Functional foods and food supplements for athletes: from myths to benefit claims substantiation through the study of selected biomarkers

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The development of the sports food market and industrial involvement have led to numerous nutritional studies to define the type of nutrients that are most suited to support energy metabolism, fluid balance and muscle function. The key question in many of these studies was: 'Does the product lead to a significant product/consumer benefit that can be used as a claim on the package?' New methods and techniques have been developed, partly with sponsorship of the food industry, with the goal of measuring the effects of specific nutrients and supplements on athletic performance and metabolism. In line with this development, a wide variety of supplements and sports foods/drinks labelled with various performance or health benefit statements have been launched on the sports nutrition market. Although a variety of products have been tested clinically, there are also many products on the market with benefit claims that cannot be supported by sound nutritional and sports physiological science. The current short review highlights some of the methods and biomarkers that are used to substantiate product/consumer benefit claims for foods and drinks that are marketed as functional foods for athletes.

Sports food: Energy metabolism: Athletic performance: Benefit claims: Biomarkers

Introduction

During the last century there have been enormous changes in the understanding of the role of diet in exercise and physical performance. Almost a century ago it was considered that protein was the most important energy source for muscle. However, since the classical Scandinavian studies on the role of diet on physical performance in the 1960s, the focus has changed completely to carbohydrate. It was shown that exercise-induced reductions in muscle glycogen correlated well with the development of fatigue and that optimizing carbohydrate stores and ingesting carbohydrate during prolonged exercise improved performance. Since then, 'carbohydrate loading' and the consumption of carbohydrate–electrolyte drinks during exercise have become common practice among endurance athletes.

This was also the beginning of a new challenge to the food industry: the development of sports-specific food products and drinks. Sports foods/drinks should provide fluid and energy (i.e. carbohydrate) rapidly and not cause any gastrointestinal discomfort, to allow consumption prior to as well as during competition. To improve the athlete's recovery from intense and exhausting exercise, other products were created to target the physiological functions that are involved in this recovery process. Specific blends of carbohydrate were studied to obtain evidence about effective energy fuels during exercise, with the aim of improving performance and delaying fatigue development. In line with these developments many products have been launched on the market to target the fitness and physical performance sector, mostly with attractive benefit claims.

Examples of such benefits are 'performance enhancement', 'more power', 'less gastrointestinal problems', 'improved recovery', 'less muscle cramps/pain' and 'reduction of body fat/increased muscle mass'. To obtain clinical evidence for a benefit, the development and use of measurement techniques that allowed measurement of real treatment effects was essential. Accordingly, techniques to measure the rate of gastric emptying, intestinal absorption, appearance of substrates in blood and their subsequent oxidation or storage, as well as laboratory exercise protocols to measure performance accurately, were developed and validated.

Using such techniques it was observed that the rates of gastric emptying, digestion and absorption are of crucial importance with respect to digestive tolerance during exercise. These factors were also found to determine the rate of nutrient/substrate supply to the active muscles and the subsequent conversion/oxidation for the generation of ATP. This has led to a large number of studies on the effect of various food applications on gastrointestinal function, tolerance and performance. Specific foods were shown to be functional in terms of benefits mentioned above. More recently the field of interest has shifted from macronutrients and fluids to isolated nutritional or non-nutritional food components (FC). Some examples are caffeine, creatine, ribose, L-carnitine, certain amino acids, antioxidants, lactate, pyruvate, glycerol, sodium bicarbonate and hydroxycitric acid (Maughan, 1999; Spriet, 1999; Wagenmakers, 1999*a*; Volek, 2000; Brouns, 2002). The present short review discusses some of the 'most favourable' claims as well as aspects of their validation.

Performance-limiting factors: a target for supplementation

There are a number of sectors in sports nutrition that are of great interest to the food industry. Basically these sectors are related to performance-limiting factors in which nutrition is thought to play a role. Research on the effect of selected FC has focused on how to improve or minimize the impact of these limitations. A number of examples are listed below. The nutrients listed have been used in studies and are given as examples only. The outcome of these studies may have been positive (P), negative (N), led to mixed results (M) or is still hypothetical (H).

1. Muscle mass ⇒ protein synthesis-stimulating FC (e.g. branched-chain amino acids in combination with a carbohydrate (P), arginine (N), creatine (H) or β-hydroxy-β-methylbutyrate (M)).

2. Fat mass ⇒ FC that induce fat loss, improving fat-free mass (e.g. hydroxycitric acid (N), caffeine (M), L-carnitine (N), chromium picolinate (N), chitosan (H), pyruvate (N) and L-tyrosine (N)).

3. Bone and joint conditions ⇒ FC to improve bone mass, cartilage thickness, synovial fluid condition (e.g. specific amino acids such as proline (H), lysine (H), mineral combinations (H), phyto-oestrogens (H), glucosamine (H), vitamin K (H) and cartilage preparations (H)).

4. Dehydration ⇒ drinks composed for rapid rehydration and fluid retention (e.g. hypotonic or isotonic drink formulation containing 30–70 g carbohydrate/litre plus 20–30 mmol Na/litre (P)).

5. Glycogen depletion ⇒ carbohydrate types to maximize muscle and liver glycogen resynthesis (e.g. glucose and glucose polymers for muscle (P), fructose for liver (P), carbohydrate in combination with amino acids (P)).

6. Carbohydrate availability and oxidation ⇒ FC such as selected carbohydrate types that are expected to be digested rapidly and absorbed completely in favour of complete oxidation (excellent energy source) during exercise (P).

7. Low fat oxidation rate ⇒ FC that influence the rate of lipolysis, and improve fat uptake in muscle and mitochondria and ultimately fatty acid oxidation rate (e.g. caffeine (M), hydroxycitric acid (N), L-carnitine (N) and oil containing medium-chain triacylglycerols (N)).

8. Adenine nucleotides depletion ⇒ FC that are thought to enhance resynthesis as well as storage of ATP and creatine phosphate (e.g. ribose (N) and creatine monohydrate (P)).

9. Immunosuppression ⇒ supply of FC that increase resistance to disease and reduce exercise-induced inflammations (e.g. specific vitamins such as vitamins C and E (H), minerals such as Zn (H), trace elements such as Se (H), colostrum extracts (H), glutamine (H), achinacea (H), polyunsaturated fatty acids (H) and probiotics (H)).

10. Neurostimulation ⇒ supply of neuromodulating FC that are thought to improve reaction times, shorten neuromuscular impulse transmission, improve the availability of precursors for hormones and brain peptides, and improve cognitive function in stress conditions (e.g. caffeine (P), specific amino acids such as tyrosine (P), γ-aminobutyric acid (H) and tryptophan (H), branched-chain amino acids (H), choline (H) and phosphatidylcholine (lecithin; H)).

11. Suppressed hormone secretion ⇒ FC thought to enhance release of hormones or improve sensitivity to hormones that are involved in protein synthesis, substrate metabolism and recovery from exercise (e.g. tryptophan (H, M), branched-chain amino acids (H, M), tyrosine (H, M), arginine (H, M), ornithine (H, M) and γ-aminobutyric acid (H, M)).

12. Blood flow ⇒ FC that are thought to enhance blood flow by vasoactive effects (e.g. arginine (M) and L-carnitine (H)).

13. Gastrointestinal distress ⇒ supply of foods and drinks that are optimally tolerated by the digestive system during exercise (e.g. hypotonic food formulas composed of rapidly digestible and completely absorbable nutrients (P)).

14. Poor nutritional status ⇒ FC to ensure optimal nutrient status of vitamins, trace elements and minerals (e.g. specific micronutrient supplements (P)).

15. Poor performance ⇒ FC that may increase endurance or maximal oxygen uptake and thereby improve performance (e.g. carbohydrate (P), coenzyme Q10 (N) and branched-chain amino acids (N)).

16. Muscle damage ⇒ FC that may reduce the occurrence of muscle damage during exercise and improve recovery from it (e.g. antioxidants like vitamin E (M) and β-carotene (M)).

17. Muscle cramps ⇒ FC to support neuromuscular events and reduce indices of cramp (e.g. Mg (H), Zn (H) and ribose (H)).

18. Injuries ⇒ FC to prevent the development of injuries and speed up the recovery process from injury (e.g. glucosamine (H) and chondroitin (H)).

Examples of claims that are made for products whose target is to improve performance-limiting factors are 'more power', 'improved performance', 'more muscle mass', 'less body fat', 'faster recovery', 'quick rehydration', 'reduces muscle cramps' and 'improved hormone release'. Clearly, the food and supplement industry tries to make such claims on the product packaging as well as in advertising by promoting the product 'as a solution with a proven benefit'. The ultimate goal is to give athletes the feeling that consumption of the product will improve performance, or result in a benefit that is of importance to health and well-being.

The basic question in this respect is: 'What is the evidence?' There are valid examples of nutritional interventions in exercise and sport that indeed resulted in significant effects on the study endpoint of interest. However, there are also a very large number of products available on the market promoted with benefit statements that have never been validated by sound scientific studies.

Below, a short discussion is given on some of the above-mentioned examples. Test methods and biomarkers thought to be suitable for the measurement of interventions in which any of such nutritional factors are tested against a suitable placebo are discussed briefly. References are given for the reader who is interested in detailed information.

Muscle/fat mass

The importance of a relatively high muscle mass and low fat mass for most power and strength events is based on the observation of a significant relationship between muscle cross-section and maximal muscle strength measured either as maximal strength or dynamic strength. The relative strength or power of an athlete declines with increasing fat mass. As such, all athletes who move/replace their own body weight will benefit from having a high lean body mass/low fat mass. Therefore, athletes involved in strength and power events will benefit from training regimens and nutritional factors that boost muscle protein synthesis and reduce fat mass (Lemon, 1993). Athletes involved in sports in which low body fat is a key factor, such as bodybuilding, will do almost anything to reduce fat mass and increase visible muscle mass (Kleiner *et al.* 1990; Walberg-Rankin *et al.* 1993).

Methods used to measure muscle mass/fat mass and muscle protein synthesis concern the following biomarkers.

1. Selected muscle mass by muscle circumference measurements (surrogate marker, not reliable due to a number of confounding factors, e.g. level of subcutaneous fat, muscle glycogen and water content).
2. Site-specific fat mass by circumference measurements (as above, not reliable).
3. Selected muscle mass or site-specific fat mass by scanning techniques such as computed tomography (CT) and magnetic resonance imaging (MRI; reliable but complex and expensive, CT is invasive, MRI is non-invasive; Gadian, 1995).
4. Total muscle mass by body composition techniques. The reliability depends strongly on the method of choice. So-called mechanistic methods, such as underwater weighing, isotope dilution or both methods

combined, are the most reliable (non-invasive, although underwater weighing may be problematic in some groups, is time-consuming, requires special laboratory facilities and is relatively expensive). The descriptive methods, such as dual X-ray absorptiometry (DXA), bio-impedance analyses (BIA) and skin folds (SF), which are derived from the mechanistic ones, are less reliable with respect to the absolute values (non-invasive although DXA uses a small doses of X-rays, fast, DXA relatively expensive, BIA and SF are cheap). Body composition changes resulting from the intervention are also detected most accurately with mechanistic methods. Finally, imaging techniques, such as CT and MRI, are used increasingly (accurate but interpretation of images may be complicated, CT invasive, MRI non-invasive, special laboratory facilities needed, often time-consuming, expensive). For more comprehensive overviews of methods and systematics, see Wang & Heymsfield (1995) and van Marken Lichtenbelt & Fogelholm (1999).
5. Protein synthesis, muscle protein degradation and turnover rates by measuring the incorporation rate and quantity of selected markers such as stable isotope-labelled amino acids: leucine, glycine, phenylalanine (reliable but complex and expensive; Wagenmakers, 1999*b*; Rennie & Tipton, 2000).

Dehydration/hydration

The importance of appropriate hydration and rehydration stems from a number of observations. With continuous exercise the water content of all body compartments will decrease as a result of fluid loss by sweating and insensible water loss from the lungs. Depending on the exercise intensity, training status, climatic circumstances and body size, sweat losses may range from a few hundred millilitres to more than two litres per hour. Such large sweat losses will reduce circulating body fluids. Because a normal plasma volume is of prime importance in maintaining an appropriate blood flow through 'exercising tissues', it may be deduced that a significant decrease in plasma volume will impair blood flow. This will in turn lead to a reduced transport of substrates and oxygen, which are needed for energy production, to the muscles. Also, the transport of metabolic waste products and heat, from the muscle to 'eliminating organs' such as the liver and skin, will be impaired. The decreased heat transfer from the muscles to the skin results in an increased core temperature in all cases where heat production exceeds heat dissipation from the body. This may lead to a decreased energy-production capacity, fatigue, impaired performance and health threats (Brouns, 1997).

Clearly, not only the total amount of body water (TBW) is important, but also the water distribution. Therefore determination of intracellular water (ICW) and extracellular water (ECW), or the ratio between these two, is often practised.

Methods used to measure the hydration status of the whole body concern the following biomarkers.

1. TBW content by dilution of stable isotopes, deuterium or the more expensive oxygen-18 (accurate, non-invasive, time-consuming, special laboratory facilities

needed, moderately expensive; Westerterp *et al.* 1995; Westerterp, 1999).

2. ECW content by bromide dilution (accurate but bromide dilution space is not identical to ECW and a correction factor is needed, non-invasive, time-consuming, moderately expensive; van Marken Lichtenbelt *et al.* 1996).
3. TBW and ECW by bio-impedance spectroscopy (accuracy is still a matter of controversy but non-invasive, fast and cheap; van Marken Lichtenbelt, 2001).

Methods used to measure shifts in body fluids concern the following biomarkers.

1. Changes in plasma volume by determination of haemocrit and haemoglobin values (reliable and easy to measure; Dill & Costill, 1974).
2. ECW:ICW with the dilution techniques mentioned above (unsuitable for short-term interventions).
3. Bioelectrical impedance (see above; Boileau & Horswill, 2000; van Marken Lichtenbelt, 2001). The method may pose problems during the study of short-term (sports) intervention, because of its sensibility to changes in body temperature and electrolyte concentrations in body fluids.
4. Muscle biopsy analysis (not reliable for rehydration effects).

Methods used to determine fluid uptake rate by the body concern techniques to measure biomarkers of gastric emptying and intestinal absorption. It should be noted that gastric emptying alone does not give a valid answer with respect to rehydration efficacy since it is only the first factor in a chain of determinants of bioavailability. For example, water is emptied rapidly from the stomach but is absorbed slowly in the gut. The best data are obtained from a battery of tests in which gastric emptying, gut segmental absorption and fluid retention measurements are combined.

Gastric emptying may be measured by:

1. Intubation techniques applying the double sampling technique of George (invasive, needs routine, reliable to quantify during exercise, cheap; Beckers *et al.* 1988).
2. Use of stable isotopes (non-invasive, complex, can be used during exercise but quantification not possible, only qualitative differences can be shown, expensive; van Nieuwenhoven *et al.* 1999).
3. Gamma scintillation (non-invasive, complex, isotopes required, reliable but not suitable during exercise, expensive; Leiper & Maughan, 1988; Beckers *et al.* 1992).

Intestinal absorption may be measured by:

1. Intubation/perfusion techniques using non-absorbable markers (invasive, routine required, difficult during exercise, reliable to quantify for the test segment but not for the whole intestine, relatively cheap; Leiper & Maughan, 1988).
2. Determining the appearance of labels in blood using stable isotopes (non-invasive, but complex and diffi-

cult to quantify due to label shifts; Leiper & Maughan, 1988).

Glycogen depletion/muscle glycogen content

Since the classical Scandinavian studies using muscle biopsy techniques, a lot of work has focused on the importance of muscle glycogen for energy homeostasis and performance. Several lines of evidence show that intense and lasting muscle work cannot be performed without appropriate availability of carbohydrate. As soon as specific muscles or muscle fibres become glycogen-depleted, they will be impaired in their ability to perform repeated high-intensity contractions. Glycogen depletion, caused either by exercise or a combination of exercise and low carbohydrate intake, leads to a reduction in work capacity to a level of about 50 % of the normal maximal working capacity. Alternatively, when the carbohydrate stores in muscle and liver are increased by diet manipulation, athletes are able to perform longer at high exercise intensity. Thus, the availability of carbohydrate and the size of the glycogen stores are important and limiting factors for endurance performance.

Methods used to measure muscle and liver glycogen concern the following biomarkers.

1. Muscle biopsy analysis for muscle glycogen (reliable and easy but invasive, cheap; Hultman, 1967).
2. NMR for muscle glycogen (reliable for qualitative changes, difficult for quantification, expensive, complex but non-invasive; Price *et al.* 1999).
3. NMR for liver glycogen (as above; Price *et al.* 1999).

Hormone-releasing agents

There are observations that intensive training may induce lower circulating levels of stress hormones, insulin and glucagon. This is most probably caused by enhanced sensitivity of tissues/cells for the hormones. It has also been observed that intense training/over-training may lower secretion of androgens and growth hormone, most probably because of endocrine disruption effects. However, the physiology of many hormones as well as the effects of exercise on hormonal adaptations are still incompletely understood (McMurray & Hackney, 2000) and make it difficult to link secretion modifications of many of them, if any, to health performance benefits. Nevertheless, a substantial number of products are promoted to enhance hormone release for athletic benefits (Brouns, 2002). For example, it has been hypothesized that the ingestion of arginine and ornithine may stimulate the release of human growth hormone, which is thought to stimulate muscle growth.

Reliable measurement of blood hormone levels after supplementation is complex. Because of diurnal effects, 24 h profiles will be required to make any statement on the physiological relevance of observations.

Gastrointestinal tolerance

A substantial number of endurance athletes (40–60 %)

may be prone to developing gastrointestinal disturbances during exercise, especially when exercising in the heat and developing dehydration (Brouns, 1991*a,b*). To avoid such problems recommendations are given to reduce the intake of poorly fermentable dietary fibres and fat-rich foods prior to competition and to ingest easily digestible and absorbable energy sources during exercise. Because the drink/food composition can have a significant influence on the occurrence of gastrointestinal symptoms (Brouns, 1991*a,b*, 1997; Brouns & Beckers, 1993; Brouns & Kovacs, 1997*a,b*; van Nieuwenhoven *et al.* 2000), the industry tries to make claims related to this aspect.

The tolerance to such products during exercise can be assessed as follows.

1. By questionnaire. Registration of the occurrence of gastrointestinal symptoms during exercise after consumption of the test product in comparison to a control product (for fluids, usually artificially sweetened and coloured water; for solids, a normal food item such as an equicaloric amount of bread). Symptoms scored usually are related to degree of fullness, stomach pains, regurgitation, intestinal cramps, borborigmy, flatulence and diarrhoea/loose stools. It has to be noticed that controlled laboratory studies do not mimic the situation in the field. Laboratory studies have the advantage of controlling variables such as work intensity, environmental temperature and humidity, and quantitative timed intake of the test product. Field conditions make these controls very difficult but are more reliable with respect to competition stress and fluctuating exercise intensities during a competition. In addition, the food intake two or three days prior to the test may be of influence on the occurrence of lower intestinal symptoms. This means that food intake should be standardized along with exercise programmes and the consumption of caffeine- and alcohol-containing beverages during the days prior to the test (easy but very labour-intensive testing, reliability of laboratory data for field circumstances is speculative).
2. By motility measurements. These can be done reliably in a laboratory but the outcome of the data in terms of relevance for competition circumstances is speculative. Moreover, data are specific for the tested biomarkers/endpoints only and do not allow one to make statements on gastrointestinal tolerance in general.
3. Oesophageal sphincter pressure/reflux episodes (invasive, needs routine, reliable, can be measured ambulatory; Schoeman *et al.* 1995).
4. Contractile activity of the stomach and duodenum by antroduodenal manometry (invasive, needs routine, reliable, can be measured ambulatory but not suitable during exercise; Penning *et al.* 2001).
5. Intestinal transit rate by breath hydrogen from small intestine (non-invasive, easy, allows ambulatory measurement, cheap; Levitt, 1969) or whole-gut transit measurements using scintigraphic techniques (non-invasive, reliable, but expensive and exposure to radioactivity and measurement takes a long time; Charles *et al.* 1995).

Fat utilization and oxidation

An enhanced oxidation of fatty acids during exercise will induce a sparing of endogenous carbohydrate stores. The latter may reduce the development of glycogen depletion and hypoglycaemia and improve endurance capacity. Many attempts have been made to modify fat metabolism (Brouns & van der Vusse, 1998; Hawley *et al.* 1998; Jeukendrup *et al.* 1998; Jeukendrup, 1999).

Biomarkers to measure fat metabolism are mostly related to (1) the rate of lipolysis, (2) the rate of fat uptake in muscle and mitochondria and (3) ultimately the fatty acid oxidation rate.

Fat lipolytic rate

Methods used to determine lipolytic rate concern the following biomarkers.

1. Determination of release of fatty acids and glycerol from adipose tissue by microdialysis (invasive, needs routine, reliable; Arner, 1999; Frayn, 1999; Henriksson, 1999).
2. Determination of the rate of appearance of glycerol (and fatty acids) in the circulation (surrogate marker, reliable, requires stable isotopes, expensive).
3. Determination of changes in plasma fatty acid and glycerol concentrations (surrogate marker, easy, reliable only in combination with stable isotopes; Coggan, 1999; Landau, 1999; van Hall, 1999).

Fatty acid uptake rate by muscle

Methods used to determine fatty acid uptake concern the following biomarkers.

1. Determination of rate of free fatty acid disappearance from blood by stable isotopes (surrogate marker, reliable, requires stable isotopes, expensive; Coggan, 1999; Landau, 1999; Rennie, 1999; van Hall, 1999).
2. Determination of net uptake by muscle using arteriovenous techniques (invasive, reliable; McDonald, 1999) or microdialysis (Arner, 1999; Henriksson, 1999).

Fatty acid oxidation rate

1. Determination of respiratory quotient (surrogate marker, easy, reliable for group means, less reliable for quantification of small changes within a subject).
2. Quantification by appearance of label in expired gas, using stable isotopes (reliable, non-invasive, expensive; Coggan, 1999; Rennie, 1999; van Hall, 1999; van Hall *et al.* 1999).
3. Quantification of intramuscular fat use by biopsy analysis (invasive, difficult to quantify, not reliable for intervention effects; Hoppeler *et al.* 1999) or by NMR (non-invasive, complex technique, not reliable for quantification of treatment effects; Boesch *et al.* 1999).

Carbohydrate oxidation

An enhanced oxidation of ingested carbohydrate during exercise will spare the endogenous carbohydrate stores and this is generally thought to enhance endurance capacity. It has been shown that when carbohydrate oxidation rates drop below critical levels during exercise, this will coincide with fatigue (Coyle *et al.* 1986). In an attempt to optimize the carbohydrate oxidation from ingested carbohydrate sources, numerous studies have been performed in which the timing, amount and type of carbohydrate were varied. These studies were reviewed recently by Hawley *et al.* (1992) and Jeukendrup & Jentjens (2000). Carbohydrate consumed during exercise is oxidized in small amounts during the first hour of exercise (~ 20 g) and thereafter reaches a peak rate of ~ 1 g/min (Jeukendrup & Jentjens, 2000). Even ingestion of very large amounts of carbohydrate will not result in higher oxidation rates. Generally, the timing of ingestion has little or no influence as long as the amount of carbohydrate ingested is sufficient (60 g/h). Glucose, sucrose, maltose, maltodextrins and amylopectin starch are oxidized at high rates (up to a maximum of 1 g/min) whereas fructose, galactose and amylose starch are oxidized at 25–50 % lower rates. Combinations of carbohydrates, however, may give higher exogenous carbohydrate oxidation rates than single carbohydrates (Adopo *et al.* 1994).

Methods to determine carbohydrate oxidation concern the following biomarkers.

1. Oxidation of ingested carbohydrate is usually measured by using a ^{13}C (or ^{14}C) isotope of the carbohydrate of interest.
2. A ^{13}C or ^{14}C carbohydrate tracer (i.e. glucose, fructose) is ingested and excretion of ^{13}C (or ^{14}C) in breath is measured. In combination with a measurement of total CO_2 production, an accurate measure of exogenous carbohydrate oxidation can be obtained. The drawback of this method is usually the cost of the tracer, although in some cases the natural ^{13}C enrichment of the carbohydrate (e.g. from cornstarch or cane sugar) can be of use for testing (reliable, complex method, expensive).

Nutritional status

There are a number of high-risk sports for marginal nutritional intakes that may impact on performance capacity and health (Brouns, 2002). The endpoints used for nutritional status in healthy athletes mostly concern circulating levels of vitamins, minerals and trace elements. Development of analytical techniques has made it possible to study 'biochemical vitamin deficits' that occur at an early stage of poor intakes. These measurements include the determination of plasma vitamin levels by HPLC and enzymatic stimulation tests (easy but invasive and also expensive). Although minerals are fixed components of tissues such as bone or muscle, this does not necessarily mean that they are freely available for metabolic purposes. The major fraction of the 'metabolic' mineral pool is present in blood plasma and interstitial fluid. The amount of

minerals circulating in body fluids is a resultant of different ongoing processes. Absorption from food, on the one hand, and uptake or release by tissues as well as losses/excretions (by sweat, urine, faeces), on the other, determine the actual mineral content in the blood. This mineral level remains within a narrow range. Any mineral shortage will, in the first instance, be compensated by reduced excretion or/and increased release from tissues. With a continuing shortage, plasma mineral levels will start to fall. The latter will influence the uptake or release of minerals by cells and thus the cell mineral status. During prolonged periods of mineral deficits, cell growth and cell function will become impaired, as will performance capacity. However, a short period with a relative shortage of one or more minerals, e.g. as may occur during an ultra-endurance event or during a multi-day cycling event, will not necessarily mean that health and performance will become affected measurably.

With respect to trace minerals, it is possible to obtain 'a certain figure' or 'a certain level' from samples such as serum, tissue, hair and nails. The question one should ask is about how representative these samples are for whole-body status and for muscle in particular. Moreover, hair and nail analyses give a figure related to intake in the past and do not necessarily correlate with current status, while in the case of hair analysis environmental factors such as water trace minerals content (swimmers, frequent showering) and use of hair products (shampoos, gels, colour agents) may be confounding factors.

Growing knowledge over the years has indicated different sample sites as being most representative for specific trace elements. This makes it practically impossible to perform a simple body fluid analysis with the goal of making a statement on trace element status, as is the case for vitamins and minerals as well.

Adenine nucleotides depletion

A number of important metabolic functions are related to the availability of appropriate levels of phosphocreatine and total adenine nucleotides (TAN; Balsom *et al.* 1994; Williams *et al.* 1999; Brouns, 2002). The maintenance of an appropriate level of ATP, through a rapid rephosphorylation of ADP from phosphocreatine, supports the creatine phosphate shuttle to enhance the exchange of high-energy phosphate from the site of the mitochondria to the site of the cytosol. It also helps reduce acidosis in muscle cells by buffering hydrogen ions, and indirectly helps regulate the activation of carbohydrate breakdown (glycolytic) processes in muscle by activation through the products resulting from the hydrolysis of phosphocreatine, which are inorganic phosphate and free creatine.

In the case of depletion of the phosphocreatine store during all-out exercise, a number of metabolic changes may occur in muscle that will lead to a change in TAN: (1) depletion of the creatine phosphate store; (2) breakdown of ATP → ADP → AMP → end products; (3) increase in muscle and blood lactate; (4) increase in muscle and blood ammonia; and (5) increase in blood xanthine, hypoxanthine, adenine and uric acid. After exercise the breakdown products mentioned might be lost from muscle.

This results in a decreased TAN content. The resynthesis of adenine nucleotides is a slow process, causing recovery to a normal level to take up to three or four days. It has been hypothesized that oral supply of ATP or ribose can lead to a more rapid recovery of the TAN pool after intensive training sessions or competitions.

Markers of TAN are:

1. Changes in plasma levels of ammonia, uric acid, xanthine and hypoxanthine (surrogate markers for qualitative changes in TAN, easy to perform, not reliable to make any statements about muscle TAN contents; Op t'Einde *et al.* 2001).
2. Muscle TAN levels by biopsy analysis (invasive, relatively easy, reliable) or NMR (non-invasive, complex methodology, only for qualitative changes, not reliable for quantification; Heerschap *et al.* 1999).

Muscle/tissue damage

There are a number of excellent reviews that highlight the effect of exercise-induced free radical formation on various tissues, as well as their measurement in biological fluids (Halliwell & Gutteridge, 1985; Jenkins, 1988; Kanter, 1994; Duthie, 1999; Jackson, 1999; Poulsen *et al.* 1999; Li, 2000). Muscle soreness after an intensive bout of exercise is caused by an inflammation process. The micro trauma (disruption at the Z band level of the sarcomeres) that results from acute overload cannot be avoided by antioxidant systems, because it is mechanical in nature. However, the repair process of the mechanically damaged muscle fibres involves an inflammatory process, which causes muscle pain, stiffness and loss of muscle strength, especially two to five days after the sport event (Smith & Miles 2000). It is suggested that free radicals play an important role during this inflammatory process and that supply with adequate amounts of antioxidants may lessen both the severity and the duration of this delayed muscle soreness.

Endurance exercise in polluted air, such as running a major city marathon on a hot summer's day in the smog, has been suggested to lead to damage to the lung tissue induced by ozone (Folinsbee, 2000). Free radical formation and reactions are also suspected here to be the mediating mechanism. Accordingly, nutritional substances such as vitamin E supplementation are suggested to reduce such damage (Evans, 1991) and lung function impairment (Folinsbee, 2000).

Biomarkers used in tissue damage assessments are listed below.

1. Free radical formation (complex assays, not reliable for tissue damage quantification; Duthie, 1999; Poulsen *et al.* 1999; Powers & Lennon, 1999).
2. Histological damage by biopsy analysis (reliable, invasive, but difficult to quantify for whole muscle).
3. Damage markers in the circulation: myoglobin, muscle enzymes (creatinphosphokinase; surrogate markers, reliable for qualitative changes, difficult as quantitative measure, long-lasting post-damage adaptation effects (up to six months) make cross-over studies

very difficult, easy to measure; Clarckson, 1992; Volfinger *et al.* 1994).
4. Strength performance and subjective pain (surrogate marker, easy to measure, no quantification of damage possible; Clarckson, 1992).

Muscle cramps

Low resting and exercise plasma Mg levels have repeatedly been reported in athletes who are involved in regular endurance exercise. This has been thought to lead to impaired energy metabolism, greater fatigue and to the occurrence of muscle cramps. Cramps are also thought to be caused by acute energy deficits as in the case of phosphocreatine depletion and reductions of ATP during very intensive, all-out metabolism. Accordingly, attempts have been made to study the effects Mg, creatine and ribose supplementation. The clinical endpoint here, 'muscle cramp', seems to occur infrequently and is very difficult to quantify. Many factors may be involved in its aetiology.

1. Mineral levels in body fluids and muscle and TAN levels do not seem to correlate with cramp occurrence (invasive, easy to measure but unreliable; Maughan, 1986).

Performance

For many products, the claim will be 'enhanced performance'. Of course, there are various forms of performance and no single laboratory or field test could be used to generalize across all sports performances. Often the distinction is made between endurance performance and high-intensity exercise or sprint performance. In addition, in the literature, the terms 'endurance performance' and 'endurance capacity' are often used as synonyms. However, endurance capacity refers to the exercise time to volitional fatigue whereas endurance performance relates to completing a certain task (running a certain distance, cycling a certain distance) as fast as possible. The latter is obviously a more realistic approach since there are very few events where athletes are asked to exercise for as long as they can. Studies have investigated the reliability of both of these test protocols and found coefficients of variation of between 1 and 3 % for performance trials and up to 26 % for time to exhaustion measurements (Jeukendrup *et al.* 1995). Performance trials have been developed and validated for the treadmill (self-paced runs for a fixed distance), intermittent running (Loughborough Intermittent Shuttle Run Test; Nicholas *et al.* 2000), the cycle (self-paced time trial; Jeukendrup *et al.* 1997), the rowing ergometer (Schabort *et al.* 1999) and various other intermittent sports such as soccer, squash (Romer *et al.* 2001) and tennis (Vergauwen *et al.* 1998). Studies that try to simulate a real event, especially in the field rather than in the laboratory, are harder to conduct. The most complicated types of performance belong to unpredictable team games or sports involving complex decision-making and motor skills. It is hard to find a way to measure adequately all components of performance, and it is complicated to design a protocol in which the same event is conducted twice, before and after an intervention, or with a treatment

and a placebo. Despite the difficulties in conducting studies, strategies that enhance carbohydrate availability have been shown to enhance cycling and running endurance, cycling and running performance, and the performance in complex games such as tennis, soccer and ice hockey.

When studying the effects of a nutrition supplement on performance, an ergometer, treadmill or similar tool should introduce negligible random error (variation) in its measurements. Random error in the performance measurement should also be minimized by choice of an appropriate type of test. Tests based on physiological measures (e.g. maximum oxygen uptake, anaerobic threshold) and tests requiring self-selection of pace (e.g. constant-duration and constant-distance tests) usually produce a random error of at least 2 to 3 % in the measure of power output (Paton & Hopkins, 2001). Random error for measures of power in 'all-out' sprints, incremental tests and very short (1–5 min) constant-power tests to exhaustion may be as low as 1 %. Measures with such low error might be suitable for tracking the small changes in competitive performance that matter to elite cyclists.

In all performance tests it is extremely important that all confounding factors are removed or standardized where possible (music, encouragement, feedback). In addition it must be kept in mind that some of these performance tests may not always pick up the small improvements in performance that are relevant to an elite athlete.

1. Sprint performance can be assessed by a Wingate test (easy to perform, reliable).
2. Endurance capacity can be assessed by a time to exhaustion test (poor reproducibility, limited practical value).
3. Endurance performance can be assessed by a constant-duration or constant-distance test (good reproducibility, needs extremely strict standardization).
4. Sport-specific performance and field tests could include measurements of performance in 'real life' such as during a soccer match or simulations of a particular sport or discipline in field conditions (very few established tests, needs extremely strict standardization; a review describing details of sport-specific tests is given by Kearney *et al.* 2000).

Claims and legal aspects

Generally, no food regulation exists (yet) to control performance or health benefit statements with respect to the supportive scientific evidence. Also, depending on the dosage, many of the FC used are in the grey zone between foods and drugs. The question whether an effective dosage of a certain FC that never will be consumed in that particular quantity with the normal diet is nutritional or pharmacological is relevant in this respect. Many of these products are sold as dietary supplements, mostly by direct mail order systems and the Internet, which are difficult to control.

Development of the sports foods and drinks market is highly attractive to the food industry. However, one should be aware of the fact that current developments in the field of consumer protection, regulatory environment and product liability aspects do call for a more careful and well defined product development and marketing programme than is usually the case. Obtaining scientific support for a product claim is an essential issue in this respect.

The establishment of a code of practice among sports food/supplement companies is another must, as long as an appropriate legislation is not in place.

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