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Biochemical Markers During and After an Olympic Triathlon Race
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ABSTRACT

Lopes RF, Osiecki R, Rama LMPL. Biochemical Markers During and After an Olympic Triathlon Race. **JEPonline** 2011;14(4):87-96. Blood biomarkers have been widely used in sports, particularly in the sports with high metabolic activity and strenuous muscular adaptation. The Olympic triathlon can create muscle damage, immune system alterations, and metabolic changes. The purpose of this study was to examine plasma levels of some muscle enzymes, and some metabolites concentrations after each stage and 1 hr after the race. Twelve male triathletes (mean \pm SE age 27.9 ± 1.73 yrs, % fat 7.3 ± 0.55) had venous blood samples drawn 1 hr before the race, after swimming, cycling, running, and 1 hr after the race. Plasma samples were analyzed for leukocytes (LEU), iron and ferritin concentrations, lipid and glucose (GLU) profiles, creatine kinase (CK) and lactate dehydrogenase (LDH) activities, and uric acid, urea, and creatinine. Immediately after swimming, there were significant ($P=0.05$) increases in CK activity, creatinine, iron, ferritin, LEU, total cholesterol, HDL-C and LDL-C. Immediately after cycling, there were significant ($P=0.05$) increases in urea and uric acid concentrations. At the end of the race there were significant ($P=0.05$) increases in triglycerides, VLDL-C, and peaks of concentration of CK (+66%), uric acid, creatinine, and LEU (+175.2%). One hr later, most parameters had returned to pre-race values. LDH and GLU concentrations remained unchanged. This study indicates that the pronounced initial systemic responses induced by the Olympic triathlon declines rapidly. Probably, a low-grade systemic inflammation persisted post-race (increased LEU 1 hr post), possibly reflecting incomplete muscle recovery (high CK 1 hr post).

Key Words: Triathlon Race, CK Activity, Urea Concentrations

INTRODUCTION

Success in triathlon depends on the ability of the triathletes to perform each stage at optimal pace, without fatigue hindering performance in the following event (25). The Olympic distance triathlon consists of 1.5 km of swimming, 40 km of cycling and 10 km of running. The first "Olympic distance" world championship was organized in 1989 (19) and, for the last 20 yrs, many scientific investigations and practical interests focused on this "new distance sport" – suggesting, for instance, that prolonged endurance exercise imposes a great impact on athletes (19), namely metabolic changes (4), significant muscle damage (31), and some immunological changes (22,25).

Several studies have examined changes in biomarkers after endurance events, such ultra marathons (26), marathons (8,23,24), ironman triathlon (22,31), and triathlon races (16,25), as well as laboratory simulations of cycling plus running events (18,20). However, triathlon is a sport with specific demands and physiological features (19), and alterations of biochemical markers during the race (immediately after swim, after cycle and after run) are currently unknown. We aimed to examine the changes in plasma levels of muscle enzymes (CK and LDH), and metabolites concentrations (urea, uric acid and creatinine) to assess a possible muscle damage after each stage and 1 hr post olympic triathlon race. Alterations in iron, ferritin, leukocytes, lipid and glucose profiles were already assessed pursuing a better understanding of the impact of an endurance event such as Olympic triathlon on body homeostasis.

METHODS

Subjects

Twelve well trained male triathletes with a minimum 4 yrs of triathlon training participated in the study. All athletes were free of acute or chronic illness, within a normal range of body mass index (BMI) and non-smokers. Athletes read and signed an informed consent form according to the statement of protection for human subjects in the declaration of Helsinki, and the study obtained approval from the Federal University of Parana Ethics Committee. Athletes were not allowed to eat during the race but were allowed to drink water *ad libitum*. Anthropometric characteristics of the subjects are shown in Table 1.

Table 1. Subject characteristics. Values are presented as Mean \pm SE.

N	Age (years)	Body mass (kg)	Height (cm)	% Body Fat	Total Race Time (min)
12	27.9 \pm 1.7	73.9 \pm 2.2	177.9 \pm 1.7	7.3 \pm 0.6	132.5 \pm 4.1

All participants of the study were required to complete a standardized 24-hr and pre race dietary recall. They followed what is recommended for athletes by Willmore and Costill (33): 55% to 65% of carbohydrate (CHO), 10% to 15% of protein (PTO) and below 30% of fats. They consumed 24 hrs prior to the race, mean \pm SE: 56.1 \pm 2.2% of CHO, 15.8 \pm 0.7% of PTO and 29.6 \pm 2.1% of fats. Before the race, the meal (breakfast) consisted of 64.9 \pm 3.6% of CHO, 13.2 \pm 1.1% of PTO and 21.4 \pm 3.0 of fats. These values did not show any significant correlation with biochemical and performance markers.

Procedures

The Triathlon event consisted of 1.5 km swimming, followed by 40 km cycling, and finally 10 km running. Environmental temperature ranged from 18.2 °C to 25 °C, with 77.1% relative humidity;

water temperature was 27 °C. Performance time for each stage and the total time were showed in Table 1.

In order to investigate the effects on biochemical markers, a cross sectional study was designed. Biochemical alterations were assessed by blood samples drawn from an antecubital vein of all athletes. The first sample was drawn 1 hr prior the race; post swimming, post cycling, post running blood samples were drawn immediately after exercise and the last one, 1 hr post race.

A field laboratory was installed at the race site to ensure the appropriate collection of the blood samples. Approximately 10 ml of blood were drawn by a standard venipuncture technique from the antecubital vein using a vacutainer, and centrifuged for 10 min to obtain plasma. The plasma samples were frozen and stored at -20 °C until analysis.

Plasma samples were analyzed for the following parameters: creatine kinase (CK), lactate dehydrogenase (LDH), urea, uric acid and creatinine, measured as muscle damage markers. Leukocytes, iron and ferritin were determined to assess the immune system and oxygen carrying functions. Glucose and Lipid profiles were also determined. CK, LDH and urea concentrations were measured by kinetic method (IFCC- *CR-NAC: unitest y AA*, LDH – *P: unitest, UREA: Kinetic AA*, respectively). Uric acid was assessed by enzymatic and colorimetric method (*QUIMIURIC: Uricase / Peroxidase*) and creatinine was analyzed by kinetic reaction (*QUIMICREA: Creatinine Picrato Alkaline*). Serum Iron was measured by colorimetric method (*Kit Fer-Color AA*). Ferritin was determined by enzymatic chemiluminescent method (*kit Ferritin Immunolite, Med Lab, EUA*). Leukocytes were assessed with an automatic counter Cell-Dyn 1400 System. Glucose, cholesterol and triglycerides were determined by enzymatic method (*QUIMIGLI-OX: Glucose Oxidase; QUIMICOL: Cholesterol Esterase-Peroxidase; TG COLOR: GPO/PAP AA*, respectively). Cholesterol fractions were assessed by spectrophotometer method (*BIOSYSTEMS SA reagent & instruments, Barcelona, Spain*)

Statistical Analyses

Values are presented as mean \pm SE. Data were tested and showed absence of normal distribution using the Shapiro Wilks test. Friedman test was used to find significant differences on repeated measurements. Wilcoxon test was conducted to asses the differences in the test variables, whereas all post stage values (swimming, cycling, running, 1h post race) were compared with pre-race values and between them. Spearman's correlation was used to examine significant relationships. All statistical analyzes were performed using SPSS 13.0 for windows. Differences were considered statistically significant at $P=0.05$.

RESULTS

Markers of Fatigue and Muscle Damage

Plasma CK activity increased significantly after swimming (+27.5%; $P=0.05$), after cycling (+50.9%, $P=0.05$) with a peak of concentration immediately post race (66%, $P=0.05$) versus pre race and remained significantly elevated (+49.3%, $P=0.05$) until 1h post-race. Urea and uric acid concentrations increased significantly after cycling ($P=0.05$), with higher values 1 hr post-race ($P=0.05$) and after running ($P=0.05$), respectively. Creatinine concentrations increased right after swimming, with higher values after cycling and the peak reached immediately after race ($P=0.05$). Plasma LDH remained unchanged during the whole event. Changes in CK and metabolites are shown in Table 2.

Table 2. Changes in markers of muscle damage and plasma biochemical variables before (pre race), immediately after swimming (after swim), immediately after cycling (after cycle), immediately after running (after run) and 1 hour after race (1h post). Values are presented as Mean \pm SE.

Variables (units)	Standard values	Pre Race	After swim	After Cycle	After Run	1h post race
CK (U·L ⁻¹)	24-195	245.8 \pm 83.5 ^a	313.3 \pm 85.7 ^b	371 \pm 104.5 ^b	408.9 \pm 114 ^c	366.9 \pm 96.8 ^{de}
LDH (U·L ⁻¹)	180-450	326.4 \pm 27.2	349.7 \pm 47.3	304.6 \pm 33.3	357 \pm 31.5	349.6 \pm 41.3
Urea (mg·dL ⁻¹)	10 – 40	37.2 \pm 2.5 ^a	37.3 \pm 2.3 ^a	40.9 \pm 3.0 ^b	42.3 \pm 3.0 ^b	43.6 \pm 2.7 ^c
Uric acid (mg·dL ⁻¹)	3.5 – 7.0	5.9 \pm 0.3 ^a	6.2 \pm 0.3 ^a	7.3 \pm 0.5 ^b	8.5 \pm 0.7 ^c	8.5 \pm 0.7 ^c
Creatinine (mg·dL ⁻¹)	0.5 – 1.4	1.1 \pm 0.04 ^a	1.2 \pm 0.04 ^b	1.3 \pm 0.1 ^c	1.4 \pm 0.04 ^c	1.4 \pm 0.04 ^c

*Significantly difference between different letters (p<0.05)

Iron, Ferritin, and Leukocytes

Iron increased significantly after swimming (+9.8%, P=0.05), after cycling (+14.9%) and after running (+19.5%), returning to pre race values within 1 hr of recovery time. Ferritin increased significantly (+11.2%, P=0.05) after the swimming stage and remained significantly (+12%, P=0.05) higher than pre race until 1 hr post-race. Total leukocyte count increased significantly after swimming (52.9%, P=0.05), after cycling (+91.6%), with maximal count reached after running (+175.2%) and 1 hr post-race (141.6%) versus pre-race values. Changes in these variables are shown in Table 3

Table 3. Changes in iron, ferritin and total leukocytes before (pre race), immediately after swimming (after swim), immediately after cycling (after cycle), immediately after running (after run) and 1 hour after race (1h post race). Values are presented as Mean \pm SE.

Variables (units)	Standard values	Pre Race	After swim	After Cycle	After Run	1 h post race
Iron (mg·dL ⁻¹)	50-150	120.3 \pm 7.1 ^a	132.1 \pm 8.4 ^b	138.2 \pm 9.0 ^b	143.8 \pm 10.6 ^b	130.5 \pm 9.2 ^{ac}
Ferritin (ng·dL ⁻¹)	18 – 370	99.1 \pm 19.6 ^a	110.2 \pm 21.6 ^b	123.3 \pm 27.4 ^b	115.3 \pm 21.3 ^b	111.0 \pm 22.0 ^b
Total Leukocytes (10 ⁹ ·L ⁻¹)	3.9-11.9	7.6 \pm 0.53 ^a	11.6 \pm 1.0 ^b	14.6 \pm 1.4 ^c	20.9 \pm 1.9 ^d	18.4 \pm 0.9 ^d

*Significantly difference between different letters (p<0.05)

Glucose and Lipid Profile

Triglycerides increased significantly (35%, P=0.05) after the running stage; however, 1 hr after the end of the race, there was a significant reduction below pre race values. Total cholesterol and HDL-C increased after swimming and remained higher until the end of the race; after one hour of inactivity,

HDL-C values had significantly decreased below pre race concentrations and total cholesterol returned to pre-race values. Glucose concentration remained significantly unchanged during the Olympic triathlon race. Glucose and lipid profiles are shown in Table 4.

Table 4. Changes in Lipid Profile and glucose concentration before (pre race), immediately after swimming (after swim), immediately after cycling (after cycle), immediately after running (after run) and 1 hour after race (1h post race). Values are presented as Mean \pm SE.

Variables (units)	Standard values	Pre Race	After swim	After Cycle	After Run	1 h post race
Triglycerides (mg·dL ⁻¹)	<150	110.2 \pm 11.7 ^a	121.5 \pm 15.3 ^{ab}	117.5 \pm 11.0 ^{ab}	138.5 \pm 13.0 ^b	102.5 \pm 10.0 ^d
Cholesterol (mg·dL ⁻¹)	< 199	180.8 \pm 18.1 ^a	196.8 \pm 14.3 ^b	193 \pm 16.6 ^b	193.8 \pm 14.9 ^b	177.2 \pm 43.2 ^a
HDL-chol (mg·dL ⁻¹)	35 - 60	57.8 \pm 4.4 ^a	62.3 \pm 4.7 ^b	60.4 \pm 4.6 ^c	61.7 \pm 5.9 ^{abc}	56.3 \pm 4.2 ^e
LDL-chol (mg·dL ⁻¹)	<130	100.9 \pm 10.6 ^a	110.2 \pm 11.8 ^b	109.1 \pm 14.2 ^b	102.8 \pm 12.1 ^a	100.3 \pm 10.5 ^a
VLDL-chol (mg·dL ⁻¹)	<40	22.0 \pm 2.3 ^{ac}	24.3 \pm 3.1 ^{ac}	24.4 \pm 2.0 ^a	28.9 \pm 2.0 ^b	21.0 \pm 2.0 ^c
Glucose (mg·dL ⁻¹)	60 – 99	92.2 \pm 8.1	108.1 (9.4)	100.5 (10.3)	95.0 (7.0)	105.9 (6.0)

*Significantly difference between different letters (P=0.05)

DISCUSSION

The primary purpose of this study was to investigate changes in markers of muscle damage and alterations in metabolic profiles. Similar to previous studies examining long distance triathlons and other endurance events, we observed increased values of fatigue markers after the race, but what happened between disciplines so far remained unknown.

A practical index of muscle damage in athletes performing heavy training is elevation of muscle proteins and enzymes (e.g., myoglobin, creatine kinase or lactate dehydrogenase) in the blood plasma (13). From the results of the present study, CK levels at rest were above reference values (CK superior reference limit: 195 U·L⁻¹), and further increased right after swimming. Since our subjects had not suffered any muscular injury or tissue damage before the experiment, our findings were supported by other studies (29,35), which have already suggested a different reference standard to athletes. Increased plasma CK activity suggests that exercise duration, in particular duration and intensity of running (31), may influence changes in this variable. Assessment of plasma creatine kinase activity is therefore potentially useful, not as a marker of impending overtraining, but as a means of identifying a state of recent muscle damage or temporary over-reaching (13).

LDH concentration has not significantly changed, but there was a tendency towards increased values during the race. Other studies have shown increased values after different kinds of exercise – such as running (1), ultra marathon (34), short triathlon (16) and Ironman Triathlon (31) – reportedly

explained by its intense and continuous release on bloodstream from heart, muscles and liver after a strenuous and prolonged exercise.

Athletes commonly display high resting urea concentrations, probably as a result of the continual stress of training (32). After a prolonged strenuous exercise, urea concentrations are generally further increased (13) and remain elevated after exercise. Usually, an increase in urea concentration may be related to a reduction in renal blood flow (and glomerular filtration rate) secondary to fluid volume deficiency (32), and increased protein catabolism (13). Uric acid may provide a measure of muscle protein breakdown in association with a catabolic state (presumably caused by chronically elevated levels of glucocorticoid hormones).

However, a temporary elevation of the plasma concentrations of uric acid and urea is markedly influenced by the dietary protein intake (13). In this study, protein intake seems to be balanced, showed by a 24 hr and pre race dietary recall done by triathletes. Creatinine concentration, the product of creatine breakdown from skeletal muscle, also generally increases after a prolonged high intensity exercise. The increase in plasma creatinine concentration is probably the result of release of creatinine from working muscles, dehydration and/or reduction in renal blood flow and glomerular filtration rate (32). All of these variables increase after prolonged high intensity exercises including events such as Ironman triathlon (31), and marathon (24, 27).

The effects of physical activity on the immune system have been investigated in many studies, using different intensities of exercise as well as strenuous and prolonged exercises (31), moderated exercise (21) and acute high intensity exercises (3,9), in different sports, disciplines and events – including marathons (8,23,24,27), ultra marathons (26,34), ironman triathlons (22,31), swimming (10,11,12), cycling (6,15) and soccer (2). In this study the major biochemical alteration was the peak value of total leukocyte count at the end of the race (+175.3% versus pre race).

This phenomenon (called leukocytosis) can result from increased cell traffic (mobilization) from bone marrow, spleen, liver, lungs, marginal pool and/or the lymphatics to blood (30), demargination from the blood vessel walls (e.g., after intense physical exercise), and decreased exit to tissues. Although a thorough understanding of the release of blood cells is still lacking, it has been suggested that the same (or similar) factors that control cell recruitment into inflamed tissues also regulate the mobilization from the bone marrow (28). There is a strong leukocyte response to this form of endurance exercise. Whether this immunological marker raises (following endurance exercise) mainly in response to muscle damage or due to other factors requires further investigation.

Most fat is stored as triglyceride in adipocytes and muscle cells. Plasma and muscular triglyceride were consumed equally during the first stage of endurance exercise, and subsequently the free fatty acid became the major source of energy (14), explaining the reduction in triglyceride (TG) after 1 hr post race. Acute reduction of TG might result from the use of body fat as the major energy source. Cholesterol and fractions (HDL-C, LDL-C and VLDL-C) were significantly lower 1 hour after the race agreeing with previous reports (34).

Glucose concentration remained unchanged during the race (although food had been prohibited) only water had been consumed *ad libitum*. Previous studies show that glucagon and catecholamine blood levels increase, and insulin level decreases to protect the body against hypoglycemia during a prolonged exercise; neural active muscle response also has an important role in increasing the glucose production during the exercise (17).

In summary, Olympic triathlon races cause substantial muscle damage, inflammation, renal and immune functions changes. The most important find of the current study was that, although initially Olympic triathlon induced marked alterations in most biochemical markers, these changes subsided rapidly (1 hr post). Nevertheless, protein enzymes and high leukocytes values were sustained after race – according to other studies, may continue high for at least 5 days more (22).

CONCLUSIONS

Few studies have extensively addressed the biochemical changes in endurance triathletes in similar conditions of competition. This investigation helps to elucidate the effects of each segment in the biomarkers' kinetics during a triathlon race.

Due to the continuous demands of triathlon competitions on training schedules, competitive athletes may not have sufficient recovery between races. Although most biomarkers returned to basal levels during the first hour after triathlon, leukocytes and CK remained elevated. Inadequate rest following prolonged, intensive exercise may cause a chronic systemic inflammatory state that could in fact lead to a syndrome of impaired performance and progressive fatigue. Thus, finding an appropriate balance between training, competition and recovery is an essential challenge to maintain a high level of performance and to minimize potential health consequences.

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