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Biomarkers of physical activity and exercise

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Abstract

Traditionally, biomarkers have been of interest in sports in order to measure performance, progress in training and for identifying overtraining. During the last years, growing interest is set on biomarkers aiming at evaluating health-related aspects which can be modulated by regular physical activity and sport. The value or concentration of a biomarker depends on many factors, as the training status of the subject, the degree of fatigue and the type, intensity and duration of exercise, apart from age and sex. Most of the biomarkers are measured in blood, urine and saliva. One of the main limitations for biochemical biomarkers is that reference values for blood concentration of biomarkers specifically adapted to physically active people and athletes are lacking. Concentrations can differ widely from normal reference ranges. Therefore, it is important to adapt reference values as much as possible and to control each subject regularly, in order to establish his/her own reference scale.

Other useful biomarkers are body composition (specifically muscle mass, fat mass, weight), physical fitness (cardiovascular capacity, strength, agility, flexibility), heart rate and blood pressure. Depending on the aim, one or several biomarkers should be measured. It may differ if it is for research purpose, for the follow up of training or to prevent risks. For this review, we will get deeper into the biomarkers used to identify the degree of physical fitness, chronic stress, overtraining, cardiovascular risk, oxidative stress and inflammation.

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BIOMARCADORES DE LA ACTIVIDAD FÍSICA Y DEL DEPORTE

Resumen

Tradicionalmente, los biomarcadores han sido de interés en las ciencias del deporte para medir el rendimiento, el progreso en el entrenamiento y para identificar el sobreentrenamiento. Durante los últimos años, cada vez hay mayor interés en evaluar los efectos relacionados con la salud que se producen en el organismo debidos a una actividad física regular y al deporte. El valor o la concentración de un biomarcador depende de muchos factores, como el grado de entrenamiento, el grado de fatiga y del tipo, la intensidad y la duración del ejercicio, aparte de la edad y del sexo. La mayor parte de los biomarcadores se miden en sangre, orina y saliva. Una de las principales limitaciones que presentan los biomarcadores bioquímicos es la falta de valores de referencia adaptados específicamente para deportistas y personas físicamente activas. Las concentraciones pueden variar considerablemente de los valores de referencia normales. Por lo tanto, es importante adaptar los valores de referencia siempre y cuando sea posible y controlar a cada sujeto regularmente, con el fin de establecer su propia escala de referencia.

Otros biomarcadores útiles son la composición corporal (específicamente masa muscular, masa grasa, peso), la condición física (capacidad cardiorrespiratoria, fuerza, agilidad, flexibilidad), frecuencia cardíaca y presión arterial. Dependiendo de la finalidad, será conveniente analizar uno o varios biomarcadores. Para esta revisión, profundizaremos en los biomarcadores que se emplean para evaluar condición física, fatiga crónica, sobreentrenamiento, riesgo cardiovascular, estrés oxidativo e inflamación.

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Palabras clave: *Condición física. Salud. Rendimiento. Biomarcador. Cortisol.*

Background

A biomarker (biological marker) is a measurable product or substance used as an indicator of the biological state, to objectively determine the body's physiological or pathological processes. In sport, biomarkers are key parameters to assess the impact of exercise on different systems, tissues and organs[1]. Therefore, we can estimate parameters for assessing the degree of fitness, muscle damage, hydration/dehydration, inflammation, oxidative damage, fatigue, overtraining, etc, which facilitate the evaluation of the response of the human body at the different levels of physical activity or training being carried out. Biomarkers can be used to measure the impact of training on the long term or the acute effect of exercise. The value or concentration of a biomarker depends on many factors, as the training status of the subject, the degree of fatigue and the type and duration of exercise, apart from age and gender, among others. Climate can also play a role, mainly temperature, humidity and wind speed. Exercise can be classified according to the duration as the following: around 20s (demand for anaerobic energy up to 90%), exercise lasting 20s to 1 min (aerobic and anaerobic energy) or exercises that extend over 1 min (aerobic energy more than 50%). Intensity is also an influencing factor on biomarker concentration. Most of the biomarkers are measured in blood, urine and saliva. In elite sports, non-invasive samples like urine and saliva have a preference. Other useful biomarkers are body composition (specifically muscle mass, fat mass, weight), physical fitness (cardiovascular capacity, strength, agility, flexibility), heart rate and blood pressure.

Depending on the aim, one or several biomarkers should be measured. It may differ if it is for research purpose or for the follow up of training.

Interest

Traditionally, biomarkers have been of interest in sports in order to measure performance, progress in training and for identifying overtraining[2]. During the last years, growing interest is set on biomarkers aiming at evaluating health-related aspects which can be modulated by regular physical activity and sport[3]. Additionally, as promotion of physical activity is supported by most of public health authorities, the evaluation of the biological response to exercise is also a need in the amateur athlete. For this review, we will get deeper into the biomarkers used to identify the degree of physical fitness, chronic stress, overtraining, cardiovascular risk, oxidative stress and inflammation.

Controversy

Controversy exists regarding if biochemical biomarkers are really useful for the monitoring of training progress and adaptation, and some trainers do not include biomarkers in their season planning[2]. Less controversy exists in regard to identifying risk situation, like overtraining, nutrient deficiencies, etc. Unfortunately, there is no gold standard for monitoring most of the processes; therefore, the analysis of several biomarkers is recommended.

Limitations

Reference values for blood concentration of biomarkers specifically adapted to physically active people and athletes are lacking. Therefore, for most of the biomarkers measured routinely in the laboratory, reagent manufactures' reference values are used. In the authors' opinion, this can lead to misclassification or wrong interpretation of the results. Our research group is currently working on reference values specifically adapted to athletes of different sports (E. Diaz, non-published data). It is important to bear in mind that highly trained people can have concentrations of biomarkers which would be pathological in non-trained people, even in routine hematology and biochemistry parameter. Therefore, it is important to adapt reference values as much as possible and to control each subject regularly, in order to establish his/her own reference scale.

Current state and perspective

Markers of Physical fitness

Physical fitness is a set of attributes that people have or achieve and it is referred to the capacity of a person to meet the varied physical demands of their activities of daily living and/or sport practice without experimenting fatigue. Physical fitness is not only a predictor of morbidity and mortality for cardiovascular disease[4]. It is nowadays considered one of the most important health markers because it integrates most of the body functions (skeletomuscular, cardiorespiratory, hematocirculatory, psychoneurological and endocrine-metabolic) involved in the performance of daily physical activity and/or physical exercise[3]. Accordingly, when physical fitness is tested, the functional status of all these systems is actually being checked. Physical fitness is in part genetically determined, but it can also be greatly influenced by environmental factors, such as physical exercise, sedentary habits, harmful lifestyles, etc.

Physical fitness components can be differentiated between a health-related and a performance-related approach that pertain more to athletic ability, being the health-related components more important to public health than are the components related to athletic ability[5]. According to Bouchard et al.[1], the health-related fitness components of a person include a cardiorespiratory component (e.g. maximal aerobic power or heart function); a muscular component (e.g. strength, power or muscular endurance); a motor component (e.g. agility, balance and co-ordination); a morphological component (e.g. body composition, bone density or flexibility); and a metabolic component (e.g. glucose tolerance, lipid and lipoprotein metabolism and substrate oxidation characteristics)[6]. There are numerous tests for measuring physical fitness, ranging from self-assessment techniques over simple field test to more sophisticated laboratory tests. One may choose to employ a different measure depending on the specific objectives of the investigation and cost constraints.

The World Health Organization (WHO), considered the maximal oxygen consumption (VO2max) as the single best indicator of cardiorespiratory fitness[7] and it can be estimated using a maximal or sub-maximal test (e.g. treadmill or bicycle tests, 2 km. walk test, 20-m shuttle run, 6 min. walk test). According to muscular component, the handgrip strength test is one of the most used tests for assessing muscular fitness being a strong predictor of morbidity and mortality[8]. For assessing power strength or muscular endurance jump, dynamic sit-up and bent-arm hand tests have been used with young, adult and older people. Agility, balance, speed or co-ordination is included in the motor component. Agility is a combination of speed, balance, power and coordination[3]. Some tests used to measure motor component are 30-m sprint test and 4 x 10-m shuttle run test for young people and 30-m walk test and 8-foot-and-go, for older adults. For measuring static balance, single leg balance, with or without open eyes, is a good alternative test. Flexibility is a morphological component; chair sit-and-reach test and back scratch test are two validated tests for measuring this capacity (Fig. 1).

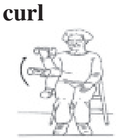
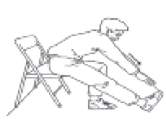
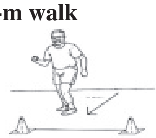
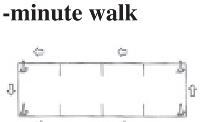
Test item	Assessment category	Description
One leg [] 	Static balance	Number of seconds during which the participant kept balance on one leg. The maximum time allowed for the test was 60 s.
Chair stand 	Lower body s strength	Number of full stands in 30 seconds with arms folded across chest.
Arm curl 	Upper body strength	Number of biceps curls in 30 seconds holding hand weight.
Chair sit -and -reach 	Lower body flexibility	From sitting position at front of chair, with leg extended and hands reaching toward toes, number of centimetres from extended fingers to tip of toe.
Back scratch 	Upper body flexibility	With one hand reaching over shoulder and one up middle of back, number of centimetres between extended middle fingers.
8 -foot -and -go 	Agility/Dynamic balance	Number of seconds required to rise from seated position, walk 2.45 metres, turn, and return to seated position on chair.
30 -m walk 	Walking speed	Number of seconds required to walk 30 metres.
6 -minute walk 	Aerobic capacity	Number of meters that can be walked in 6 minutes around a 46 meters course.

Fig. 1.—Some tests used for the evaluation of physical fitness in elderly (modified from 9).

For use in clinical practice, reference values for men and women in all age groups are needed. Some of the most important studies that have provided reference values are AVENA and HELENA[3] studies, for Spanish and European adolescents, respectively, and the senior fitness test by Rikli & Jones and the EXERNET study[9], for Americans and Spanish elderly people, respectively.

Markers of chronic stress and fatigue

Cortisol

Cortisol is a steroid hormone synthesized from cholesterol by enzymes of the cytochrome P450 located at adrenal cortex. It's expressed following a circadian rhythm: at midnight, cortisol blood levels are very low (sometimes even undetectable) and they increase overnight to reach a peak in the morning. This rhythm is regulated by the main circadian oscillator in the suprachiasmatic nucleus which is located in the hypothalamus[10]. Cortisol counters insulin effect by promoting high blood glucose levels via stimulation of gluconeogenesis, the metabolic pathway that synthesizes glucose from oxaloacetate. The presence of cortisol triggers the expression of enzymes critical for gluconeogenesis, facilitating this increase in glucose production. Conversely, it also stimulates glycogen synthesis in the liver, which decreases net blood sugar levels. Thus, cortisol carefully regulates the level of glucose circulating through the bloodstream: when blood glucose has been depleted (for example during fasting), cortisol ensures a glucose basal concentration by activating gluconeogenesis[11].

Cortisol shows other metabolic functions. Among others, it allows a correct pH regulation of extracellular liquid: when cells loose too much sodium, it accelerates the rate of potassium excretion. Therefore, cortisol regulates the action of cellular sodium-potassium pumps to reach an ion equilibrium after a destabilizing event[11]. Cortisol's weakening effects on the immune response have also been well documented. T-lymphocyte cells (T-cells) are activated by cytokine molecules (interleukins) via a signaling pathway. Cortisol prevents specific T-cells receptors to recognize interleukin signals and reducing proliferation of T-cells, which provokes a decrease of inflammation course. In the same way, it reduces inflammation due to inhibition of histamine secretion. Cortisol's ability to prevent the immune response can render people who suffer from chronic stress highly vulnerable to infection[12].

While it is important for adrenal glands to secret more cortisol in response to psychological or physical stress, it is also fundamental that cortisol levels return to normal values following a stressful event. Unfortunately, in some athletes the stress response to an intensive exercise is activated so often that the metabolic pathways do not always have a chance to return to a normal situation. This can lead to health problems, resulting, among others, in chronic stress and fatigue.

Training load as measured by the session-RPE (Rating of Perceived Exertion) is a subjective method of quantifying the load placed on an athlete. Measured session-RPE in 8 young elite middle-distance runners for 8 weeks, showed that this indicator of training load was able to detect states of overreaching[13]. In another way, measurement of the countermovement jump (CMJ) score can be used as an indicator of neuromuscular performance and therefore it has been used to assess fatigue in different kinds of athletes. Finally, salivary cortisol correlates with both physical magnitudes[14]. Thus, the measurement of session-RPE, CMJ score and salivary cortisol is used to monitor the training process in different kinds of athletes. Post-exercise salivary cortisol responses were significantly different depending on the intensity. For example, immediately after a high intensity acute resistance exercise, salivary cortisol showed a significant elevation of 97% from baseline values, while there was no difference when the intensity of exercise was very low[15]. In addition to the intensity, another factor that may affect the salivary cortisol response is the training status of the subjects[16]. Highly-trained strength athletes show an inverse and significant correlation with neuromuscular performance. Kraemer et al. [17] through their study on cortisol and performance of a group of highly-trained soccer players throughout a season, concluded that athletes starting the season with elevated cortisol values may experience significant reductions on performance during the season. Similar results have been obtained with middle and long distance runners[18]. Individuals presenting higher long-term salivary cortisol levels showed a significant tendency to be those with lower CMJ scores throughout the season. However, when correlation was studied in a shorter period of time, a significant positive trend was observed between runners with higher weekly salivary cortisol concentrations with higher CMJ scores. Further studies are needed to explain these opposite tendencies depending of measure time.

Testosterone

This steroid hormone belonging to the group of androgens in the body facilitates increased muscle mass and strength, increased combativeness and aggression of athletes and allows greater reduction of muscle fat. Reference ranges are 300 - 1000 ng/dL for men and 15-70 ng/dL for women. A disproportionate rise in the physiological stress response induces an increase in cortisol secretion which could in turn inhibit the synthesis of testosterone. The cortisol/testosterone ratio is an index used to measure chronic fatigue in athletes[16].

Markers of overtraining

Lactate

Muscles always produce lactate, even at rest (0.8 – 1.5 mmol/L), but lactate increases incrementally with exercise intensity. At a certain intensity lactate increases exponentially, this is called the lactate threshold, which occurs on average at a blood lactate concentration of 4.0 mmol/L. Fatigue onset appears fast above the lactate threshold limit, while efforts just below this limit can be sustained for hours when athletes are well trained. This training allows also raising the lactate threshold to the highest genetic potential of each subject. However, to perform too much training at or above the lactate threshold can result in overtraining. Thus, blood lactate measurement is used to determine not only the lactate threshold, but also the correct intensity of the exercise and the time needed for recovery. Lactate testing is used all over the world by researchers and athletic coaches. It can be considered as the current gold standard for determining exercise intensity and for determining whether or not training is producing the desired physiological effect. Briefly, muscle contraction starts on an electrical impulse from the brain, which is transmitted to muscle cells by means of the acetylcholine liberated at the motor neuron synapses. This produces a change in the membrane potential due to the leak out of potassium ions to the extracellular space, allowing calcium ions to be released from the endoplasmic reticulum and finally to trigger contraction of the muscle fiber. But during high-intensity or long-time exercises, potassium ions continuously leak out of the muscle cell into the extracellular space, causing a depolarizing effect in the membrane, as the charge difference between inside and outside cell decreases. As a consequence, electrical currents have a harder time getting in and muscle contractions become weaker. Recent studies have shown that far from causing fatigue in the exercising muscle, lactate production actually prevents fatigue by counteracting the effects of depolarization produced by the potassium ions outflow[19].

Creatine (phospho) kinase (CK or CPK)

CK is used as a marker of muscle fiber damage. Blood concentrations increase with increasing exercise intensity and duration. There is an adaptation due to training, enabling the levels in trained people to rise less than in sedentary people. Elevated baseline values indicate trauma or overtraining and its concentration can be used to monitor activity around athletes who have got a muscle injury[20] (Table I).

Table I
Training status depending on creatine kinase concentrations

CK concentration	*Interpretation*
200UI	Training adaptation
200-250UI	Elevated training levels
>300UI	Possible overtraining and muscle damage

Creatinine

This metabolite is an end product of muscle metabolism. It originates from muscle creatine degradation which in turn is produced by hydrolysis of creatine phosphate, by the action of creatine phosphate kinase (CPK). Creatinine clearance in the human body occurs almost exclusively by glomerular filtration, which is an important indicator of renal function. Renal excretion, unlike urea, does not depend on diuresis. Concentrations are substantially constant in each individual independent of diet, muscle mass being the main determinant. Commonly, creatinine is measured to assess whether renal function is adequate or not. In sports medicine, creatinine is typically used to assess the overall health of the athlete but normal values based on the normal population are not adequate for athletes. Normal references range from 0.7-1.3 mg/dl in adult men. In athletes, levels are usually high, depending on training, time of the season, which can induce changes in creatinine levels due to changes in the homeostasis of the body, which can lead to errors in biochemical and hematological parameters[2]. No specific references have been defined for athletes. Therefore, most commonly elevated creatinine is an indicator of a high degree of training or overtraining rather than a case of renal pathology. Creatinine concentration should be taken with caution, as it can be up to 1.4 mg/dl without suffering from renal disease. The interpretation of creatinine values should be done individually, taking into account gender, age and weight of the athlete.

Ammonia

In athletes, the accumulation of ammonia in the blood is dependant on the effort intensity. During physical exercise, the two main mechanisms by which ammonia accumulates are resynthesis of ATP from the breakdown of phosphocreatine (PC) and the deamination of amino acids. Rising ammonia is related to fast twitch muscle fibers. Therefore, the analysis of the values of ammonia can serve both as a marker for this type of exercise and as a marker of intense muscular effort fibers. The normal range of ammonia is 15 to 45 μg/dL. Elevated blood ammonia levels indicate a physiological response in sprinters (purely anaerobic metabolism), while lower rates correspond to medium or long distance runners (predominantly aerobic metabolism)[21].

Lactate dehydrogenase (LDH)

LDH is a catalytic enzyme found in most tissues of the body, and specifically in heart, liver, kidneys, muscles, blood cells, brain and lungs. LDH plays an important role in anaerobic energy metabolism, reducing pyruvate to lactate at the end of the glycolysis. When there is muscle damage or muscle fiber destruction, LDH serum levels are significantly increased. In addition, LDH has a variety of isoenzymes, which are specific to different tissues, which provide additional information on the origin of muscle damage[20].

Uric acid

Uric acid is the end product of purine metabolism, which increases after intense exercise. Its concentration should be stable during the competition season. Elevated levels may be due to intense training, to high energy demands and small muscle damage as a result of overtraining. The increase may also be related to intake of purine-rich foods and food supplements, and body weight changes in athletes[2].

Urea

Urea is mostly formed in the liver as the waste product from the breakdown of proteins (amino acids). Normal blood urea concentrations for optimum training loads are 5-7 mmol/L. Very long training sessions cause an increase in the urea concentration in blood, liver, skeletal muscles, urine and sweat. It is used as a marker of protein catabolism. Thus, the measurement of urea allows to evaluate the degree of protein utilization as an energy substrate, the degree of effort in a competitive test session in particular, and the level of athlete's overtraining[21].

Markers of cardiovascular risk

Homocysteine

Homocysteine (Hcy) is an intermediate sulfhydryl-containing amino acid derived from methionine. In the methionine cycle, methionine converses to S-adenosyl methionine and to Hcy. Hyperhomocysteinemia (high Hcy blood levels) can be classified depending of total serum plasma Hcy concentration in three stages: moderate (for concentrations between 16 and 30 μM/L), intermediate (31–100 μM/L), and severe (for concentrations higher than 100 μM/L). Hyperhomocysteinemia can be the result of disturbed Hcy metabolism, principally when deficiencies in folic acid, vitamin B6, or vitamin B12 are present, as these vitamins are coenzymes of several regulating enzymes[22]. Hyperhomocysteinemia can be also caused by other factors independent of diet like genetic disorders in methionine and homocysteine metabolism, including mutations in cystathione b-synthase, methionine synthase and methylenetetrahydrofolate reductase (MTHFR)[21]. Increased Hcy levels are associated with several disorders, like cardio and cerebrovascular diseases[22] and neurodegenerative diseases that affect the central nervous system, such as epilepsy, stroke, Alzheimer's disease and dementia[23].

There are several proposed mechanisms to explain the toxicity of Hcy. For this review, we will only describe the two main ones. The first one is related to oxidative stress. Oxidation of the thiol terminal group of Hcy, when Hcy binds proteins (by forming a disulphide bridge), low-molecular plasma thiols or a second Hcy molecule, produce an increase of production of reactive species. Those free radicals induce the subsequent oxidation of proteins, lipids and nucleic acids[24] and can lead to the endothelial dysfunction and damage to the vessel wall, followed by platelet activation and thrombus formation. Homocysteinylation represent the second main mechanism of Hcy toxicity and it consists on modification of protein structure due to disulphide bond. Degree of the protein homocysteinylation increases with increased plasma Hcy and causes immune activation, autoimmune inflammatory response, cellular toxicity, cell death and enhanced protein degradation[22].

Because physical activity contributes to reduce cardiovascular risk factors and Hcy is one of such factors, theoretically Hcy could be used as biomarker of cardiovascular health when physical activity is performed. However, results obtained from several studies are contradictory and sometimes inconclusive, may be due to different kind of exercise, intensities, duration, with or without prior training, etc. A recent study carried-out by Iglesias-Gutierrez et al.[25] about serum Hcy concentration during an acute bout of exercise showed an increase at the beginning of exercise and a subsequent decrease at the end, the basal value being recovered 19 hours post-exercise. Due to this long period to reach again the initial Hcy levels, differences in timing of post-exercise sample collection could explain these discrepancies on Hcy levels variations after exercise described in the literature. An important point to be considered is the maximum Hcy concentration reached during exercise and how long high Hcy concentration persists in blood. In this study, authors did not observed any concentration above 15 μmol/L, the upper limit of Hcy normal range, beyond which it falls in hyperhomocysteinemia. However, this does not mean that the transient increase observed in Hcy is lacking of physiological relevance. A meta-analysis has shown that an average Hcy increase of 1.9 μmol/L was associated to a 16% higher risk of isquemic heart disease[26]. Nevertheless, this increase in cardiovascular disease risk observed by Wald et al.[26] refers to a sustained elevation of Hcy throughout lifespan, while Iglesias-Gutierrez et al.[25] refers to an elevation as response of an acute exercise.

In a recent review on effects of physical activity on Hcy levels, authors underscored that a high daily physical activity can help to control Hcy levels and thus reducing cardiovascular disease risk[27]. However, an intensive and acute exercise tends to increase Hcy blood levels[28]. The effect of aerobic training is more controversial: resistance training seems to reduce Hcy levels while intensive training increases them. Authors suggest to carry-out further studies to study changes in homocysteine induced by combined exercise programs (i.e., aerobic and resistance).

Cardiac troponin

Cardiac troponin consists of two protein complexes *(cTnI and cTnT)* which regulate muscle contractile function. They are present in skeletal and cardiac muscle. Increased concentration of the cardiac isoforms (TnI and TnT) indicates that there has been muscular heart damage. Therefore, both markers are useful parameters to assess a cardiac event. However, the increase after intense or prolonged exercise in the absence of cardiac symptoms, suggests muscle lesions, due to adaptation of training.

Markers of oxidative stress

Malondialdehyde (MDA) and protein carbonyls (PC)

Malondialdehyde (MDA) is a marker of oxidative degradation of the cell membrane caused by lipid peroxidation of unsaturated fatty acids. Protein carbonyls (PC) come from the oxidation of albumin or other serum proteins and are used as a marker of oxidative damage of proteins. Reference limits for PC are 0.30 to 0.36 nmol/mg[29]. PC and MDA are lower in trained individuals. An increase can be due to stress caused by increasing training loads. However, after adaptation to training, concentrations decrease and return to normal values.

Superoxide dismutase (SOD) and glutathione peroxidase (GSH)

These are antioxidant enzymes modulated by physical activity. Resistance training increases moderately enzymes activity[29].

Reactive oxygen species (ROS)

There is growing evidence that the continued presence of high concentrations of free radicals is capable of inducing antioxidant enzymes and other defense mechanisms. In this context, free radicals can be viewed as beneficial rather than as harmful, since they act as signals to improve the defenses when cells are exposed to high levels of ROS. This is mainly due to the regulation of endogenous antioxidant enzymes such as glutathione peroxidase, manganese superoxide dismutase (MnSOD), and γ-glutamylcysteine synthetase. Therefore, we could state that training increases the expression of antioxidant enzymes that in turn keep decreasing ROS levels. The stimulated high levels of ROS create more antioxidant enzymes, which do not contribute to the oxidation of the body's cells[29].

Markers of inflammation

C-reactive protein

C-reactive protein (CRP) originates in the liver. There are many stimuli that can cause an increase in CRP concentrations, such as infection, trauma, surgery, chronic inflammatory conditions, etc. In the field of sports, intense physical activity can cause an increase in CRP. However, continuous training causes a reduction of CRP levels compared to baseline. This is due to different mechanisms and processes taking place while the body is adapting to training (improved endothelial function, reduced inflammatory cytokine production, antioxidant effects, increased insulin sensitivity, etc). A higher CRP level after training indicates lack of adaptation or overtraining, probably due to oxidative stress (inflammation). However, after adaptation to training, values are normalized[30].

Interleukin-6

Interleukin-6 (IL-6) is considered an anti-inflammatory cytokine that regulates acute inflammatory response. Receptors are located in adipose tissue, skeletal muscle and liver. IL-6 increases lipolysis in adipose tissue and improves insulin sensitivity in the liver, increases glycogenolysis in skeletal muscle. Intense exercise training increases plasma concentrations of IL-6 up to 100 times, indicating the beneficial effect of physical exercise[30].

Leukocytes

The white blood cells are part of the immune system, produced in the bone marrow and lymphoid tissue. After being synthesized they are transported by the blood to the different parts of the body. The fundamental value of leukocytes is that they are specifically transported into areas where there is inflammation, to provide rapid and vigorous defense against any possible infectious agent. Exercise causes a transient leukocytosis, which magnitude is directly related to the intensity of the exercise: it is more pronounced in response to maximal exercise, and in untrained or poorly trained individuals. An increased leukocyte value due to exercise reaches again normal values within 24 hours.

Conclusions

Biomarkers are useful tools for assessing and monitoring health, training status and performance. As there is some controversy in the literature, depending on the process to be monitored, a combination of biomarkers could be useful. However, controversy exists regarding which parameters are most relevant for monitoring fatigue. The most researched and applied to muscle fatigue are cortisol, lactate and IL-6. Also gaining increasingly more importance is the measurement of ammonia, leukocytes and oxidative stress parameters. The biomarkers of muscle fatigue could be a prognostic tool to identifying subjects who are at increased risk of poor adaptation to training. Exercise, in particular, has a major influence on the most widely used inflammatory biomarkers, including C-reactive protein and interleukin-6. Additionally to biochemical biomarkers, the measurement of physical fitness components should be included in order to monitor progress and adaptation to training, as fitness is considered one of the most important markers of health.

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