BRIEF REVIEW

OPEN

BIOMARKERS IN SPORTS AND EXERCISE: TRACKING HEALTH, PERFORMANCE, AND RECOVERY IN ATHLETES

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ABSTRACT

Lee, EC, Fragala, MS, Kavouras, SA, Queen, RM, Pryor, JL, and Casa, DJ. Biomarkers in sports and exercise: tracking health, performance, and recovery in athletes. *J Strength Cond Res* 31 (10): 2920–2937, 2017—Biomarker discovery and validation is a critical aim of the medical and scientific community. Research into exercise and diet-related biomarkers aims to improve health, performance, and recovery in military personnel, athletes, and lay persons. Exercise physiology research has identified individual biomarkers for assessing health, performance, and recovery during exercise training. However, there are few recommendations for biomarker panels for tracking changes in individuals participating in physical activity and exercise training programs. Our approach was to review the current literature and recommend a collection of validated biomarkers in key categories of health, performance, and recovery that could be used for this purpose. We determined that a comprehensive performance set of biomarkers should include key markers of (a) nutrition and metabolic health, (b) hydration status, (c) muscle status, (d) endurance performance, (e) injury status and risk, and (f) inflammation. Our review will help coaches, clinical sport professionals, researchers, and athletes better understand how to comprehensively monitor physiologic changes, as they design training cycles that elicit maximal improvements in performance while minimizing overtraining and injury risk.

KEY WORDS hydration, muscle quality, endurance performance, injury prevention, inflammation

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31(10)/2920–2937

Journal of Strength and Conditioning Research

INTRODUCTION

Proteins, metabolites, electrolytes, and other small molecules may serve as biomarkers for athletes and recreationally active individuals. Advances in big data approaches to assessing health and performance of athletes suggest that using the newest technology with intrinsic data such as biochemical, hematological data can be powerful in identifying the balance between training and recovery in each unique individual. Indeed, many commercially available services are offering biochemical and genetic testing for athletes, and professional athletes are reportedly exploiting technology and biomarker testing to track performance and recovery during training. Numerous biomarkers may assess different aspects of health, sport performance, and recovery, but when tracking athletes, even useful biomarkers have limitations. Biomarker testing/analysis poses many challenges: (a) single biomarkers are not definitive for diagnosing broad physiological function such as "recovery" in sport, (b) sensitivity of single biomarkers to detect overtraining or injury risk is limited, (c) reference ranges for athletes and specific subgroups of athletes are not well defined, (d) interindividual variance in absolute values and relative changes in biomarkers, and (e) highly contextualized nature of biomarker testing and analysis.

A single measurement of a biomarker does not allow for precise determination of an individual's health status. For example, although the immune signaling molecule interleukin-6 (IL-6) is a cytokine that can indicate inflammation alone, it provides little diagnostic information about chronic inflammation during overtraining in an athlete; it has both pro inflammatory and anti-inflammatory roles and responds to many stimuli acutely and chronically. There seems to be evidence for the usefulness of IL-6 as a potential biomarker of overtraining. However, researchers agree that multiple cytokines should be measured together when attempting to detect chronic inflammation in athletes, and that other variables related to physiological/physical function and upstream stimuli for chronic inflammation should be measured simultaneously (80,114). Data on multiple inflammatory cytokines, endocrine markers of long-term dysregulation and overtraining

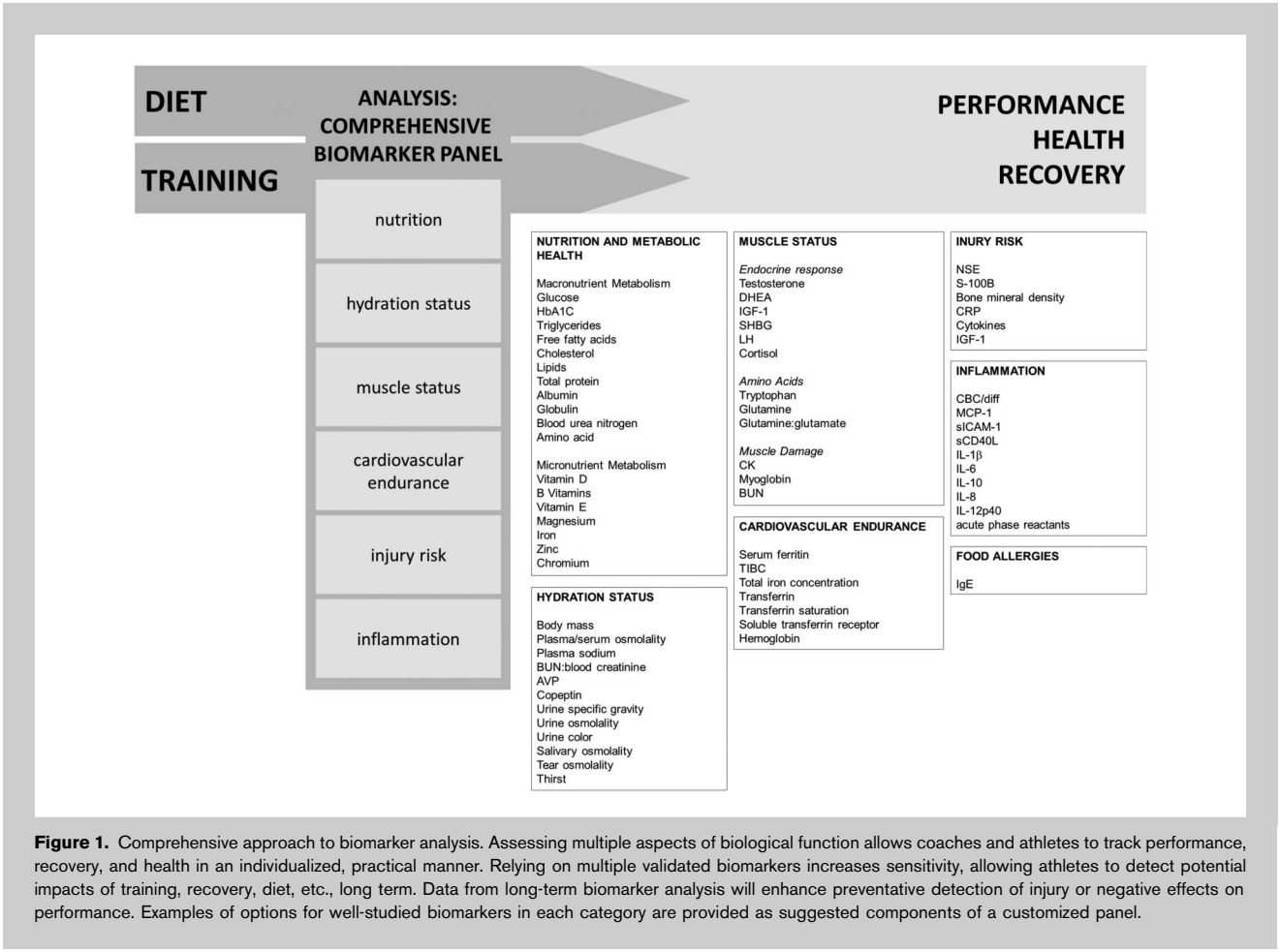

Figure 1. Comprehensive approach to biomarker analysis. Assessing multiple aspects of biological function allows coaches and athletes to track performance, recovery, and health in an individualized, practical manner. Relying on multiple validated biomarkers increases sensitivity, allowing athletes to detect potential impacts of training, recovery, diet, etc., long term. Data from long-term biomarker analysis will enhance preventative detection of injury or negative effects on performance. Examples of options for well-studied biomarkers in each category are provided as suggested components of a customized panel.

like testosterone and cortisol, and muscle damage markers like creatine kinase (CK) can be integrated to provide precise and accurate information about an athlete's health and overtraining status. Relying on a single marker to sensitively and precisely detect overtraining is overly simplistic given the pleiotropic nature of most biological markers. Figure 1 outlines markers of hydration state, nutrition/metabolic health, oxygen transport, muscle status, inflammation, injury risk, and food allergies that can be integrated to help athletes interpret their blood biomarker data to meaningful practical application.

Further complicating biomarker analysis is the fact that isolated or infrequent testing of biomarkers provides limited information. There are few athlete-specific reference ranges for most biomarkers, and this is in part because there is large interindividual variance in biomarker values, and that measurement of biomarkers can vary by context. Again considering the example of overtraining biomarkers, people generally exhibit high variability in serum/plasma cytokine levels and responsiveness (68,74,130), and athletes could exhibit greater rates of variability or different ranges of values (compared with average/sedentary individuals) entirely as they do for other markers such as muscle damage marker CK (86). Markers such as the many inflammatory cytokines are elevated after exercise in healthy individuals and return to baseline values within minutes to hours after exercise (126). Absolute values of cytokines in a one-time blood sample might be meaningful if values are elevated or decreased outside of the large range of interindividual variability observed, but perhaps more meaningful might be the responsiveness of circulating cytokine levels to a challenge such as an acute training bout or weeks of training. The absolute resting levels of biomarkers may not change while the response to stress could be abnormal. Thus, timing of the measurement and an individual's average resting levels over multiple days are relevant to interpretation and important to understanding the normal fluctuation in biomarkers for a given individual over a short period of time and in response to exercise and recovery over the course of hours, days, or weeks.

Time course for when to take measurements and how frequently are included in the discussion of specific biomarkers. Although we do not recommend a precise testing schedule that is suitable for all athletes under any training conditions, we recommend 4 main considerations for determining frequency/timing of biomarker testing (Figure 2). The first recommendation is to test at the beginning and end the key points of training transitions. For example,

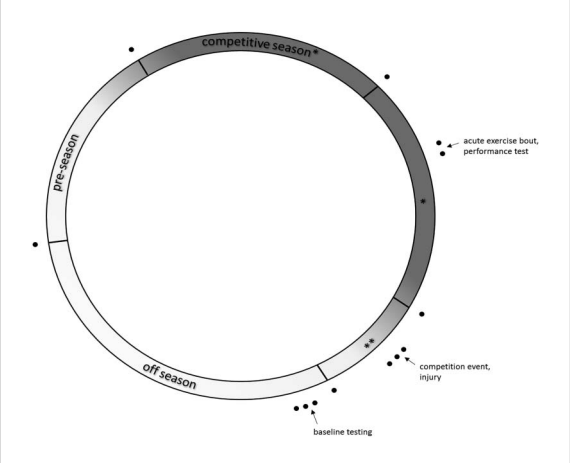

Figure 2. Suggested biomarker testing time points. ● represents suggested biomarker panel testing at diagnostic opportunities throughout off-season and during-season training. * represents competitive season during which both training and competition occur. ** represents peak competition season during which championship matches might occur. Suggested time points for biomarker testing include before and after each major shift in training. Frequent (2–3 tests) testing during rested, healthy periods in the off-season will provide baseline values for many biomarkers and will be important for providing individualized biomarker data. Testing is also recommended before and after at least 1 acute exercise bout or performance test in the middle of a training season to acquire data around optimal performance. Testing before and after an acute bout of exercise will also allow athletes to analyze variables that might be more meaningful as responsive to an acute bout rather than as a single value at rest. It is also recommended that flexibility be built in to test biomarkers around a major championship competition or acute injury event. Testing not just before and after such an event, but also at additional time points after the competition or injury will allow the athlete to assess a recovery response. Data from biomarkers should be analyzed with physiological and physical data to contextualize results. This approach will optimize sensitivity, precision, and accuracy.

testing before and at the end of preseason training will provide important information about the athlete coming out of off-season or rest periods and how preseason training has prepared the athlete for the competitive season without ideally, inducing any overtraining or injury. Second, it is recommended that during the competitive season, which may have training subcycles, that biomarker testing be completed around a single bout of exercise. Testing can be administered before and after (a) a bout of exercise during a particularly challenging training week, (b) a performance test, or (c) a bout of exercise after recovery from an injury or after some shift in training. This type of testing will elucidate any deficiencies or defects in biomarker responses to an acute stress. This would be valuable when resting values of biomarkers might not reveal any concerns, but the response and recovery from a bout of exercise would more sensitively detect concerns. A third recommendation is to test before and multiple times after a major competition event or injury. In this case, there is a severe stress imposed by either the competitive event or an injury and biomarker testing multiple times after the event will allow an athlete to determine whether recovery has occurred on a biochemically measurable level. This case highlights the potential of biomarker testing to precisely detect potential health/recovery concerns when an athlete might feel ready, but may not actually be ready at the tissue/cellular level. Finally, a recommendation to establish standards for each individual and address the variability in most biomarkers, is that biomarker testing be done on multiple days during off-season when an athlete is fit, healthy, and rested to determine the athlete's average resting values for all biomarkers to be tested under training conditions. Flexibility should be built into biomarker testing schedules to account for testing that can be associated with an athlete's subjective feelings of fatigue, measurable decreases in performance, and injury incidents.

Accurately and precisely assessing health and performance of athletes requires a more comprehensive, integrative, and dynamic approach to biomarker analysis. A simplistic approach to using molecular biology/biochemistry in applied/practical sport science will not be appropriate in maximizing the benefit of biomarker testing to diagnose and make training decisions. The application of biomarker testing to traditional sport assessment/coaching requires thoughtful selection of multiple biomarkers and schedule of biomarker testing, and informed interpretation of both biochemical results and physiological/physical data about athletes. Through this review, we present an example holistic approach to tracking athletes using biomarkers that assess nutritional health, metabolic health, hydration status, muscle status, endurance performance, injury status and risk, and inflammation. Diet and training affect these key aspects of health and performance that can be assessed with biomarkers that have been relatively well studied; examples of evidence-based biomarkers for each specific aspect of health/performance are suggested based on our review of the literature (Figure 1). We suggest ideally, a comprehensive approach to biomarker analysis, but markers are presented based on their respective physiological relevance for individuals seeking a more focused approach to hematological assessment.

Biomarkers of Nutrition and Metabolic Health

Athletic performance and recovery from exercise are enhanced by optimal nutrition according to a joint position stand by the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine. Functional performance is impaired when nutritional intake is inadequate and a high prevalence of disordered eating in athletes, especially female athletes contributes to concerns about general health. Specific nutritional deficiencies are common in athletes particularly for vitamin D and iron, for which studies have reported deficiency rates of 73% (27) and 22–31% (in female athletes) (37,104). Other, less common nutritional deficiencies in nutrients such as folate, vitamin B12, or magnesium may result in reduced endurance work performance and muscle function. Individual nutritional needs depend largely on sport- and training-specific

bioenergetic demands as well as on an athlete's metabolic tolerance, needs, and preferences. Frequent monitoring of macronutrient and micronutrient intake may help identify individual deficiencies and track changes, especially as training volume and nutritional demands increase. Nutritional assessment by objective biomarker testing eliminates bias associated with more traditional and subjective nutritional assessments (e.g., recall, questionnaire).

Macronutrient Metabolism

Glucose functions as the primary energy source. Unlike fats and proteins (e.g., ketones), which the body uses as energy sources in some conditions, glucose is the only energy substrate in the body that functions solely for providing energy to cells. Circulating glucose levels during exercise depend on energy status, food intake, event intensity, and glycogen storage levels. Reduced glycogen availability is commonly associated with fatigue. With glucose-depleting events, carbohydrate consumption before or during prolonged exercise has been shown to replenish glycogen, maintain blood glucose levels, and enhance performance, especially for high-intensity activity (136). Tracking and monitoring fasting and longer-term blood glucose through biomarkers such as glucose may help individual athletes monitor the nutritional adequacy of their diet. Although fasting blood glucose is not often related directly to performance, athletes tend to have lower fasting blood glucose (76), where levels are associated with the intensity of the training regimen (76). Adequate nutrition for a given training volume can reduce the risk of exercise-induced hypoglycemia in athletes. In addition, exercise training may reduce vulnerability to hypoglycemia in athletes because of a shift in substrate metabolism. However, overtraining may reverse this adaptation, making athletes more vulnerable to hypoglycemia in the over-trained state.

Fats are used as a primary energy source in endurance events or when carbohydrate availability is low. In particular, medium-chain fatty acids are preferred for oxidation, as they enter circulation more rapidly and are primarily absorbed by the liver. Fat utilization during exercise impacts lipid profiles by reducing resting levels of total cholesterol and triglycerides (59), thereby improving cardiovascular health profiles. In addition to providing energy, some types of fats play important roles in recovery. Omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) reduce inflammation, muscle soreness, and the perception of pain from exercise (14,63,129). Moreover, omega-3 fatty acids may influence performance through their effects on neuromuscular function (123), nerve conduction velocity (123), and neuromuscular sensitivity of the acetylcholine receptor. In addition, omega-3 fatty acids may support increased training volume (63) and support adaptations to exercise training. Levels of omega fatty acids measured in the blood reflect their clinical role more so than dietary intake. Nevertheless, the recommended daily intake of omega-3 fatty acids (EPA + DHA) is $\leq 3\ g \cdot d^{-1}$ for average individuals or those moderately physically active, but recommendations may be as high as 6–8 $g \cdot d^{-1}$ (2:1 ratio of EPA:DHA) for elite athletes. Greater training demands may increase requirements for omega-3 fatty acid intake.

Proteins serve as the building blocks of hormones and enzymes used in all cells and tissues in the body, including muscle. Generally, protein intakes of 1.3–2.0 grams per kg body mass per day are recommended for athletes to support muscle protein synthesis, facilitate training adaptations, and prevent lean muscle mass loss. An imbalance between dietary protein intake and dietary protein needs may result in net protein loss in athletes. With protein deficiency, tissue protein breakdown becomes a source of essential amino acids needed to maintain critical body functions. While it is generally recognized that athletes require higher protein intake than the recommended daily allowance, defining individual needs is challenging. As outlined below, a combination of biomarkers including total protein, albumin, globulin (calculated), urea nitrogen (blood urea nitrogen [BUN] or urinary urea nitrogen), nitrogen balance (calculated), and amino acid analysis may help athletes to gauge their protein status and make dietary alterations to improve training outcomes.

Protein deficiency decreases blood proteins, especially of albumin, and low protein intake seems to decrease the rate of albumin synthesis (61). However, albumin may also serve as a marker of other aspects of athlete health and performance, and this measure requires contextualization when assessed in athletes. The need for contextualization is a common feature of many biomarkers in a comprehensive panel approach to tracking biomarkers and performance. Contextualizing albumin levels, traditionally defined for sedentary or nonathlete populations, for athletes training or competing is important for interpreting the implications of either decreased or elevated plasma albumin. In addition, it is important to understand that measures such as albumin may also be related to performance and recovery through nontraditional functions or signaling in athletes. For example, albumin has been associated with human growth hormone (GH) concentrations in the blood (101), and although the mechanism by which these 2 markers are related is unknown, this type of result suggests that albumin may require additional interpretation when tracking athletes.

Similarly, while urea nitrogen (blood or in urine) is a product of protein degradation and suggests protein breakdown, elevations can be due to a variety of factors such as protein intake, endogenous protein catabolism, fever, infection, glucocorticoids, state of hydration, hepatic urea synthesis, and renal urea excretion. Lower urea nitrogen may be due to low protein intake, malnutrition starvation, or impaired metabolic activity in the liver. Higher urea nitrogen may be due to exhaustive exercise training, catabolism (59), and high dietary protein intake (148). As maintaining a positive protein balance is essential to facilitating optimal recovery and training adaptations, protein status should be optimized to avoid nutritional insufficiencies and excessive

protein catabolism. In the absence of disease, low blood protein, low albumin, and elevated urea nitrogen may be indicative of insufficient protein intake in athletes. In circumstances where protein intake seems to be sufficient for an athlete's estimated needs, albumin and urea nitrogen may indicate other relevant athlete health issues.

Many athletes follow nontraditional diets, such as low-carbohydrate or ketogenic diets. Athletes are able to sustain performance on diets comprising as little as 7% carbohydrates without effects of gluconeogenesis (146), but dramatic effects on fat oxidation to maintain similar muscle glycogen use and repletion to that of athletes on traditional high carbohydrate diets (139). As with all biomarkers, we recommend contextualizing nutritional biomarkers with each individual's habitual diet in a dynamic fashion. In other words, absolute values for certain biomarkers may not direct action for a given athlete, but changes with training that coincide with reduced capacity to recover and decreased performance should be monitored on an individual basis. This approach to biomarker monitoring will allow coaches and staff to better monitor groups of highly variable athletes who will inevitably have highly different diets and other behaviors that affect performance.

Micronutrient Metabolism

A variety of vitamins and minerals support physiological processes that underlie performance. For example, vitamin D, in addition to being involved in bone maintenance, has a role in muscle function and protein synthesis. Many athletes monitor vitamin D with a goal of achieving levels of greater than 50 $ng \cdot ml^{-1}$ because of the many potential ergogenic effects of vitamin D on sport performance rev. in Dahlquist et al (31). Although some studies have determined that specific vitamin D supplementation regimens do not affect power-specific performance variables, there is promising evidence that vitamin D supplementation enhances aerobic performance (62) and that vitamin D levels are correlated to aerobic performance (31). B complex vitamins (thiamin, riboflavin, niacin, pyridoxine, folate, biotin, pantothenic acid, and choline) also play an important role in performance by regulating energy metabolism by modulating the synthesis and degradation of carbohydrate, fat, protein, and bioactive compounds. Other vitamins play important supporting roles in recovery processes. For example, vitamin E functions as an antioxidant in cell membranes and subcellular structures (65). Deficiencies in vitamin E may relate to neurologic damage and erythrocyte hemolysis, as well as muscle degradation (79). Similarly, beta-carotene, a precursor of vitamin A, acts as antioxidants in reducing muscle damage and enhancing recovery after exercise (65). Low calcium, iron, B-vitamins, and vitamin D have been associated with increased injury risk, specifically lower extremity stress fractures (81).

Several essential minerals, such as magnesium and iron, affect physical performance (79). For example, magnesium is important for energy metabolism as well as nerve and muscle function (79). Deficiencies may lead to muscle weakness (79), muscle spasms (43), and altered CK and lactate response to exercise (49). In addition, specific nutrients including iron, folic acid, and vitamin B12 (cyanocobalamin) are essential to hemoglobin synthesis and subsequently oxygen transport (79). Deficiencies may lead to fatigue, anemia, cognitive impairment, and immune deficiencies (79,81). Iron deficiency is prevalent in athletes from a variety of sports (79,104), with prevalence as high as 31% in some sports (104). In addition to decreased iron concentrations, other biomarkers are useful in the assessment of iron deficiency including ferritin (concentration less than 12 $\mu g \cdot L^{-1}$) and transferrin (saturation less than 16%) (79). Moreover, red blood cell indices may provide early indications of nutritional deficiencies. For example, hematocrit, hemoglobin, and red blood cell indices may suggest iron, vitamin B12, or folate deficiency (79).

Other micronutrients including zinc and chromium also have important supporting roles in athletes. Zinc is required for a variety of functions including protein synthesis, cellular function, glucose use, hormone metabolism, immunity, and wound healing (135). Low zinc is prevalent (22–25%) in endurance athletes (35). Chromium is a provisionally essential mineral that functions broadly in the regulation of glucose, lipid, and protein metabolism by potentiating the action of insulin at the cellular level. Athletes excrete higher amounts of chromium (3), which may result in increased nutritional needs. Monitoring micronutrient levels may help athletes to identify deficiencies and increase nutritional needs early to reduce the potential performance-impairing impact of nutritional deficiencies.

Food Allergies

One additional aspect of nutritional and metabolic health that may have value in tracking athletes is that of an athlete's unique responses to certain foods. Blood-based biomarkers are available for testing food allergen sensitization that may or may not be known to the athlete. Adverse reactions (food allergy/intolerance) to specific foods may result from immunoglobulin E (IgE)-mediated mechanisms, where IgE is produced against specific food components in food-allergic individuals (38). Immunoglobulin E triggers immune responses within minutes to hours after consuming the food by way of mast cell degranulation resulting in the release of vasoactive and proinflammatory mediators. In adults, allergies to peanuts, tree nuts (walnut, hazel, cashew, pistachio, Brazil nut, pine nut, almond), fish, shellfish (shrimp, crab, lobster, oyster, scallops), fruits, vegetables, seeds (cotton, sesame, psyllium, mustard), milk, egg and spices are most prevalent (38). Reactions vary from aggravation of the skin, nose, eyes, lungs, and gastrointestinal tract to severe cardiovascular effects. Symptoms may be noticed as swelling and itching of the lips, tongue, or palate, abdominal pain and cramping, nausea, vomiting, diarrhea, respiratory challenges, and asthma. While a variety of methods exist to assess food allergies, IgE-based testing is considered an acceptable

approach to assess suspected food allergies. Immunoglobulin E–based blood tests measure IgE directed against specific antigens, where levels can predict reactions to certain foods with greater than 95% certainty (112). Specific IgE levels higher than 0.35 $kU \cdot L^{-1}$ suggest sensitization (112). The accurate identification of causative foods is important for creating effective treatment plans in athletes, especially when pharmaceutical interventions are subject to the World Anti-Doping Agency regulations. Food allergen testing may be performed under resting conditions as part of athletes' preseason physicals. Identification of potential food allergens is particularly important due to a condition known as food-dependent exercise-induced anaphylaxis (85). In this condition, exercise in combination with ingestion of the food agent triggers the allergic response (85), possibly because of altered absorption from the gastrointestinal tract (85), or altered IgE levels from exercise (2), or hyperosmolar conditions (93). Although the direct effects of IgE-mediated responses on exercise tolerance and performance are yet to be examined, symptoms like anaphylaxis, eosinophilic inflammation, bronchial hyperresponsiveness, urticaria-angioedema, dermatitis, rhinitis or asthma, and gastrointestinal disorders (oral allergy syndrome, colic, nausea, vomiting, diarrhea, abdominal pain) may present a barrier to exercise tolerance.

Biomarkers of Hydration Status

Water is the most essential nutrient of the body undergoing continuous recycling, functioning as a solvent, and regulating cell volume, while playing a critical role in thermoregulatory and overall function. Water balance is mainly regulated by thirst and antidiuretic hormone, known also as vasopressin, through its renal effect. Acute decrease in body weight has been used as the gold standard to evaluate the degree of dehydration because it reflects mainly a decrease in total body water and not energy substrates (e.g., fat, protein). In this case, we assume that both cutaneous (sweating) or renal (urination) water losses have a specific gravity of approximately 1.000, resulting in a 1-gram change in body weight for every milliliter of sweat and urine output. This technique is accurate, assuming there is no bowel movement or food consumption and of course body weight is taken before exercise in a euhydrated state. A hypohydrated state of greater than −2% body mass has been linked to decreases in exercise/sport performance, cognitive function, mood, and increases in risk of exertional heat illness or exertional heatstroke for individuals exercising in hot and humid environments (107).

During exercise, especially in the heat, most people tend to drink less than what they lose through sweating, resulting in a water deficit (involuntary dehydration). Because sweat is hypotonic, exercise-induced dehydration leads mainly to a decrement of the extracellular fluid volume. This state is described as hypertonic hypovolemia due to water loss from the plasma. Blood biomarkers of hemoconcentration have thus been widely used as an indexes of dehydration.

Blood Markers

Both blood osmolality and sodium levels have been used for hydration assessment because both values increase in a linear fashion with the levels of dehydration. Blood osmolality is considered by many as the gold standard for the assessment of hydration state, especially for acute and dynamic changes of hydration state (6,25). Even a small degree of dehydration (e.g., −1% of body weight) can significantly increase plasma osmolality. Most studies suggest that the threshold of dehydration for blood osmolality is 295 $mmol \cdot kg^{-1}$ of plasma water (25,45,108). In addition, because dehydration has a negative impact on kidney function, the ratio of urea nitrogen to creatinine has been used as a strong indicator of hydration state with a suggested threshold of 20 for dehydration (45).

Timing of blood hydration biomarkers depends on the intent. Pre-exercise hydration state may be used to assess whether an athlete is hypohydrated before a training session or competition; in this case, the result will define fluid consumption recommendations to optimize training benefit or performance during an event. Postexercise hydration state will also define fluid consumption recommendations for an individual, but for the purposes of promoting optimal recovery. Tracking hydration state over a number of days can elucidate whether fluid and food intake is providing sufficient hydration to maintain a hydrated state during critical times in training and before and after competition.

Urine Markers

The hemoconcentration-driven elevation in plasma osmolality and decrement in plasma volume stimulate arginine vasopressin (AVP) secretion by osmotic receptor stimulation and unloading of the baroreceptors. Even though AVP could be used as a marker of dehydration, its analytical process is laborious and expensive. Although this 8–amino acid molecule is very sensitive, it tends to degrade quickly, making its measurement challenging. Luckily, AVP has a strong effect on the renal system by increasing water reabsorption in the nephron tubules. As a result, urine volume is smaller and more concentrated. Therefore, urinary markers of concentration have also been widely used as an index of hydration. Urine-specific gravity (USG) and urine osmolality (UOsm) are sensitive to changes in hydration state. Both the American College of Sports Medicine and the National Athletic Training Association recommend cut-off points for dehydration of ≥1.020 for USG and ≥700 $mmol \cdot kg^{-1}$ for UOsm, respectively (22,108). Based on the link between dehydration and urine concentration, urine color has been shown to be a valid practical marker of hydration assessment both in adults and in children (5,66). Based on the 8-point urine color scale developed by Dr. Lawrence Armstrong, the threshold of dehydration is color 4, with 1 being the lightest and 8 the darkest color. A urine color of 5 or above is consistently and reliably associated with dehydration (83).

Thirst

Thirst has also been suggested as a surrogate perceptual marker of dehydration. Thirst and AVP are similarly stimulated by decreases in plasma volume and increases in plasma osmolality (100). However, both are more sensitive to small osmotic stimulation than to baroreceptor activation. What is interesting is that the osmotic threshold for thirst activation is significantly greater than the one for AVP secretion (100). Mild dehydration-induced increase in plasma osmolality will rapidly increase AVP and elevate urinary markers of dehydration, even in the absence of thirst. Of course, because thirst is stimulated by significant dehydration, people may already be dehydrated by the time they notice thirst. This phenomenon could also explain why both recreational and professional athletes often start their training or competition in a suboptimal hydration state, as indicated by their elevated urine hydration markers. We therefore recommend that although thirst be a useful measure of hydration state particularly at rest, blood or urine biomarkers may be more precise and accurate.

Other Measures of Hydration

Researchers have studied novel biomarkers including saliva, sweat, and even tears as possible biological samples in which to measure hydration state. Saliva osmolality and saliva flow rate have shown promise as hydration markers (142); other studies raise doubts about the usefulness of salivary osmolarity as a biomarker except in highly controlled conditions, during physical activity (89) or in special clinical populations (45). Similarly, sweat osmolality, electrolytes, and other variables, as well as tear osmolality (44) show promise as potential biomarkers, but research is limited. Although these options currently cannot serve as valid biomarkers of hydration status for athletes, when considering biomarkers to select for a comprehensive panel, it is critical to consider newly studied markers as potential options. Table 1 provides options for well-validated hydration biomarkers.

Table 1. Indexes of hydration assessment with their threshold values.

Hydration assessment technique	Threshold value
Practical self-test	
Acute decrease in body mass (kg)	−2%
Dark urine color (color chart rating)	4
Thirst sensation (thirst scale rating)	+
Diagnostic laboratory tests	
Urine	
Urine-specific gravity	1.020
Urine osmolality ($mOsm \cdot kg^{-1}$ or $mmol \cdot kg^{-1}$)	700
Blood	
Urea nitrogen/creatinine ratio	20
Blood osmolality ($mOsm \cdot kg^{-1}$ or $mmol \cdot kg^{-1}$)	295
Sodium concentration ($mEq \cdot L^{-1}$ or $mmol \cdot L^{-1}$)	145

Biomarkers of Muscle Status

Skeletal muscle tissue quality (size, structure, composition, metabolic capacity, and contractile indices) is an important aspect of athletic health and performance. Strength, power, fatigue, and endurance in athletes are directly affected by muscle status or the fatigue and recovery state of the muscle. Also, insufficient recovery from exercise-induced muscle damage caused by training impairs performance, likely because of increased sense of effort, reduced exercise tolerance, reduced strength, and reduced power. Monitoring indices of muscle status will help athletes to tailor their training/competition and recovery regimens to optimize performance. Blood-based biomarker muscle status assessment should focus on endocrine regulation of muscle repair/adaptations, metabolic homeostasis (anabolic-catabolic balance, protein/amino acid deficiencies, substrate availability), muscle damage, and muscle excitability. There are well-validated markers (Table 2) related to fatigue, recovery, protein synthesis, or fueling strategies, which are all major athlete concerns. Because hormone and amino acid concentrations in the blood are highly variable among individuals, these types of biomarkers are best assessed by analyzing progressive increases/decreases away from a baseline measure for each person (Table 2). This requires monitoring for these types of biomarkers at multiple time points throughout training, off-season, and competition cycles. To monitor chronic changes across a season, athletes may be tested every 4–6 weeks under similar conditions (i.e., fasted, in the morning, before training, the day after a rest day or similar training day).

Endocrine Response

Proper hormonal signaling is essential for the physiological adaptations to exercise training. Dependent on the magnitude of the training stimulus, often defined by acute program variables such as load, volume, duration, modality, and rest, hormones elicit specific training adaptations. Testosterone, cortisol, dehydroepiandrosterone (DHEA), GH, insulin-like growth factor 1 (IGF-1), sex-hormone binding globulin, and luteinizing hormone (LH) are among the key hormones demonstrated to be critical to athletes.

Testosterone is required for promoting protein synthesis, red blood cell production, and glycogen replenishment and for reducing protein breakdown. Decreased testosterone levels accompanied by decreased performance, energy, or strength observed during a training season may indicate that that training volume is too high. In this case, an athlete may benefit from temporarily reducing training volume. Cortisol works antagonistically to testosterone, inhibiting protein synthesis by interfering with testosterone's binding to its

Table 2. Markers of muscle status and trends to monitor in athletes.

Biomarker	Role	Potential indication	References
Testosterone	Protein synthesis Reduces protein breakdown Red blood cell production Glycogen replenishment	Chronic ↓ may indicate that training volume and intensity exceeds body's tolerance or reduced anabolic potential	(47)
Cortisol	Catabolic Immune suppressive	Chronic ↑ may indicate impaired capacity for recovery, impaired capacity for protein synthesis, or overreaching	(24,128,133)
T:C Ratio	Anabolic-Catabolic balance	Chronic ↓ in ratio may reflect increased proteolysis or suppressed protein synthesis	(8,133,138)
Dehydroepiandrosterone (DHEA)	Precursor hormone Body composition	Chronic ↓ levels may reflect susceptibility to overtraining	(16,42,56)
Growth hormone	Protein synthesis Reduces protein breakdown	Chronic ↓ levels may reflect reduced potential for adaptations to training	(20,52,53,71)
Insulin-like growth factor 1 (IGF-1)	Mediator of anabolic actions GH in skeletal muscle	Chronic ↓ levels may reflect overreaching or impaired muscular adaptations to training	(94,128,137)
Sex-hormone binding globulin	Transporter for testosterone and estradiol	Chronic ↑ or ↓ may indicate insufficient recovery, overreaching, or suboptimal ability to adapt to training	(40,75,128,133)
Luteinizing hormone	Reproduction	Chronic ↓ levels may reflect susceptibility to overtraining	(54,133,145)
Creatine kinase	Muscle enzyme	↑ levels may indicate muscle damage	(69,86)
Urea nitrogen	Metabolic product of protein degradation	↑ levels may indicate catabolic state	(7,59)
Tryptophan	Amino acid	↑ levels may indicate fatigue or suboptimal training adaptation	(23,67)
Glutamine	Amino acid involved in neural plasticity and protein synthesis	Chronic ↓ levels may reflect fatigue or suboptimal training adaptation	(67)
Glutamine: glutamate ratio	Ratio of amino acid glutamine to glutamate, a product of glutamine breakdown	Chronic ↓ levels may reflect suboptimal training adaptation and catabolism	(115)

Biomarker	Role	Monitor for	Potential indication	References
Testosterone	Protein synthesis Reduces protein breakdown Red blood cell production Glycogen replenishment	↓	Training volume and intensity exceeds body's tolerance Reduced anabolic potential	(47)
Cortisol	Catabolic Immune suppressive	↑	Impaired capacity for recovery Impaired capacity for protein synthesis Overreaching	(24,128,133)
T:C Ratio	Anabolic-Catabolic balance	↓	Increased proteolysis Suppressed protein synthesis	(8,133,138)
Dehydroepiandrosterone (DHEA)	Precursor hormone Body composition	↓	Susceptibility to overtraining	(16,42,56)
Growth hormone	Protein synthesis Reduces protein breakdown	↓	Potential adaptations to training	(20,52,53,71)

(continued on next page)

Insulin-like growth factor 1 (IGF-1)	Mediator of anabolic actions GH in skeletal muscle	↓	Overreaching Impaired muscular adaptations to training	(94,128,137)
Sex-hormone binding globulin	Transporter for testosterone and estradiol	↑ or ↓	Insufficient recovery Overreaching Suboptimal ability to adapt to training	(40,75,128,133)
Luteinizing hormone	Reproduction	↓	Susceptibility to overtraining	(54,133,145)
Creatine kinase	Muscle enzyme	↑	Muscle damage	(69,86)
Urea nitrogen	Metabolic product of protein degradation	↑	Catabolic state	(7,59)
Tryptophan	Amino acid	↑	Fatigue Suboptimal training adaptation	(23,67)
Glutamine	Amino acid involved in neural plasticity and protein synthesis	↓	Fatigue Suboptimal training adaptation	(67)
Glutamine-glutamate ratio	Ratio of amino acid glutamine to glutamate, a product of glutamine breakdown	↓	Suboptimal training adaptation Suboptimal adaptations to training Catabolism	(115)

androgen receptor and by blocking anabolic signaling through testosterone-independent mechanisms. When chronically elevated, cortisol is catabolic and immunosuppressive leading to circumstances that make it more difficult for an athlete to build/maintain muscle mass and recover from training.

In addition to monitoring testosterone and cortisol separately, monitoring their relative levels (T:C ratio) during a training season may provide a relative indication of anabolic-catabolic balance, especially in male athletes (133). T:C ratio is considered more sensitive to training stresses than either measure alone. A prolonged decrease in T:C is associated with detriments to performance through increased proteolysis (muscle protein breakdown) and decreased protein synthesis. A 30% decrease in T:C has been suggested as an indicator of insufficient recovery (8,138), whereas a value of 0.35×10^{-3} has been considered to be the threshold of overtraining (138). Poor performance outcomes and suboptimal training adaptations have been reported in both soccer athletes (70) and tactical athletes (26) with a low T:C ratio.

As other hormones moderate physiological adaptations to training, especially in female athletes, monitoring other hormones, such as SHBG or DHEA-S in relation to cortisol may provide additional insights into the anabolic to catabolic balance in both male and female athletes. Dehydroepiandrosterone is a precursor hormone to both estrogen and testosterone. In addition to affecting body composition (56) in athletes, changes in DHEA in relation to cortisol have been reported to be a useful marker of susceptibility to overtraining in the female athlete (16,42). Similarly, SHBG is a useful indicator of training status and performance (strength and rate of force development) (40). SHBG transports hormones such as testosterone in the body and increases in response to exercise training in both male and female athletes. Increased SHBG is believed to protect sex hormones from being degraded by protecting the biologically active free sex hormones in circulation. Increased SHBG and decreased testosterone may indicate insufficient recovery (53). Low SHBG may merely represent an individual's chronic diet (1); diets high in fat and protein may be associated with low levels of SHBG and high levels of sex hormones (1) and may be considered a sign of suboptimal capacity to adapt to training (133).

Other key hormones inform us about training adaptations. These include GH, IGF-1, and LH. Growth hormone stimulates anabolism by promoting muscle protein synthesis and inhibiting protein breakdown. Growth hormone concentrations have been correlated to exercise volume and intensity. Growth hormone increases levels of circulating IGF-I, both of which hormones are involved in muscle mass regulation, making IGF-1 and GH together potentially useful biomarkers. Luteinizing hormone is associated with reproductive function in men and women. Luteinizing

hormone may be another useful marker to detect overtraining or insufficient energy intake.

Amino Acids

Athletes require greater daily intakes of protein (in the range of 1.3–1.8 $g \cdot kg^{-1} \cdot d^{-1}$) to maximize muscle protein synthesis as compared to the general population. As discussed, markers of nitrogen balance (e.g., urea nitrogen) are important for assessing the nutritional status of an athlete, but a number of specific amino acids can reveal information about protein synthesis, nutrition, and fatigue. For example, the branched-chain amino acids (BCAA), leucine, isoleucine, and valine, increase the rates of protein synthesis and degradation in resting human muscle (13). Branched-chain amino acids levels have been informative about whether BCAA supplementation is directly affecting skeletal muscle protein synthesis signaling (4). With some special considerations for measurement (131), BCAA can also indicate whether diet, stress, or disease states are affecting an athlete's skeletal muscle. There are a few other examples in which specific amino acids may indicate muscle status based on their unique roles in skeletal muscle. The amino acid taurine is not incorporated into protein, but is abundant in muscle tissue and is needed for the differentiation and growth of skeletal muscle. Taurine deficiency can impair muscle development, structure, and function (118). Researchers have interpreted elevated taurine levels, perhaps because of release from muscle fibers, as a marker of damage or impaired muscle function (29,144). Others have used urine excretion of taurine as a biomarker in athletes (28). Another amino acid, glycine, is involved in the biosynthesis of heme, creatine, nucleic acids, and uric acid (143), deficiencies in which may affect various aspects of the metabolic pathways. Other amino acid patterns (e.g., elevated tryptophan, decreased glutamine) have been associated with fatigue and suboptimal training capacity in athletes (23,67,115) and suggest specific amino acids that may serve as biomarkers of muscle quality/status. While some amino acids change in response to acute exercise (103), monitoring resting amino acids across a season as part of a comprehensive panel under similar conditions (i.e., fasted, in the morning, before training, the day after a rest day or similar training day) may provide insights into training (39) and fatigue (67).

Recovery (Urea Nitrogen and Creatine Kinase)

After muscle-damaging exercise, the enzyme CK leaks from the muscle into the circulation (69,86). It is typical for athletes to have elevated CK during training, with reference ranges of 82–1,083 $U \cdot L^{-1}$ in male and 47–513 $U \cdot L^{-1}$ in female athletes suggested as athletic norms (86). Monitoring CK levels during training in comparison with baseline levels may help athletes to monitor muscle status. Creatine kinase levels peak approximately 24 hours after damaging exercise such as heavy strength training, but may remain elevated up to 7 days after exercise. Chronically elevated CK may indicate insufficient recovery. Because other components of muscle such as myoglobin may leak into circulation during muscle damage (peak 1–3 hours after exercise), and urea nitrogen can indicate overall protein synthesis vs. breakdown (59), using all 3 markers to determine an athlete's muscle status during training and recovery will be useful to athletes, coaches, and clinicians.

Biomarkers of Cardiovascular Endurance Performance

Iron is an important mineral in oxygen transport and oxidative phosphorylation which are fundamental physiological processes required for aerobic metabolism and cardiovascular endurance performance (60). Endurance athletes, especially females (113), are particularly susceptible to iron deficiency because of one or a combination of the following factors: menstrual bleeding, poor dietary intake, exercise-related gastrointestinal tract bleeding, hematuria, sweating, poor intestinal iron absorption due to subclinical exercise-induced inflammation (97), and erythrocyte destruction through repeated foot striking (98), elevated intramuscular pressure in swimmers and cyclists (110), and increased mechanical loading and hepcidin release in response to subclinical exercise-related inflammation (97,105). Other factors affecting iron status biomarkers in athletes include regular nonsteroidal anti-inflammatory drug (NSAID) use, blood donation, and chronic alcohol consumption (12). Athletes with compromised iron status may experience decreases in performance because of the inability to optimally metabolize substrates into energy (51). Iron deficiencies also prevent adaptations to endurance and altitude training (11,60). Also, iron deficiency with anemia may have a role in the greater prevalence of upper respiratory tract infections in marathon runners (88). Given the physiological role of iron and its association with aerobic performance, health, and adaptation, athletes and coaches should consider tracking iron, iron binding capacity, transferrin saturation, and ferritin levels during training. Approaches to timing and frequency of iron status testing for individual athletes can be customized to address issues with when cardiovascular endurance performance may be affected by changes in training programs/cycles or general health (e.g., during infection or personal stress experienced during training). Iron status assessments acutely before competition will also be contextually useful. Practical considerations of cost of biomarker assessments may define frequency of testing.

The compliment of widely used biomarkers includes iron, total iron binding capacity (TIBC), transferrin saturation, and ferritin, with more recent biomarkers such as soluble transferrin receptor and hepcidin peptide assay possibly improving diagnosis. Iron status markers should be interpreted in the context of recent events (e.g., competition season, recent training intensity, frequency, and duration, inflammation state, and diet changes). Changes in iron status markers indicate a number of well-studied, potential effects on performance (Table 3).

Table 3. Potential indications from reductions in iron status markers.

Biomarker	Monitoring for	Potential indication	Reference
Iron status	↓	Reduces time trial performance	(57,82,106)
	↓	Impaired $\dot{V}O_2$peak/$\dot{V}O_2$max	(33,46,58,73)
	↓	Reduced energy efficiency	(33,34,46,57,58,151)
	↓	Lower training volume per day	(33)
	↓	Greater max lactate	(73,109)
	↓	Lower time to exhaustion	(58)

Iron concentration reflects total iron content with a reference ranges within 50–175 $\mu g \cdot dl^{-1}$ (9). Between and within-day variation of iron concentration is high (10–26%) and as a consequence iron concentration must be interpreted cautiously and cannot be rendered a useful measure of iron status alone (15). Serum ferritin can be falsely elevated in an inflammatory state (e.g., postexercise, infection) but inflammatory markers such as C-reactive protein (CRP) or alpha-1-acid glycoprotein can aid in the interpretation of ferritin in the assessment of iron status (9). A more stable indicator of iron status is TIBC (reference range: 250–425 $\mu g \cdot dl^{-1}$), which reflects the total number of binding sites on the blood iron transporting peptide transferrin. Daily variation of TIBC is relatively low (8–12%) and does not change before iron stores are depleted (9), thus reducing the likelihood of falsely detecting iron depleted states. Total iron binding capacity would rise in iron deficiency as more free transferrin binding sites are available. In addition, transferrin is not an acute phase reactant or affected by other diseases and therefore is a valuable biomarker panel addition for determining iron status (152). Transferrin is an iron-carrying monomeric glycoprotein within blood that transports iron to tissues. Transferrin saturation (reference range: 15–50%) is the percentage of iron to TIBC, with values under 15% consistent with iron deficiency. Because TIBC is quite stable, alterations in iron concentration will also affect transferrin saturation (9). Soluble transferrin receptor reflects iron deficiency at the tissue level and is believed to be a more sensitive measure of functional iron deficiency assessed by ferritin (152). In 2 iron supplementation studies examining aerobic training adaptation in females, improvements were only noted when soluble transferrin receptor was elevated before training (>8 $mg \cdot L^{-1}$) compared with those with adequate iron status (<8 $mg \cdot L^{-1}$) (18,19). This biomarker seems not to be affected by inflammation and has low within-subject variability in athletes undergoing training. The combination of at least transferrin and transferrin saturation, TIBC, serum ferritin, and hemoglobin is required for accurate determination of the presence and severity of iron deficiency. Including additional clinical parameters such as soluble transferrin receptor, among others, may increase the confidence in iron status diagnosis.

Endurance performance suffers when iron levels are insufficient (serum ferritin <12 $\mu g \cdot L^{-1}$) for hemoglobin (Hb) to efficiently transport oxygen to exercising muscle tissue (Hb, females, <12 $g \cdot d^{-1}$; males, <13 $g \cdot dL^{-1}$). Yet, serum ferritin stores can be depleted before hemoglobin has declined to levels required for diagnosis of anemia (32). Functional iron deficiency has been defined as ferritin <35 $\mu g \cdot L^{-1}$, Hb <11.5 $g \cdot dl^{-1}$, and transferrin (iron transport molecule) saturation $<16\%$ (96); others have used more precise serum ferritin ranges of 12–20 $\mu g \cdot L^{-1}$. Iron deficiency without anemia is more common than iron deficiency with anemia in endurance athletes, but it is critical to consider multiple aspects of iron metabolism that may affect an athlete.

Supplementation with iron is known to correct low levels of ferritin, transferrin, and hemoglobin, but in some cases may not affect endurance performance (96). However, a vast amount of research supports that tracking these variables and introducing supplementation regimens is effective in improving endurance performance in athletes with low ferritin, both anemic and nonanemic (33,34,58,73,82,106,109,151). A recent review determined that in 73% of studies, implementing low-moderate doses of iron supplementation resulted in improvements in aerobic/endurance performance in female athletes (32).

Biomarkers of Injury Status and Risk

Although biomarkers have been studied in human performance, there has been limited use of biomarkers to determine injury states (both risk for injury, severity of injury, and recovery from injury). No previous work, to our knowledge, has examined the use of biomarkers for injury prevention or for recovery after injury. Concussions are a major concern in sports. One of the major concerns in concussion is understanding when injured athletes have recovered. Previous work has examined biomarkers of concussion recovery with the goal of detecting and monitoring changes in the central nervous system to provide objective measures of when athletes are ready to return to athletic pursuits safely (111). Previous work in this area has focused on the examination of biomarkers in the cerebrospinal fluid, with specific emphasis on markers of axonal damage (total tau, neurofilament light), which have been shown to be elevated in boxers after repeated punches to the head even without a knockout (92,149). However, because of the invasiveness, difficulty, and expense of completing a lumbar

puncture, researchers began to explore the possibility of assessing blood-based biomarkers of brain injury. Two blood-based biomarkers of interest have been neuron-specific enolase and the glial cell biomarker S-100 calcium binding protein B (S-100B), with most studies focusing on changes in S-100B levels (36,90,95,119–122). Serum levels of both markers have been reported to be increased after boxing matches in which the athlete sustained direct or repetitive blows to the head (50,150). By contrast, when examining these same markers in concussed hockey players, only S-100B was found to be increased in the serum (111). Based on this work and the 2015 review article by Papa, it is clear that the study of biomarkers of concussion is beginning to identify potentially diagnostic as well as recovery markers (99). However, no biomarkers have yet been identified for clinical diagnosis or tracking of concussions in athletic populations. This remains an active area for examination.

Another area of musculoskeletal health that has received substantial attention is stress fractures, specifically female stress fractures. Women had a 10-fold higher risk of sustaining a stress fracture when compared with men in a study of military recruits, and the risk has been reported to be as much as 50% higher in female athletes (10,41). Stress fractures are known to result in significant medical costs, lost duty time in the military, and lost game time for athletes. The female athlete triad is a medical condition that affects physically active females and is characterized by 3 components: (a) low-energy availability with or without disordered eating, (b) amenorrhea or menstrual dysfunction, and (c) low bone mineral density (BMD) (91). This condition has been associated with osteoporosis and low BMD, which have been proposed as risk factors for stress fracture development (41,102). Although specific biomarkers have not been associated with the female athlete triad, some biomarkers of bone breakdown have been associated with poor bone quality or bone density. Insulin-like growth factor (IGF-I), one biomarker associated with bone quality, has been reported to be significantly lower in osteoporotic women with poor bone quality and to be positively associated with BMD (87,116). In addition, reduced concentrations of IGF-I have been associated with fracture risk in women (64,125). Only a few studies have examined the use of biomarkers to assess stress fracture risk, none of which have identified a single set of bone turnover biomarkers that could be used for stress fracture prediction (124,147). However, Strohbach et al. (124) did report that serum IGF-I was decreased in subjects who sustained a stress fracture when compared with their noninjured control subject. The results of these few studies as well as an improved understanding of the female athlete triad will allow for the continued exploration of biomarkers that could potential identify individuals at risk of stress fracture development.

Anterior cruciate ligament (ACL) injuries have been reported to result in the development of osteoarthritis (OA) in up to 50% of patients (77). The development of OA in ACL patients has been reported to occur within 10–15 years of the primary injury (77,78,141). While the examination and exploration of both inflammatory biomarkers as well as markers of cartilage breakdown have been extensive in the study of OA, very few studies have explored these markers in ACL patients after injury and surgery; no studies, to our knowledge, have determined biomarkers that can be used to predict ACL injuries. The biomarkers of greatest interest in the early postoperative recovery period after ACL reconstruction have been serum concentrations of collagen type I and type II cleavage products as well as inflammatory responses in both human and animal models (55,127,132). Immediately after ACL injury, the serum concentration of these biomarkers indicates an imbalance between cartilage breakdown and synthesis that could be indicative of posttraumatic changes in cartilage metabolism and signal the onset of posttraumatic OA (127,132). Haslauer et al. examined changes in the IL-6, IL-8, markers of tissue damage (CRP), as well as vascular endothelial growth factor (VEGF), and transforming growth factor β (TGFβ) in Yucatan minipigs to examine the immediate response after ACL transection (55). The results of this study indicate that in the early postinjury period, there is an increase in IL-6 and IL-8 in the synovium as well as an increase in CRP in the ligament, whereas there was no change in TGFβ or VEGF. Similar to human studies, the CRP returned to normal levels by 15 days after injury or after surgery, wheras IL-6 and IL-8 returned to normal levels by approximately 5 days after injury or after surgery (21,55). In a study of ACL reconstruction patients, similar results were found regarding CRP, but this study reported an increase in TGFβ and myostatin in the early postoperative period and then returned to normal by approximately 12 weeks after surgery (84). Although these studies have identified biomarkers that change with injury and after surgical intervention, no studies to date have examined the potential for using biomarkers to identify individuals at increased risk. Thus, further research is required before these biomarkers should be assayed as a standard, clinical approach for injury assessment; it is important to note, that contextualized with results from other biomarkers, of muscle status for example, potential markers of injury like certain cytokines or CRP may be indicative of simply, exercise-induced muscle damage, or more seriously, overtraining. The overlap of biomarkers in many areas of diagnosis is one of the reasons that we suggest panels that will help define the true reason for changes in intersecting biomarkers.

Biomarkers of Inflammation

Muscle damage is an expected part of exercise training, as are the physiological and immune responses that occur during and after muscle tissue damage. Athletes monitoring their performance during training may track inflammation indirectly through key components of the inflammation process that can enter systemic blood circulation. Chronic

inflammation that persists after damage results from positive feedback of multiple signals indicating injury or stress from overtraining, or results from infection/illness can also be tracked in specimens by assessing proteins and other molecules that control inflammation (Figure 3). Chronic inflammation can also result from infection, autoimmune disease, cardiovascular disease, or other major health concerns. In both instances, chronic inflammation is a positive feedback phenomenon that can impact health and performance of an individual. Creatine kinase, for example, is released in response to skeletal muscle damage or cardiac muscle damage during myocardial infarction. Creatine kinase levels have remained a valuable biomarker for muscle damage despite several limitations, including individual variability in CK response to damaging exercise (140), the need for information on CK isoforms to determine whether elevated CK is due to cardiac or skeletal muscle damage (30), and other complicating factors. In addition to CK, myoglobin released is a more of a short-term marker of damage measurable in blood (117). More specific markers including skeletal muscle troponin I, skeletal muscle specific enzymes, and markers indicating an oxidative stress-antioxidant response during muscle damage have also been extensively reviewed and used to track muscle damage during exercise (17). Concurrently measuring muscle damage markers when assessing biomarkers of inflammation in an athlete is critical to contextualizing the potential source of inflammation and define the subsequent action required for athlete health and optimal performance.

As markers of muscle damage are released into circulation, at the tissue level resident or locally surveying naive immune cells migrate to the site of tissue injury and differentiate into mature proinflammatory macrophages that function to phagocytose and clear debris and degenerate damaged tissue. Mature activated macrophages also release a number of growth factors, cytokines, and other signaling molecules to promote the inflammatory process by recruiting other cells required for skeletal muscle regeneration to differentiate and function in repair. As inflammation progresses, macrophages convert to anti-inflammatory profiles and release different growth factors, cytokines, and have distinct effects to encourage the progression of repair stages. Shifts in circulating immune cells as cell populations move in and out of tissue vs. systemic circulation can be measured through a complete blood count with differential (CBC/diff). Although the CBC/diff assay cannot be used alone to assess an athlete's level of inflammation, it is another assay that provides valuable information about shifts in immune cell populations that may occur during muscle damage-induced inflammation. Another benefit of assessing CBC/diff profiles in athletes is that CBC/diff can be used to diagnose potential infection or disease that might also cause

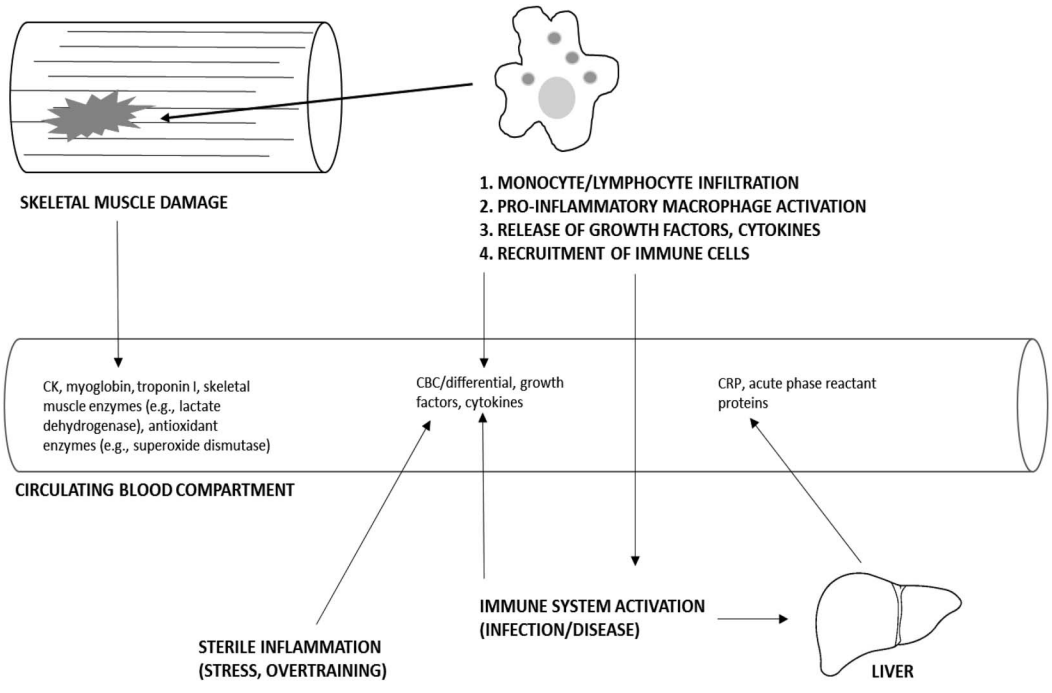

Figure 3. Exercise-induced muscle damage and inflammation are physiologically integrated. Biomarkers of skeletal muscle damage and inflammation often increase concurrently during exercise-induced muscle damage or injury that will negatively affect performance. Inflammation is a process that will follow initial tissue damage and lag during recovery.

inflammation and increases in biomarkers that are common with muscle damage-induced inflammatory biomarkers. Additional recruitment of monocytes or other immune cells during inflammation can be tracked using blood measures of chemicals that attract immune cells to an area (e.g., monocyte chemoattractant protein-1 or soluble intracellular adhesion molecule-1) while activation of other immune cell types can be measured through cellular components like CD40 ligand (CD40L, CD154) that are only expressed or released as soluble factors by activated immune cells.

The blood biomarkers that indicate proinflammatory macrophages activity include growth factors and cytokines released by macrophages. The most standard of these are generalized signaling molecules termed "cytokines." Cytokines are numerous and diverse in function, making it difficult to use the presence of these alone as a direct measure of inflammation in an athlete. However, we can assess inflammation through increases from an individual's normal baselines in cytokines classically considered proinflammatory such as IL-1β, TNF-α, IL-6, IL-10, IL-8, and IL-12p40. There is no recommendation for a threshold above which increases in inflammatory markers are universally interpretable as "elevated." The recommendation is to use repeat testing at rested, healthy baseline states to establish individual reference ranges for normal values, and also test at key changing points in training, health status, performance status during competition, and heavy training periods to determine what are normal fluctuations in inflammatory markers, and which are fluctuations and values associated with physical concerns. This dynamic approach to biomarker testing begs coaches and athletes to use biomarker testing to observe normal changes in biomarkers during healthy states and document dramatic changes in biomarkers that are associated with performance effects. This may require a period of adjustment during which biomarker testing is essentially calibrated to each individual, but this approach will provide the greatest accuracy and precision independent of the biological diversity that we know occurs among all individuals.

Hallmarks of prolonged, severe inflammations include markers of tissue damage associated with chronic inflammation. One aspect of prolonged or severe inflammation involves hepatic signaling by circulating cytokines. During inflammation, liver tissue may be stimulated to produce an acute phase response. The acute phase response and acute phase reactant proteins produced by the liver trigger a systemic inflammation response that recruits vascular tissue activation, systemic immune response, endocrine function, and other multiorgan involvement in positive feedback of inflammation. Classic acute phase reactant proteins that are measured include CRP, serum amyloid A, E-selectin, von Willebrand factor (endothelial dysfunction), plasminogen activator inhibitor-1, fibrinogen, P-selectin, and inflammatory cytokines.

Because muscle damage, inflammation, and acute phase response may normally occur during exercise training designed to optimize performance, it is critical to contextualize assessment of inflammation biomarkers with other assays concurrently. For example, the assessment of CBC/diff could indicate the presence of an infection that is temporary and requires no long-term change in an exercise training program. Chronic or prolonged inflammation should be evaluated with such markers that might indicate chronic disease states that will direct long-term and dramatic changes in training. Additional markers that overlap with other aspects of an athlete's health will also provide valuable information about action from insight. An athlete that consistently and chronically exhibits high levels of inflammatory markers should also, for example, be evaluated for chronic stress, which can be tested for by physical assessment of fatigue or performance decrements, subjective perceptual scales, or assays measuring levels of stress hormones such as cortisol (48,72,134). We reiterate the recommendation that repeat testing during rested, healthy states will provide average values for each individual, as markers of inflammation may be highly varied person to person, and establishing per-individual reference ranges will be most practical and useful.

Practical Applications

To better understand the dynamic and integrative aspects of how diet, hydration, training, and competition affect athletes, assessment of biomarkers should include select, diverse, and well-validated markers of performance (muscle status and oxygen transport), health (nutritional and hydration status, allergies), and recovery (inflammation, injury risk, muscle damage) (Figure 1). Because many validated biomarker reference ranges are appropriate for generalized populations rather than for athletes, repeat measurements will allow each clinician/coach to establish personalized reference ranges; from these individualized "normal" values that may fluctuate day-to-day or week-to-week, an athlete or sports professional can track chronic changes in directions that are associated with risk of injury, overtraining, or decreased performance. We have provided examples of useful biomarkers. It is important that the coach and athlete determine priorities for tracking training and competition and adapt their biomarker panels accordingly. As new biomarkers are being tested and validated, researchers will identify more universal, consistent biomarkers of multiple aspects of athlete health and performance.

Acknowledgments

E. C. Lee, S. A. Kavouras, R. M. Queen, J. L. Pryor, and D. J. Casa are members of the Quest Diagnostics' Sports Science and Human Performance Medical and Scientific Advisory Board. M. S. Fragala is an employee of Quest Diagnostics. No grant support contributed to the development of the manuscript. The content of the manuscript does not constitute endorsement of a product by the authors or the NSCA.

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