, total protein, glucose, triglycerides, total-, HDL-, LDL-cholesterol, albumin, uric acid, urea, creatinine, calcium, magnesium, sodium, potassium, chloride and phosphorus in Olympus AU5400® equipment (Beckman-Coulter, Olympus, Hamburg, Germany). Myoglobin was measured by a

chemiluminescent analyser, Abbott® Architect i2000 (Abbott Diagnostics, Lake Forest, IL, USA).

In order to understand if there were significant changes in plasma volume between M1 and M2, haemoglobin, red blood cells, white blood cells and haematocrit [4,12] were analysed using the equipment Sysmex® XE-5000 (Sysmex Europe GmbH, Norderstedt, Germany). Plasma volume changes were calculated according to Dill and Costill [13].

To identify if any participant had already been in a muscle damage process at the beginning of the study, the reference value of 1083 U/L for creatine kinase (CK) was used as a cut-off [14].

Statistical analysis

Descriptive data are reported as median (interquartile range) due to small number of participants (n=13). Non-parametric Wilcoxon related sample test were used to compare mean differences between M1 and M2 moments. The % of change was compared with expected acceptable bias according to Westgard QC [15]. Whether the differences between two moments exceed, or not, desirable bias specifications based on the biological variations of each parameter [Mean Difference (M2-M1)/Mean (M1) × 100%] was also evaluated.

All statistical procedures were completed using the SPSS software (SPSS® Inc., Chicago, Illinois, 20.0). The level of significance was set at $P<0.05$ for a confidence interval of 95%.

Results

Since there were no significant changes in haematocrit (P=0.208), haemoglobin (P=0.214), red blood cells (P=0.202), and white blood cells (P=0.116), absolute data were used for statistical analysis. Descriptive data of analytical biomarkers differences between moments M1 and M2 are shown in Table 1.

None of the participants were excluded from the study. As expected, an increase is observed in muscle markers values between M1 and M2 moments with significant differences for CK (P=0.023), CRP (P=0.033) and myoglobin (P=0.002). Myoglobin also exceeded greatly the acceptable bias (66% of bias). ALP and LDH increased after exercise, but with no statistical significance (0.207 and 0.152, respectively). From the previous markers, only LDH exceeds the acceptable bias. Significant differences between both moments were observed in total protein (P=0.003), glucose (P=0.012), albumin (P=0.003), uric acid (P=0.001), magnesium (P=0.039) and phosphorus (P=0.001) also exceeding the biological acceptable bias. Total-, HDL-, LDL-cholesterol and GGT (P=0.015, 0.009, 0.033 and 0.006 respectively) increased significantly in moment M2, but within acceptable values of biological variability. Although potassium is slightly above the acceptable bias, it did not show significant differences between both moments of evaluation.

Remaining parameters did not vary significantly with the exception of phosphorus ion that decrease significantly and exceed the biological bias (-16.3%). Magnesium and chloride ions decreased in Moment 2.

Discussion

Our results clearly point out that after an intense exhaustion physical exercise, performed twice by the same individuals, only a small group of parameters showed changes that challenged the acceptable biological variation bias. Sanchis-Gomar and Lippi [5] already described clinical implications on biomarkers and parameters depending on level, type, intensity and duration of training. Brancaccio et al., [16] also described in their study that after exercise, a structural damage of muscles fiber occurs, followed by a plasma release of intracellular biochemical markers.

Increases in Myoglobin and CRP (M2>M1) are consistent with other studies in response to an exhaustion exercise [5,8]. However many studies only described increases after a dynamic type of exercise. Takara et al., [17] reported that myoglobin peaked 2 h after a rugby match and Bird et al., [18] also described elevations in myoglobin a few hours after a marathon. Despite myoglobin being of muscle origin and CRP an inflammatory marker, in our study, the observed increase immediately after exercise may be explained by the ECC protocol that involves a greater strenuous muscular efforts and a severe micro-injury [19]. An increase in hepatic and cardiac damage biomarkers when the exercise bout exceeds the limit of muscle strength would be expected. Sanchis-Gomar and Lippi [5] refer in their review many studies which described increases in liver and cardiac biomarkers after a marathon and even after a rugby match. Consistent with these findings is CK increase observed in our study. Studies report that serum CK peaks 8 h after a strength training [16], 24 h to 36 h after a dynamic mode like a marathon [18] and 96 h after an eccentric exercise [20]. Furthermore, additional bouts of exercise only produces small increases, probably from accelerated enzyme clearance, and daily training may result in a persistent increase in serum CK (resting CK values are higher in athletes) [20]. Less significant increases were observed in AST and ALT. A possible explanation for this may be the ECC exercise protocol used since it was performed under short and anaerobic conditions until exhaustion [21]. AST and ALT were described to peaked 24 h to 48 h after ECC exercise [22]. It is important to note that although ALT and AST levels, which bias exceeded the biological variability, are usually considered as markers of liver function; the clinical interpretation of their serum concentration in athletes should regard their release from muscles into circulation and may reflect an adaptation to a regular training. In fact, the participants in this study are national-level athletes [23].

ECC exercise may also explain the less significant increase in LDH, despite exceeding the acceptable bias range. Sietsema et al., [22] described that ECC induces LDH increases between the third and fifth day after exercise. They also observed that aldolase, which can be used as muscle damage marker, have a significant increase in activity 24 h to 48 h after ECC exercise [20]. This may explain the slight decrease of aldose observed immediately after exercise (Moment M2). Aldolase regulates the cell contraction and its degradation during a stress conditions is related to tissue damage and the maintenance of normal blood levels.

This exercise protocol also appears to influence total protein, glucose, albumin, uric acid, magnesium and phosphorus which exceed the acceptable bias range, although without clinical meaning. That has already been reported by Lippi et al., [24]. Creatinine and urea concentration were unaffected by this exhaustion protocol. Banfi and Del Fabbro [25] compared serum creatinine values in 8 different sports with sedentary people. They conclude that serum creatinine is significantly lower in professional athletes than in sedentary individuals. Also, Lippi et al., [26] described significantly higher serum creatinine concentration in sedentary individuals than in professional cyclists.

Calcium, sodium, potassium and chloride concentration remained basically unchanged after the ECC protocol. This exhaustive protocol

did not alter significantly the electrolyte homeostatis and it was already described by Bird et al., [18] stating that electrolyte homeostasis is well maintained during most exercises bouts, even in post-marathons.

It is well known that a regular or a strenuous and prolonged exercise is associated with a reduced risk of cardiovascular disease. Studies describe either an increase, for HDL-cholesterol and slight for Total-cholesterol, or a decrease or no change for LDL-cholesterol and triglycerides, respectively, after a marathon [18]. We observed that total, HDL- and LDL-cholesterol increased immediately after the exercise and the triglycerides decreased. Nikolaidis et al., [27] studies the favourable changes in blood lipid and lipoproteins profile after an eccentric knee flexions repeated after 3 weeks. Their findings reveal that lipid and lipoprotein profile was a favorably affected by the muscle-damaging, but apparently this effect is less pronouncing after repeated sessions of eccentric knee flexions. These apparently contradictory data, point to the need to consider, as others already suggested, that the effects of exercise on laboratory tests should be separated in short-term acute adaptations to high-intensity exercise and long-term chronic adaptations to regular exercise [5].

Conclusion

In conclusion, we expect that this study contributes to a better understating of the effects of an intense exhaustion physical exercise on common analytical biomarkers.

Conflict of Interest

None of the authors have any conflict of interest to report.

Acknowledgment

This study was conducted in Centre of Research, Education, Innovation and Intervention in Sport, Faculty of Sport, University of Porto with collaboration of Clinical Pathology Department, São João Hospital Centre of Porto.

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