

Laboratory and Sports medicine

Cod: T306

PREVENTIVE NONINVASIVE ANALYSIS OF URINARY PROTEINS IN 13-24 YEARS OLD INDIVIDUALS IN CORRELATION WITH PHYSICAL ACTIVITY

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Background: Continuous preventive examination of the renal function in correlation with physical activity is important for identifying of inborn or acquired diseases that might lead to irreversible lesions. The purpose of our study was to evaluate the results obtained from the preventive noninvasive examination of renal function in 13-24 years old sportsmen actively involved in the training process in comparison with individuals not actively included in the training process.

Methods: The examination included 520 male active sportsmen in basketball, tennis and volleyball clubs and 410 healthy male subjects, recruited from the general population. All examinees sportsmen should have been actively involved in the training process for more than six months. For the evaluation of changes in qualitative and quantitative content of urinary proteins, five urinary samples were used excreted during the day: two samples of first morning urine, two samples of daily urine and one sample excreted after the increased physical effort. Total urinary proteins concentration in all urinary samples was determined with Meulemans' spectrophotometric method. The evaluation of changes in qualitative and quantitative composition of urinary proteins with 4-22% gradient horizontal SDS-PAG electrophoresis, enabled us detection of individuals with exercise induced proteinuria, so called sports proteinuria. Coomassie Blue R-250 staining technique was used.

Results: The functional proteinuria of type sports proteinuria was detected in 2.2% of examinees from the sportsmen group and in 1.9% of examinees from the control group. There was a significant difference in the excretion of total proteins ($p < 0.001$) and in SDS-PAG electrophoretic profiles of urinary proteins between individuals with and without sports proteinuria.

Conclusions: The preventive noninvasive examination of the renal function in correlation with physical activity are useful for ontime detection of individuals with increased risk for developing of pathological response to increased physical effort.

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BIOMARKERS OF HEART DAMAGE AFTER EXERCISE IN ATHLETES

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INTRODUCTION

The role and a degree of elevation of biomarkers indicating myocardial injury in professional athletes is not defined. Interpretation of results of a new generation of tests for determination of highly sensitive troponin is causing a debate among cardiologists and doctors of laboratory diagnostics. These tests are hardly known among coaches and specialists on sports medicine.

The aim of this study was to examine changes in the levels of cardiac biomarkers before and 1 hour after the maximum physical stress in professional athletes of high class.

METHODS

10 male athletes aged 21-28 years were examined during one day. Venous blood was taken in the morning before the exercise 2-3 hours after a light breakfast and 60 min after a simulated cross-country skiing with sticks (distance - 13 km). We determined CRP, high-sensitive troponin I, CK (MB)-mass with the use of reagents, calibration, control materials and analyzers "Architect i1000, c4000" (Abbott).

RESULTS

CRP levels before the exercise were within 2 mg/l in all athletes, indicating the absence of systemic inflammation. Concentrations of troponin I and CK(MB) were within normal values for all athletes and amounted to 2.8 ng/l and 4.76 ng/ml respectively. After 60 minutes of rest after running levels of cardiac markers in the blood increased significantly, average CK level was 40% higher than baseline values, levels of troponin I increased 2.1 times.

CONCLUSION

There are significant differences in the release of cardiac markers into the blood during myocardial infarction, cardiac surgeries and extreme physical exertion, typical for sport of high achievements in terms of time and the degree of increase of their levels in the blood.

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EFFECT OF POMEGRANATE ON THE ACUTE AND DELAYED BIOLOGICAL RESPONSE: RECOVERY OF MUSCLE DAMAGE AND AND INFLAMMATORY MARKERS

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Background

The aim of this study was to investigate the effect of pomegranate juice supplementation on biomarkers of muscle damage and inflammatory markers after an intensive strength training session.

Methods

Healthy, nonsmoking, and professional athletic male (21 ± 0.5 years) were recruited to perform two training-sessions (snatch, clean and jerk, and squat) after either placebo (PLA) or natural pomegranate juice (POM) supplementations. Blood samples were collected before the training and 5min then 48h after each session for the measurement of muscle damage markers (CK, LDH, ASAT, PAL) and C-reactive Protein (CRP) spectrophotometrically, on Architect Ci 4100, ABOTT.

All statistical tests were processed using STATISTICA 10.0 Software.

Results

Compared to the PLA, POM decreased the acute responses for the majority of the tested parameters in the nine recruited men: 5 minutes after training sessions the PLA showed a significant increase for CK ($p < 0.001$) and LDH ($p < 0.01$). A lower rate of increase was registered when using POM supplementation, -8.75% and -1.64% respectively.

The acute effects were also evaluated by an increased rate of ASAT (+16.59%), PAL (+04.51%) and CRP (+12.59%) with a significant difference ($p < 0.05$) in pre-post training session with PLA. The increase was no longer significant when using POM supplementation.

As for the delayed effects (after 48h), the comparison of the pre-post training session values showed a significant increase for CK ($p < 0.001$), LDH ($p < 0.001$), ASAT ($p < 0.001$), CRP ($p < 0.05$) and PAL ($p < 0.05$) with PLA. Three markers (CK, LDH, ASAT) showed significantly lower results ($p < 0.05$) with POM supplementation.

Moreover, a significant interaction was found between POM supplementation and CK ($p < 0.05$).

Conclusions

The present study showed that natural pomegranate supplementation was able to accelerate the recovery of the muscle damage by improving the kinetics of its damage markers after an intensive training session. The interaction found between pomegranate supplementation and CK suggests a dose response effect. Our findings encourage further studies to explore potential therapeutic applications and the dose response effect.

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THE PLASMA LEVELS AND DIAGNOSTIC UTILITY OF MMP-3 AND TIMP-3 IN PSORIATIC PATIENTS

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BACKGROUND. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are thought to be associated with the pathogenesis and spread of psoriatic disease. This study was designed to investigate plasma levels of MMP-3 and TIMP-3 and diagnostic power of these parameters in plaque psoriasis patients.

METHODS. Plasma samples of 60 patients suffering from plaque psoriasis and 30 healthy volunteers were evaluated. Concentration of MMP-3 and TIMP-3 was determined using enzyme-linked immunosorbent assay (ELISA), while Psoriasis Area and Severity Index (PASI) was used to define disease advancement.

RESULTS. Plasma levels of MMP-3 (15.03 ng/ml) and TIMP-3 (565.25 ng/ml) were significantly elevated in psoriasis patients compared to healthy individuals (12.97ng/ml; 412.30 ng/ml - respectively). Furthermore, the concentration of selected tissue inhibitors was negatively correlated with baseline PASI score. The areas under the ROC curve (AUC) for MMP-3 (0.6401) were lower than the AUC of TIMP-3 (0.7423) in total psoriatic patients group. Moreover TIMP-3 showed a very high diagnostic power in individuals with psoriasis of early onset (0.9689).

CONCLUSIONS. These results highlight the meaningful role of MMP-3 and TIMP-3 in psoriasis pathogenesis. Furthermore, our findings indicate the usefulness and high diagnostic power of TIMP-3 in the early detection of psoriasis.

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COMPARISON OF CARDIAC BIOMARKERS FLUCTUATION IN RUNNERS OF MARATHONS, SEMI-MARATHONS AND UNTRAINED RUNNERS

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Background: Regular exercise is important for the prevention program of cardiovascular disease. There are several studies on biomarker changes during marathons especially cardiac biomarkers have been studied and mild to moderate elevations have been described as a results of a running exercise Exact underlying mechanism for these elevations reflecting physiological or even pathobiological changes is unknown and less trained athletes might exhibit a higher risk compared to well trained The aim of our study was to compare three cardiac biomarkers for ischemic condition (Troponin), cardiac stretch (natriuretic peptides) and fibrosis(Galectin-3) in different type of runners, trained marathon and semi-marathon runners and untrained runners at 3 different time.

Methods: 23 (mean age 41 ± 8.8 yo) marathon runners, 15 semi-marathon runners(44.1 ± 8.4 yo) and 17 healthy sedentary subjects (37 ± 4.4 yo) (race of 10 km, <2h of sport/week).

Blood samples were taken just before (T0), just after (T1) and 3 hours (T3) after the race,, centrifuged , aliquoted and stored frozen at -80C before further analysis. The study was approved by the Ethical Committee of our University Hospital. The analyses were performed on the Abbott ARCHITECT i2000SR (Abbott Laboratories, Germany) for the hs cTnI, BNP and Gal-3 and on the C8000 (Roche Diagnostics,Switzerland) for hs-cTnT and NT-proBNP according to the manufacturer's instructions for use.

Results: In all 3 running groups there is an increase of cardiac biomarkers Troponin I, BNP, Galectin-3 and NT-ProBNP after completion of the physical exercise. Biomarkers increase is depending on the intensity and duration of the exercise and is higher in long distance marathon and semi-marathon runners compared to the control group with a 1 hour run. Cardiac biomarker levels between trained marathon and semi-marathon runners were not statistically different in the pre-exercise baseline samples for BNP, NT-Pro-BNP and Galectin-3. Compared to untrained runners only Troponin I levels were higher in baseline sample of marathon runners (hs-cTnI, $p < 0.03$) when compared to controls, cardiac Troponin T (hs-cTnT, $p < 0.29$) was less significant.

Conclusions: Our study demonstrates that exercises of different intensity can be associated with biochemical abnormalities that may reflect adverse consequences on cardiac structure as fibrosis and biology. With exeption of Troponin I there was no difference in the pre-exercise baselines samples.

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IMPACT OF AN ULTRA-MARATHON OF 330 KM ON CARDIAC BIOMARKERS CONCENTRATIONS

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Background: While moderate exercises produce beneficial effects on the cardiovascular system, consequences of supraphysiological efforts are not yet clear. The aim of our study was to evaluate the consequences of such effort on cardiac, inflammation and renal function biomarkers. This project also studied the evolution of new biomarkers of cardiac fibrosis such as the ST2 and Galectin-3.

Methods: 51 people attending the Tor des Géants (330 km, with an altitude range of 24,000 meters) were followed. The study was conducted on 33 participants having reached at least half of the race (148.7 km). Blood and urine samples were collected at four different times: before the race, halfway, at the finish and three days after the arrival. Several biomarkers as High sensitive Troponin T (hsTnT), N-terminal pro B-type natriuretic peptide (NT-proBNP), copeptin, Heart Fatty acid binding protein (H-FABP), Soluble tumorigenicity 2 (ST2), Galectin-3 (Gal-3), creatin kinase (CK), myoglobin (MYO), C-reactive protein (CRP), creatinine and Neutrophil gelatinase-associated lipocalin (NGAL) were assayed on different analyzers such as COBAS®, KRYPTOR®, VIDAS® and ETIMAX®. Meanwhile, the ST2 was measured manually with the Presage ST2 (Critical Care).

Results: During this ultra-endurance effort, the plasma levels of cardiac markers (hsTnT, NT-proBNP, copeptin, H-FABP, ST2, Gal-3), muscle (CK, MYO) and inflammation (CRP, GB) have increased significantly to halfway (148.7 km). Meanwhile, the markers of renal function (urinary NGAL and plasma and urinary creatinine) have only slightly varied, excepting plasma creatinine.

Conclusion: Our study suggests that there is no permanent structural damage at the myocardium level during an ultramarathon. Concerning the kidney, it exists a transient but reversible renal suffering, reversible after rehydration. However, the low pace adopted by the runners, due to fatigue, caused an inflammatory response as well as muscle damage less important than shorter races. Nevertheless, an ultraendurance race as the Tor des Géants means an intense physical and psychological effort.

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THE EVALUATION OF HIGHLY QUALIFIED SPORTSMEN'S BIOCHEMICAL HOMEOSTASIS

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BACKGROUND-AIM

Nowadays biochemical screening is of vital significance within the modern framework of common medico-biological examinations and noninvasive control of the fitness level of highly professional sportsmen. Saliva is put forward as an informative biological environment. The aim of this study was to evaluate the energy status and free radical intensity in sportsmen's saliva before and after physical activity.

METHODS

23 track athletes took part in the research. Saliva samples acquisition was performed with the help of test-tubes through spitting without further stimulation before and after physical activity consisting in a series of 3x100m flat race distances. Each saliva portion was diagnosed according to the level of glucose, free fatty acids, lactat dehydrogenase and creatine phosphokinase. Free radical processes intensity were measured by induced biochemiluminescence method (BChL). The level of products of lipid peroxidation: dien conjugate (DC), trien conjugate (TC) and Schiff Based (SB) were determined. The statistical analysis of the received data was carried out employing Microsoft Excel 2013 # Statistica 13.0 software applications.

RESULTS

Statistically relevant changes in the glucose and free fatty acids levels in sportsmen's saliva after physical activity were not observed. Change in enzymes activity tended to increase though carried no significance statistics-wise. The research revealed statistically relevant decrease in all the BChL markers in comparison with a quiet state. Thus, S and I_{max} indicators significantly decreased by 22,3% and 14,7%. Despite a lower potential capacity to free-radical oxidation TC and SB presence in oral fluid being in the post-physical-load state is statistically significantly higher by 40,1% and 89,2%.

CONCLUSION

The saliva can be used as a highly informative biological marker of the non-invasive research when assessing a number of biochemical indicators in the practice of sports medicine.

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EVALUATION OF TRANSFERABILITY OF hGH AND IGF1 REFERENCE RANGES, CALCULATED ON A NORMAL HEALTHY POPULATION, TO ELITE FEMALE VOLLEYBALL PLAYERS

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Background. Peak concentrations of hGH/IGF-1 axis hormones increase immediately after exercise in athletes and a strong positive correlation between hGH levels and intensity of training has been demonstrated. Moreover, hGH levels are significantly higher in elite athletes than in non elite athletes and sedentary people. The wide range of IGF-1 levels between athletes reflects the inter-individual variability of the marker, without difference between athletes younger and older than 18 years in both genders. **Aim.** To assess the transferability of basal serum hGH and IGF1 reference ranges used in our laboratory to female elite volleyball players. **Methodology.** A longitudinal, retrospective, observational clinical trial enrolled 58 female elite volleyball players belonging to the same team. Their state of health was evaluated 3/4 times per sportive season, from 2013 to 2016. A blood sample was collected during each physical examination after an overnight fast (36 hours of rest) for routine hematological and hormonal laboratory test, including hGH and IGF1. The robust method was applied to the calculation of the 2.5 and 97.5 percentiles. Laboratory tests: hGH and IGF1 levels were measured with two immunoassays performed on two fully automated platforms, the DXI 600 (Beckman Coulter Inc, USA) and the Liaison XL (Diasorin S.p.a. Italy). **Results.** Number of reference measures= 132. Mean age 23,36 +/- 5.7 years, median of age 22.8 years. Mean value of hGH= 3.05 ng/ml +/- 0.99, median value= 4.47 ng/ml. Percentage of data exceeding the laboratory reference range (0.01 – 3.6 ng/ml)= 23.50%; Reference range [ratio calculated/ suggested] calculated on players population: 0.06 [6] - 31.08 [8.6] ng/ml. Mean value of IGF1= 288.98 ng/ml +/- 97.2, median value= 265.6 ng/ml. Percentage of data exceeding the laboratory reference range (109 – 293 ng/ml)= 39.4%; Reference range [ratio calculated/ suggested] calculated on players population: 146 [1.3] - 541 [1.8] ng/ml. **Conclusion.** Here we found that, as regards the hormonal status, female volleyball players represent a population that differs from the healthy population of female subjects. As consequence, the laboratory reference ranges are inadequate to the assessment of the state of health in professional athletes. The establishment of proper reference ranges for hematological parameters which are known to increase accordingly to physical and psychological stress should be considered.

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TROPONIN-I, GALECTIN-3 AND NTproBNP IN HALF-MARATHON RUNNERS BEFORE AND AFTER A RACE

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INTRODUCTION: Troponins (cTnI and cTnT), NTproBNP and galectin-3 (GAL) can mirror cardiomyocyte injury and stretch, and fibrosis, which represent the most important and prevalent injuries to myocardial tissue. However, the significance of these biomarkers in parapsycho-physiologic states, as endurance exercise, is less known.

AIM: to evaluate the trend of cTnI, NTproBNP and GAL in trained runners before an half-marathon run and a 48-h recovery period.

METHODS: Trained runners (n=18, 15 males, 46±6 years) participated to the half-marathon run with serial evaluation of cTnI and GAL (Architect, Abbott), cTnT and NTproBNP (Cobas e411, Roche) at rest, immediately post-run and at 24 and 48 h post-exercise.

RESULTS: Concentration of cTnT (4±2, 12±7, 6±3, 5±2 ng/L, p<0.01), NTproBNP (32±30, 58±47, 50±43, 31±25 ng/mL, p<0.001) and GAL (12±4, 18±5, 12±3, 14±7 ng/mL, p<0.001) transiently increased after post-race as compared with baseline values, but normalized at 24h (GAL and cTnT) or 48 h (NTproBNP). Instead, cTnI values did not significantly vary (10±16, 14±13, 14±17, 8±11 ng/L). The frequency of values exceeding the diagnostic threshold at baseline and after the race did not differ for cTnI (chi2=1.1, p=ns), and NTproBNP (chi2=6, p=ns), but significantly increased for cTnT (chi2=23, p<0.001) and GAL (chi2=6.3, p<0.05).

CONCLUSION: The transient increase in cTnT, NTproBNP and GAL levels may suggest a temporary stress on the myocyte. However, being the increase in these biomarkers moderate and reversible, although more slowly for NTproBNP, it may represent a physiological response to acute exercise.