SPECIALIZED MOVEMENT ON THE ROWING ERGOMETER AND POST-WORKOUT CHANGES IN SELECTED PERIPHERAL BLOOD PARAMETERS – A CASE REPORT

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Abstract Rowing is a sport discipline, which requires extreme physical strength and endurance and appropriate aerobic and anaerobic capacity as well. However, when the workout intensity and load is very high, exercise is associated with temporary changes in cellular metabolism and the immune system. The study included one male rower aged 28 years – the highly-skilled and experienced athlete. We determined basic cardiorespiratory fitness measures, complete blood count, and 24 clinical chemistry parameters including relevant biochemical and haematological parameters and matrix metaloproteinases activities. Maximal exercise on the rowing ergometer induced 2-fold increase in absolute counts of all leukocytes subsets. There was observed an increase in C-reactive protein concentrations as well. MMP-9 activity increased 1.3-fold compared to the baseline value. Exhaustive exercise caused significant changes in creatinine and urea serum levels, but the most prominent changes were found in total and direct bilirubin concentrations. Maximal exercise induced also a decrease in the iron and magnesium levels. No changes in ALT, GGT and ALP activity were observed, while increase in CK, AST and LDH activity in post-exercise time and the decrease during the recovery was found. Therefore acute specialized movement on the rowing ergometer is not the cause of muscular damage, but rather indicate efficient adaptation to the physical exercise. Moreover, it seems that maximal exercise induces an inflammatory response characterized by greater count of all subpopulations of leukocytes, elevated levels of CRP and MMP-9 serum activity.

Key words rowing, biochemical markers, haematological markers, matrix metalloproteinases

Introduction

Physical exercise is aimed to significantly disturb the current homeostasis in athletes' skeletal muscles resulting in collagen type IV regulation and enhancement of basement membrane, which leads to improvement of skeletal muscle mass and strength. However, under certain conditions, when the workout intensity and load is very high, exercise can cause a damage of skeletal muscles and tendons. Acute physical activity may lead to sarcomere damage, inflammatory responses and activation of satellite cells in order to replace the lost muscle fibres. The degradation of the extracellular matrix (ECM) structure often occurs in the damaged skeletal muscle and connective tissue. This kind of disturbances lead to the activation of collagen degradation enzymes – tissue matrix metalloproteinases (MMPs) as well. MMPs, including MMP-9, are also present in many types of leukocytes, including monocytes and lymphocytes (Baumert, Lake, Stewart, Drust, Erskine, 2016; Lo Presti, Hopps, Caimi, 2017).

Rowing is a sport discipline, which requires extreme physical strength and endurance and appropriate aerobic and anaerobic capacity as well. Minimising the influence of injuries and overtrained states and maximising the training efficiency are the main aims for coaches in preparing elite athletes for competitions. There are groups of well described metabolic parameters measured in blood, that are very useful tools in athletes' physical condition assessment, for example, creatine kinase activity, lactate dehydrogenase, and blood lactate concentration (Banfi, Colombini, Lombardi, Lubkowska, 2012; Chamera et al., 2015; Chamera et al., 2014). Intensive, long-term endurance training and participation in sporting competitions lead to the damage of muscle fibres and release of the inflammation factors and cellular enzymes into the peripheral blood.

Ever-changing environmental conditions typically found during on-water rowing testing cause significant standardisation problems. For this reason, we use the rowing ergometer, which simulates properly the action of on-water rowing in our research (Akça, 2014; Sforza, Casiraghi, Lovecchio, Galante, Ferrario, 2012).

Therefore, the aim of this study was to analyze the relationship between high-intensity physical exercise and changes in selected haematological and biochemical parameters levels of the highly-skilled rower, which was tested on a rowing ergometer.

Methods

Study design

To investigate body metabolism response to acute exercise in elite rower, we designed an experiment to evaluate these processes. We determined cardiorespiratory fitness measures ((maximum oxygen uptake (VO_{2max}), maximum heart rate (HR_{max}) and anaerobic threshold (AT)), complete blood count, and 24 clinical chemistry parameters, including relevant biochemical and haematological variables and matrix metalooproteinase 9 activity. The study was performed in the biochemistry, physiology and genetic laboratories of the Centre for Human Structural and Functional Research, Szczecin, Poland.

Participant

The study included one male rower aged 28 years. The participant was top-athlete, multiple medallist of high-profile competitions with 13 years of training experience. The rower trained 9 hours a week. The characteristics of the study participant are presented in Table 1. The participant was non-smoker, free of any health problems and

was not taking any drugs, supplements or medications known to affect metabolism for 2 weeks prior to the test. He had no history of any metabolic syndrome or cardiovascular diseases. The participant was under no dietary restrictions.

Table 1. Subject characteristics

Parameter	Value
Age (years)	28
Height (cm)	194.0
Weight (kg)	98.9
Fat mass (kg)	13.4
Body mass index (BMI) (kg/m^2)	26.3
VO_{2max} (mL/kg/min)	57.4
HR_{max} (beats/min)	192.0
AT (beats/min)	146.0

VO_{2max} – maximum oxygen uptake, HR_{max} – maximum heart rate, AT – anaerobic threshold.

The Local Ethics Committee at the Szczecin approved the study protocol. Participant provided written informed consent.

Procedures

Aerobic capacity evaluation

The progressive exercise test until exhaustion was carried out on Concept2 model D rowing ergometer (Concept2, Morrisville, VT, USA) according procedure described previously by Nowak et al. (2017).

Blood sampling

Blood samples from the athletes' elbow vein were obtained three times: (1) at rest (in the morning, the day before testing, after an overnight fast), (2) immediately after the exercise test (5 min after completing the test), and (3) after a 17-h recovery period (after an overnight fast). For the safety reasons the test protocol required the participant to be after a light breakfast. Therefore, blood sample collected after the exercise test was not fasting blood.

Venous blood samples were collected into S-Monovette tubes containing clot activator and dipotassium EDTA (SARSTEDT AG & Co., Nümbrecht, Germany) for serum and anticoagulated whole blood preparations, respectively. The tubes for serum preparation were centrifuged at 2000 × g for 10 min at room temperature. The part of serum collected for zymographic assays was stored at – 80°C.

Biochemical, haematological and zymographic analyses were performed before the progressive test (pre-exercise), after the test (post-exercise) and at the end of recovery period (17 hours after completion of the test). The biochemical and haematological analyses were performed immediately after blood collection.

Complete blood count

Blood samples were taken for the analysis of red blood cells (RBCs), white blood cells (WBC), mean corpuscular volume (MCV), haemoglobin (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular

haemoglobin concentration (MCHC), total platelets level (PLT) and haematocrit (HTC) in a haematology analyser ABX Micros 60 (Horiba ABX, Warsaw, Poland).

Biochemical analyses

Biochemical tests have been carried out with the use of an Auto Chemistry Analyser BM-100 (BioMaxima SA, Lublin, Poland) in case of clinical chemistry variables or an Ion Selective Analyser BM ISE (BioMaxima SA, Lublin, Poland). Blood serum was used to determine metabolites (creatinine, urea, uric acid and bilirubin, both total and direct), albumin, total protein, C-reactive protein (CRP), lipid profile (triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein (LDL-C) levels), enzymes activities (aminotransferases: aspartate (AST) and alanine (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH)) and selected ions, namely iron, magnesium, phosphorus (Auto Chemistry Analyser BM-100, BioMaxima, Poland) and calcium (Ion Selective Analyser BM ISE, BioMaxima, Poland). All studied parameters were determined using a diagnostic method according to the appropriate manufacturer's protocol (BioMaxima SA, Lublin, Poland). Moreover, the CRP level was determined using two different turbidimetric assay kits according to the manufacturers' protocols (BioMaxima SA, Lublin, Poland and Quimica Clinica Aplicada SA, Amposta, Spain) to confirm the results obtained during the study. All analyses were verified with the use of multiparameteric control serum, as well as control serum of a normal level (BioNorm) and a high level (BioPath) (BioMaxima SA, Lublin, Poland).

Gelatin zymography

The serum samples collected were centrifuged at 1600 × g for 15 min at 4°C and were diluted 40-fold. To each sample a loading buffer (Tris-Base containing 10% glycerol, 2% sodium dodecyl sulphate (SDS) and 0.05% bromophenol blue (pH 6.8)) was added. Then, samples were loaded in a 7.5% polyacrylamide-SDS gel containing 0.1% gelatin and separated by electrophoresis at 110 V for 2 h at 4°C. Thereafter, gels were washed three times for 60 min with 2.5% Triton X-100 to remove SDS. Gels were then incubated for 48 h at 37°C in catalytic buffer (50 mM Tris-HCl, 10 mM $CaCl_2$, 200 mM NaCl, pH 7.5) and stained with 0.1% Coomassie brilliant blue G-250 in methanol : acetic acid : water (9 : 2 : 9, v : v : v) mixture for 1.5 h. Finally, gels were destained with methanol : acetic acid : water (1 : 1 : 8, v : v : v) mixture for 3 h. Proteolytic activity of MMPs was visualized as clear bands on a dark blue background. The bands were identified basing on the molecular weights of MMPs: 72 kDa and 92 kDa corresponding to MMP-2 and MMP-9, respectively. Proteolytic activity was determined using an imaging system (ChemiDoc™ XRS+ System, Bio-Rad, USA) and a software package (Image Lab™ Software, Bio-Rad, USA).

Results

Maximal exercise on the rowing ergometer induced changes in absolute counts of all leukocytes subsets. Athletes' total leukocyte count was 2-fold increased after maximal exercise (Table 2). Elevation of circulating lymphocytes and neutrophils was the main reason for the change in total white cells count. There was no increase in haemoglobin concentration and haematocrit (Table 2). There was observed an increase in C-reactive protein concentrations after maximal exercise as well (Table 3).

Table 2. Selected haematological parameters determined before (pre-exercise) and after the progressive exercise test on rowing ergometer until exhaustion (5 minutes post-exercise) and during recovery (17 hours after the test)

	Pre-exercise	Post-exercise	Recovery time
WBC (10^9/L)	4.10	8.30	5.90
RBC (10^{12}/L)	4.63	4.79	4.72
HGB (mmol/L)	8.70	9.00	9.20
HTC (%)	45.00	46.00	46.00
PLT (10^9/L)	172.00	201.00	164.00
MCV (fL)	97.00	97.00	97.00
MCH (fmol)	1.88	1.88	1.94
MCHC (mmol/L)	19.40	19.40	20.00
Lymphocytes (%)	39.10	48.50	33.70
Lymphocytes (10^9/L)	1.60	4.00	1.90
Monocytes (%)	5.70	6.90	5.00
Monocytes (10^9/L)	0.20	0.50	0.20
Neutrophiles (%)	55.20	44.60	61.30
Neutrophiles (10^9/L)	2.30	3.80	3.80

Table 3. Serum selected metabolite levels determined before (pre-exercise) and after the progressive exercise test on rowing ergometer until exhaustion (5 minutes post-exercise) and during recovery (17 hours after the test)

	Pre-exercise	Post-exercise	Recovery time
Creatinine (µmol/L)	111.00	132.00	104.00
Urea (mmol/L)	6.50	7.10	6.90
Uric acid (µmol/L)	280.00	261.00	218.00
Total bilirubin (µmol/L)	11.32	8.46	6.91
Direct bilirubin (µmol/L)	17.56	15.47	9.11
CRP (mg/L)	0.10	2.80	2.50
Albumin (g/L)	49.00	52.20	46.90
Total protein (g/L)	60.18	66.60	60.22

Exhaustive exercise caused significant changes in creatinine, urea and bilirubin levels. The most prominent changes were found in total and direct bilirubin concentrations in participant (Table 3). The activity of gelatinases (MMP-2 and MMP-9) were examined by gelatine zymography assays. MMP-9 activity increased 1.3-fold compared to the baseline value and then decreased below the baseline value in the recovery time. No detectable activity of MMP-2 was determined (Table 3 and Figure 1). CK activity in rowers' serum was far higher than in general population, however post-exercise increase did not remain at that high level in the recovery time. No changes in ALT, GGT and ALP activity in athletes' serum were observed, while increase in AST and LDH activity in post-exercise time and the decrease during the recovery period in rower was found (Table 4). It was also noted that maximal exercise induced a decrease in the iron and magnesium levels and post-exercise raise in the phosphorus level (Table 5). No changes in the lipid profile of the participants (Table 6).

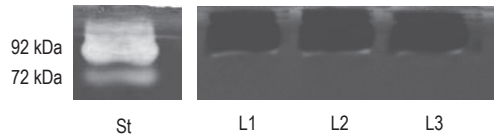

Figure 1. Zymographic profile showing relative serum activity of MMP-9 (92 kDa) and MMP-2 (72 kDa) in athlete determined by gelatine zymography before (pre-exercise) and after the progressive exercise test on rowing ergometer until exhaustion (5 minutes post-exercise) and during recovery (17 hours after the test). St – standard, L1 – pre-exercise, L2 – post-exercise, L3 – recovery time

Table 4. Serum enzyme activities determined before (pre-exercise) and after the progressive exercise test on rowing ergometer until exhaustion (5 minutes post-exercise) and during recovery (17 hours after the test)

	Pre-exercise	Post-exercise	Recovery time
Amylase (U/L)	62.30	70.20	67.40
AST (U/L)	46.40	64.40	35.40
ALT (U/L)	33.60	40.80	31.80
CK (U/L)	502.60	578.00	304.90
GGT (U/L)	13.40	14.70	13.20
LDH (U/L)	365.00	353.00	99.00
ALP (U/L)	61.48	66.64	55.51
MMP-9 (relative activity coefficient)	1.00	1.31	0.67

AST – aspartate aminotransferase, ALT – alanine aminotransferase, GGT – gamma-glutamyltransferase, LDH – lactate dehydrogenase, CK – creatine kinase, ALP – alkaline phosphatase, MMP-9 – matrix metalloproteinase 9.

Table 5. Serum selected ions determined before (pre-exercise) and after the progressive exercise test on rowing ergometer until exhaustion (5 minutes post-exercise) and during recovery (17 hours after the test)

	Pre-exercise	Post-exercise	Recovery time
Iron (μmol/L)	29.20	34.90	24.60
Magnesium (mmol/L)	0.75	0.69	0.62
Calcium (mmol/L)	2.53	2.58	2.37
Phosphorus (mmol/L)	0.96	1.55	0.93

Table 6. Serum lipid profile determined before (pre-exercise) and after the progressive exercise test on rowing ergometer until exhaustion (5 minutes post-exercise) and during recovery (17 hours after the test)

	Pre-exercise	Post-exercise	Recovery time
TG (mmol/L)	0.43	0.77	0.42
TC (mmol/L)	4.76	5.04	4.80
LDL-C (mmol/L)	2.32	2.28	2.27
HDL-C (mmol/L)	2.25	2.41	2.30

TG – triglycerides, TC – total cholesterol, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol.

Discussion

Exhaustive exercise is associated with temporary change in cellular metabolism and the immune system (Desgorces, Testa, Petibois, 2008; Reihmane, Jurka, Tretjakovs, 2012). We observed some changes in blood parameters responses to a characteristic rowing maximum exercise in athlete, which was tested.

First of all, we noted that white blood cell total count was twice as high as baseline values with no significant increase in haemoglobin concentration and haematocrit. This suggest lack of dehydratation effect on haematological parameters in athlete examined. Moreover, it was found, that C-reactive protein concentration was elevated, which suggests induction of an inflammatory response. This hypothesis is supported by transient increase of the serum activity of another protein linked with inflammation state, such as MMP-9. Previously Danzig and Madden with co-workers has shown that the degree of the muscle damage and the intensity of exercise are important factors that positively influence the release of MMP-9 (Danzig et al., 2010; Madden, Byrnes, Lebin, Batliner, Allen, 2011). Further, Reihmane et al. showed that maximal exercise-induced MMP-9 release correlated with the increase in pro-inflammatory cytokine – interleukin-6 (IL-6) level suggesting a close relationship between inflammatory and proteolytic processes in tissues (Reihmane et al., 2012). In general, MMP-9 is considered to be the marker of skeletal muscle remodelling and regeneration (Lo Presti et al., 2017). However, it remains unclear which tissues were the original source of this enzyme after exhaustive exercise in our study? Since we observed 2-fold increase in leukocyte count this may reflect accelerated release of MMP-9 into the circulation from neutrophiles rather than an increased extracellular matrix damage (Koskinen, Hoyhtya, Turpeenniemi-Hujanen, 2001). Moreover, MMPs are the major components of neutrophilic granules (Chen, Fan, Poon, 2006), which supported hypothesis, that neutrophils could be the major source of increased MMP-9 activity.

The progressive exercise test on rowing ergometer until exhaustion induced also increase of bilirubin serum level. In these conditions, the primary source of bilirubin in peripheral blood is the red blood cells haemolysis. The greater destruction of erythrocytes is mainly caused by mechanical factors and the harmful effects of free radicals (Witek et al., 2017). The main reason for this were elevated body temperature, metabolic acidosis, hypoglycaemia and hemoconcentration which all took place during physical exercise (Reeder, Wilson, 2001; Robinson, Cristancho, Boening, 2006). Yusof et al. suggested that intravascular haemolysis observed during long-term exercise results from the injury of older erythrocytes, which are less elastic (Yusof et al., 2007). On the other hand, Hwang et al. have described anti-inflammatory properties of bilirubin, expressed through a negative correlation with the concentration of C-reactive protein (Hwang, Lee, Kim, 2011). Therefore, some researchers tried to explain out of range levels of bilirubin concentration in the highly-skilled athletes by the training adaptation (Witek et al., 2017). Moreover, in the recovery period, decrease in the serum iron concentration was noticed. Given the foregoing, this decline was not related to dehydratation state of athlete after the training. It seems, that the main reason of iron level lowering is the accelerated breakdown of red blood cells.

Another metabolite – serum creatinine level has been related to muscle mass and renal function. The maximal effort caused raise of this parameter value suggesting a decrease of renal function. However, since creatinine is a by-product of muscle contraction the post-exercise increase of this parameter could be due also to muscle tissue damage (Colombini et al., 2014). It is known, that acute exercise could initiate amino-acid metabolisms in highly trained subjects (Desgorces et al., 2008). Initiation of this metabolic pathway could provide an explanation for post-exercise increase of the urea level. This clinical variable could be good diagnostic marker for the evaluation of physical fitness and condition of athletes as well (Nowak, Buryta, Kostrzewa-Nowak, 2016).

CK is frequently described as the best indirect marker of damage of muscle tissue, especially after resistance exercise (Callegari et al., 2017). In our investigation, CK and LDH activity were at the very high levels in the post-exercise period of time, considerably exceeding the reference values established for general population. However, this state did not persist during the recovery time, which suggested that specialized movement on the rowing ergometer did not cause the muscular damage and athlete was well adapted to the acute effort. Great elevation in enzymes activity could be partially explained by research of Amorim et al., who found significant negative correlation between CK activity and the eGFR indices of renal function (Amorim et al., 2014). Similar changes in the AST serum level, which seems to be another marker of skeletal muscle damage (Banfi et al., 2012), supported this hypothesis.

Conclusions

Specialized movement on the rowing ergometer causes changes in selected clinical parameters in high-level athlete. The observed increase in CK and ASAT activity did not maintain at the end of recovery, which suggest that specialized movement on the rowing ergometer is not the cause of muscular damage. This observation is supported by decrease in the activity of another skeletal muscle damage marker – lactate dehydrogenase at the recovery time. Moreover, it seems that maximal exercise induces an inflammatory response characterized by greater count of all subpopulations of leukocytes, elevated levels of CRP and MMP-9 serum activity.

In conclusion, the different changes in biochemical and haematological parameters are part of the adaptive mechanisms to acute physical exercise. Therefore, monitoring of these parameters permit the alignment of the training process to the athletes' condition and could directly improve the athletes' performance.

Acknowledgments

The authors would like thank a rower and his coach who volunteered to participate in the study.

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Cite this article as: Proia, P., Amato, A., Contró, V., Thiel, P., Vostatková, D., Buryta, B., Kolbowicz, M., Lewandowski, T., Nowakowska, A. (2018). Specialized Movement on the Rowing Ergometer and Post-workout Changes in Selected Peripheral Blood Parameters – a Case Report. *Central European Journal of Sport Sciences and Medicine*, *4* (24), 5–13. DOI: 10.18276/cej.2018.4-01.