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Laboratory medicine in sports

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Role of biocemical diagnostics in sports

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Introduction: Intensive exercising may significantly damage muscles which is reflected in pain, fatigue and the increase of muscle proteins concentrations in blood such are creatinin kinase (CK), lactic dehydrogenase (LD), myoglobin (MB) and other biochemical parameters including urea serum concentration (SU). Biochemical markers vary with age, sex, race, muscle mass, physical activity and climate conditions. They also assist us in determining the limit between the capacity for adaptation to given training process which results in supercomepensation and in condition of overtraining (OT), in the case of load that exceeds the physiologic potential of regeneration. Concerning the problem of diagnosis and explanation of the symptoms of overtraining, markers that can apply reliably and with sufficient sensitivity and simplicity of interpretation in the praxis are sought. It is critical to take into account difference among individuals and groups that could hamper the interpretation.

The most frequently used markers: The most frequently used biomarkers that provide us with the information on physical activity and on the amount of load through exercise are CK, SU and LD. Level of serum retaining kinas has been measured and interpreted for years as part of different scientific and professional investigations and presents one of basic parameters for determining the level of muscle damage. It reaches maximal concentration of the fourth day of exercising which depends on the type of exercise and the nature of stress triggered by exercise but also on individual characteristics.

The level of serum urea presents marker of nitric compounds metabolism and is the principle chemical substance in the urine of mammals. It is thus possible to draw a parallel between the increases of serum urea concentration on increased degradations of proteins. Significant fall of serum amino acid levels occurs after sixty to seventy minutes of exercising with the increase of urea and free tyrosine and these changes have high correlation with the duration and intensity of.

LD changes are important index of well-trained sportsmen and their capability to withstand the pace and force during strain in the training process. The level of LD is a good index of exercise intensity and marker of metabolic exchange in tissues whose concentration in serum is dependent of cell damage.

Conclusion: There is not a single, unique parameter that would provide enough valuable information for the estimation of the quality of exercising, amount of load and identification of overtraining. Delayed measurement of biomarkers is far from ideal, but it is obvious that the amount of stress/load in training is the most important factor for the development of state of overtraining. Daily body weight control, diet, biochemical indices values and the input of water should be known and standardized before measurements. For the most of parameters determination of basal levels are needed in specific populations for more accurate interpretation and evaluation of results. The sampling process itself should be under the most strict conditions of standardization by repeating measurement at least every third day. Dependence of mentioned parameters (SU, CK, LD) on exercise intensity varies among individuals and without these additional measurements and subpopulation evaluations it is difficult to come to conclusions with certainty as well as to come to conclusions on causative correlations of training load and dynamic in biochemical parameters.

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Common sports injuries

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Sports injuries are injuries that occur in athletic activities and can be broadly classified as either traumatic or overuse injuries. Traumatic injuries because of the dynamic and high collision are nature

of some sports. Overuse injuries cause wear and tear on the body, particularly on joints subjected to repeated activity.

At every age, competitive and recreational athletes sustain a wide variety of soft tissue, bone, ligament, tendon and nerve injuries, caused by direct trauma or repetitive stress. Different sports are associated with different patterns and types of injuries, whereas age, gender and type of activity influence the prevalence of injuries. Sports trauma commonly affects joints of the extremities or the spine.

The hip, knee and ankle are at risk of developing osteoarthritis (OA) after injury or in the presence of malalignment, especially in association with high impact sport. Spine pathologies are associated more commonly with certain sports. Upper extremity syndromes caused by a single stress or by repetitive micro-trauma occur in a variety of sports.

Random control trials expose some subjects, but not others, to an intervention. This is more clinical in nature and not typically appropriate for the study of injury patterns. Cohort studies monitor both injured and non-injured athletes, thereby providing results on the effects of participation. Case-control studies monitor only those athletes who suffered an injury. The Ideal study would be Cohort design conducted over several teams, with longitudinal prospective data collection and one recorder where possible, as well as uniformity of injury definition across sports so comparisons between studies can be made accurately.

Physical injury is an inherent risk in sports participation and, to a certain extent, must be considered an inevitable cost of athletic training and competition. Injury may lead to incomplete recovery and residual symptoms, drop out from sports, and can cause joint degeneration in the long term.

Advances in arthroscopic techniques allow operative management of most intraarticular post-traumatic pathologies in the lower and upper limb joints, but long-term outcomes are not available yet. It is important to balance the negative effects of sports injuries with the many benefits that a serious commitment to sport brings.

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Determination of sample size and number of study groups in sport studies

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Sample size refers to the number (N) of subjects in a sample, and minimal sample size is defined as the minimal number of subjects *per* study group(s) needed to support a statistical significance of association or difference with appropriate power. Sample size should be determined before the start of the study by using power analysis. In statistical data analysis, the power of a test is defined as a probability of finding the statistical significance of association or difference between the variables in study groups (namely, an effect) if the same effect is present in the population (rough statistical vocabulary defines power as probability to reject the null-hypothesis when alternative one is true). Effect describes the relationship, and effect size measures its quantity (e.g., the difference in blood glucose levels of 4.5 ± 1.2 and 5.6 ± 1.2 mmol/L between two groups has an effect size of 1.1 mmol/L). While effect size in typical biomedical research is usually presented using non-standardized measures (an absolute effect size, such as the exact difference between the two groups in the previous example, and Pearson, Spearman or similar correlation coefficients as absolute association measures), the use of standardized measures of effect (e.g., Cohen's d) or effect size categories (small, medium or large effect) is most often suggested in sport and social sciences. In a typical experiment comparing measurements performed in two or more study groups, the effect size is determined using a 5% significance level (alpha error = 0.05) and at least 80% power (beta error = 0.2; power defined as 1–beta). It should be noted that the significance of an association/difference (alpha in hypothesis testing) is not the same as the strength (beta); therefore, both values should be known along with the expected effect size to calculate a

minimal sample size. These calculations may be performed using free Internet sources (e.g., *http://www.stat.ubc.ca/~rollin/stats/ssize/*).

The number of study groups does not depend on the study type and research field and determines the choice of appropriate statistical test. For example, if high and low glucose groups are compared with a control group, there are three groups in the study (for example, ANOVA test is required) rather than two, as might be reported (control vs. study groups, compared using *t*-test; and low vs. high glucose group, compared using another t-test).

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Biochemical markers of muscle damage in active training

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Competitive demands on professional athletes affect musculosceletal system which can be damaged as a consequence of mechanical injury as well as some metabolic factors. Mechanical injury represents a direct damage to the muscle tissue, while an example of an indirect damage is limited vasodilatation and ischemia due to hypokaliemia subsequent to excessive sweating. Regular and moderate exercise provides beneficial health effects, but maximal exercise shows some adverse effects. Increased exercise intensity results in "blebs" creation on the myocytes plasma membrane surface, and release of cytoplasmatic contents without necrosis. In intensive training or in exercise of untrained people, when ischemia lasts longer, the blebs grow and cell necrosis occurs.

The most useful markers of direct muscle damage are markers of muscle tissue functional status like creatine kinase (CK), lactate dehydrogenase (LD), myoglobin, aldolase, and carbonic anhidrase III (CA-III).

Usual increase of markers after exercise are up to 4-fold for CK and myoglobin, 2-fold for LD and AST while troponin shows a minimal increase.

Daily training may result in increase in serum CK apparent as a higher baseline value in athletes. After exercise, CK and LD increase is higher in untrained subjects than in trained ones. The highest post-exercise serum enzyme activities are found after long competitive excercise like marathon. AST levels decrease within 24 hours, while level of CK and LD varies depending on the training type and rest protocols included in preparing for the next competition.

Aldolase is used to evaluate muscle adaptation to training together with CK, especially because CK has no significant linear correlation with muscle functions following exercise.

In training, myoglobin increases within 30 minutes and remains increased for 5 days so it is a good marker for monitoring the effectiveness of workload on muscle.

Troponin T is released after exercise, and intense exercise shows bigger increase than prolonged exercise at moderate intensity.

CA-III is a very good marker, present in skeletal muscle and not in myocardium, and its decrease is more rapid than for all mentioned enzymes.

Markers of indirect damage are linked to oxidative stress (malondialdehyde, reduced glutathione, superoxide dismutase) and are not determined routinely.

Urinary markers complete the estimation of muscle stress after exercise as myoglobinuria and haemoglobinuria appear 24-48 hours after excercise and usually disappear after 72 hours of rest.

Using blood and urinary markers for assessing the muscle damage gives us a better understanding of beneficial and adverse effects of training.

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Inflammatory markers in monitoring training and sports injuries

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Every exercise lasting at least six minutes causes an acute phase inflammatory response.

Exercise induced inflammatory response is mediated by an increase of pro-inflammatory cytokine IL-6. Levels of IL-6 increase up to 100-folds during exercise and decrease during post-exercise period. Inflammatory response is also characterized by an increase of anti-inflammatory cytokines, cytokine inhibitors IL-1 receptor antagonist (IL-1RA) and soluble TNF receptor (sTNF-R). Summarized, exercise induces increase of IL-6 followed by an increase of IL-1RA and IL-10 and causes balance between pro- and anti-inflammatory exercise responses.

Furthermore, recent studies have shown that IL-6 from miocytes inhibits endotoxin induced TNF-alpha production.

The effects of different types and duration of exercise on inflammatory markers were examined in numerous studies.

Reported serum CRP levels immediately after training were significantly higher in relation to baseline levels before the start of the exercise. The effect of exercise on CRP depended on the type of exercise but the levels after exercise were significantly lower in professional athletes than in untrained control subjects (except in soccer players). Furthermore, CRP levels in circulation could be a useful serum marker for estimation of athletes' physical condition during the training program as well as the balance between physical capacity and intensity of exercise. Intensive aerobic exercise despite of CRP results in significant increase of sedimentation rate and leukocyte count with neutrophile predomination. Elevated levels of fibrinogen and IL-6 were also reported. All markers tend to decrease even below baseline levels during resting period.

Stated above is the proof that regular physical activity reduces the risk of diseases associated with chronic systemic inflammation.

Regular and moderate exercise therefore provide beneficial health effects, but exhausting exercise may cause overtrain syndrome and musculoskeletal sports injuries which impair homeostatic inflammatory responses to exercise.

Overtrained athletes have low blood leukocyte count with depletion of neutrophils. CBC monitoring could provide a useful screening tool to determine when exercise is becoming too stressful. Exercise causes a large release of neutrophils from the bone marrow and too too intense, training program could deplete the bone marrow of its reserves of mature neutrophils. This is likely to make overtrained individuals more susceptible to infection.

The homeostasis between beneficial and harmful inflammation that inhibits muscle regeneration after injury may depend on the local environment and presence of oxidative stress.

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Oxidative status – laboratory monitoring in athletes

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During the past three decades, the field of redox biology of exercise has witnessed many remarkable developments and our knowledge about the biological implications of exercise-induced oxidative stress has expanded rapidly.

Oxidative stress is defined as "a disturbance in the prooxidant/antioxidant balance in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage". A common approach to assess exercise-induced oxidative stress in biological systems involves the measure-

ment of the increase or decrease in a redox-sensitive molecule that responds to oxidative stress.

In general, reliable laboratory markers of exercise-induced oxidative stress possess the following qualities: 1) chemically unique and detectable, 2) increased or decreased during periods of oxidative stress, 3) possess relatively long half-lives, and 4) not impacted by other cellular processes.

Thus exercise alters redox homeostasis across body fluids, organs and tissues it is often characterized by the following parameters: 1) increase in the formation of radicals and other

oxidants, 2) decrease in small-molecular-weight and/or lipid-soluble antioxidants, 3) disturbance in cellular redox balance, and 4) oxidative damage to cellular components. Hence, laboratory biomarkers of exercise-induced oxidative stress typically fall into one of four categories.

Indeed, during exercise reactive oxygen species attack of lipids, protein, or DNA generates uniquely oxidized biomolecules that can be used as "fingerprints" to detect oxidative stress in cells. Common measures of bio-oxidation include the measurement of protein carbonyls as an indicator of protein oxidation; assessment of isoprostanes, malondialdehyde, and 4-hydroxyl- 2-nonenol as signs of lipid peroxidation; and evaluation of DNA oxidation by assaying the levels of the oxidized base, 8-hydroxy-2'-deoxyguanosine.

With the necessary modifications, exercise is capable of inducing redox homeostasis alterations in all fluids, cells, tissues and organs studied so far. More importantly, exercise-induced oxidative stress is not specifically associated with a particular type of exercise, tissue or species. Rather, oxidative stress constitutes a ubiquitous fundamental biological response to the alteration of redox homeostasis imposed by exercise. Indeed, it is now appreciated that while high levels of free radicals can damage cellular components, low-to-moderate levels of oxidants play multiple regulatory roles in cells such as the control of gene expression, regulation of cell signaling pathways, and modulation of skeletal muscle force production.

Redox biology of exercise, by nature multidisciplinary with integrative and comparative approaches, has been recognized as one of the key themes that will drive the exercise science in the future.

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