PROMOTING TRAINING ADAPTATIONS THROUGH NUTRITIONAL INTERVENTIONS

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ABSTRACT

Training and nutrition are highly interrelated in that optimal adaptation to the demands of repeated training sessions typically requires a diet that can sustain muscle energy reserves. As nutrient stores (i.e. muscle and liver glycogen) play a predominant role in the performance of prolonged, intense, intermittent exercise typical of the patterns of soccer match play, and in the replenishment of energy reserves for subsequent training sessions, the extent to which acutely altering substrate availability might modify the training impulse has been a key research area among exercise physiologists and sport nutritionists for several decades. Although the major perturbations to cellular homeostasis and muscle substrate stores occur during exercise, the activation of several major signalling pathways important for chronic training adaptations take place during the first few hours of recovery, returning to basal levels within 24 h after exercise. This has led to the paradigm that many chronic training adaptations are generated by the cumulative effects of the transient events that occur during recovery from each (acute) exercise bout. Evidence is accumulating that nutrient supplementation can serve as a potent modulator of many of the acute responses to both endurance and resistance training. In this article we review the molecular and cellular events that occur in skeletal muscle during exercise and subsequent recovery, and the potential for nutrient supplementation (e.g. carbohydrate, fat, protein or combinations) to affect many of the adaptive responses to training.

Key words: AMPK, carbohydrate, glycogen, genes, fat, MAPK, mTOR, protein

Introduction

The capacity of human skeletal muscle to adapt to repeated bouts of physical activity over time so that subsequent exercise capacity is improved is termed physical training (Booth and Thomason 1991). The goal of such training for the soccer player is to induce multiple physiological and metabolic adaptations that enable the working muscles to increase the rate of ATP production from both aerobic and O_2-independent pathways, maintain tighter metabolic control (i.e. match ATP production with

ATP hydrolysis), minimise cellular disturbances and improve fatigue resistance during exercise (for review see Hawley 2002a). Although the major perturbations to cellular homeostasis and muscle substrate stores occur during exercise, the activation of several major signalling pathways important for chronic training adaptations take place during the first few hours of recovery, returning to basal levels within 24 h after exercise (Hildebrandt et al. 2003; Pilegaard et al. 2000). This has led to the paradigm that many chronic training adaptations are generated by the cumulative effects of the transient events that occur during recovery from each (acute) exercise bout (Pilegaard et al. 2000; Widegren et al. 2001; Williams and Neufer 1996).

Training and nutrition are highly interrelated in that optimal adaptation to the demands of repeated training sessions typically requires a diet that can sustain muscle energy reserves (Coyle 2000). As nutrient stores (i.e. muscle and liver glycogen) play a predominant role in the performance of prolonged, intense, intermittent exercise (McInerney et al. 2005; Nicholas et al. 1999) typical of the patterns of soccer match play (Hargreaves 1994), and in the replenishment of energy reserves for subsequent training sessions (Burke et al. 2004; Jentjens and Jeukendrup 2003), the extent to which acutely altering substrate availability might modify the training impulse has been a key research area among exercise physiologists and sport nutritionists for several decades. Here we review several nutritional interventions that modify the acute responses to exercise and thus have the potential to influence subsequent training adaptations. Specifically, we discuss the molecular and cellular events that occur in skeletal muscle during exercise and subsequent recovery and show that diet is a potent modulator of many of the adaptive responses to training. The cardiovascular and other adaptations taking place outside the skeletal muscles are not discussed here.

The training stimulus, response and adaptation

The acute metabolic responses associated with a single bout of exercise and subsequent training-induced adaptations are highly specific to the mode, intensity and duration of the stimulus (Hildebrandt et al. 2003; Nader and Esser 2001) and the corresponding pattern of muscle fibre recruitment (Gollnick et al. 1973). Although long-term muscle adaptations are likely to be the result of the cumulative effect of repeated bouts of exercise, the initial responses that lead to these chronic changes occur during and after each training session (Pilegaard et al. 2000; Widegren et al. 2001; Williams and Neufer 1996). Consideration of the molecular and cellular events that occur in skeletal muscle in response to a single bout of exercise is essential to understand how nutritional interventions might modulate these responses and promote (or inhibit) subsequent training adaptations. When such a view on training is taken, it becomes clear that any chronic training-induced adaptation is merely the consequence of increases in exercise-induced proteins (Hansen et al. 2005). The coordinated series of events that allows for these changes in protein levels is pivotal to any training adaptation.

Figure 1 illustrates the events that take place during and after a single bout of exercise and with repeated exposure to that stimulus. Contractile activity produces a multitude of time-dependent physiological, biochemical, and molecular changes within the muscle cells. With sufficient time, and in accordance with the dominant stimulus, this sequelae of events produces mitochondrial biogenesis (Hood 2001) and/or muscle hypertrophy (Glass 2003) and concomitant alterations in muscle phenotype that serve to improve cellular function and thereby enhance exercise capacity.

FIGURE 1 HERE

At the onset of exercise there are rapid (within milliseconds) increases in cytosolic and mitochondrial [Ca^{2+}] and Na^+/K^+ ATPase activity and, depending on the relative intensity, changes in metabolite concentrations (i.e. increases in [ADP] and [AMP]). There may also be increases in muscle [lactate], accompanied by decreased muscle (and blood) pH, and impaired O_2 flux. With an increase in exercise duration, endogenous muscle substrates (principally glycogen) become depleted. These contraction-induced metabolic disturbances in muscle, along with the accompanying mechanical stress (particularly muscle damage caused by physical contact and/or eccentric work) activate several key kinases and phosphatases involved in signal transduction. Chief among these are the 5'-adenosine monophosphate-activated protein kinase (AMPK), several of the mitogen-activated protein kinases (MAPK), and the mammalian target of rapamycin (mTOR).

AMPK is a critical signalling protein involved in the regulation of multiple metabolic and growth responses in skeletal muscle in response to exercise. This 'fuel sensing' enzyme is involved in acute exercise-induced events and also plays an obligatory role in adapting skeletal muscles to repeated bouts of exercise during training programmes (for review see Ashenbach et al. 2004; Winder 2002). The AMPK cascade is turned on by cellular stresses that deplete ATP (and consequently elevate AMP) either by accelerating ATP consumption (e.g. muscle contraction) or by inhibiting ATP production (e.g. hypoxia, ischaemia). Once activated, the AMPK cascade switches on catabolic processes both acutely (by phosphorylation of downstream metabolic enzymes such as acetyl coenzyme A carboxylase) and chronically (by effects on gene expression), while concomitantly switching off ATP-consuming processes (Hardie and Hawley 2001). Activation of AMPK is rapid (<30 s) and occurs in an intensity-dependent and isoform-specific fashion (Chen et al. 2003; Fuuji et al. 2000; Wojtaszewski et al. 2000). Pharmacological activation of AMPK (an "exercise-like" effect) enhances the protein expression of GLUT4, hexokinase, and several oxidative enzymes, as well as increasing mitochondrial density and muscle glycogen content (Ashenbach et al. 2004). Accordingly, many of the chronic training-induced adaptations in skeletal muscle have been proposed to involve AMPK. In this regard, cross-sectional studies reveal that muscle from endurance trained athletes has increased AMPK protein levels (Nielsen et al. 2003), while in highly-trained subjects AMPK activation during exercise is blunted compared to untrained individuals when exercising at the same relative intensity (Frosig et al 2003; Nielsen et al. 2003; Yu et al. 2003), an observation consistent with the maintenance of a better phosphorylation potential of the muscle (as reflected by the difference in [PCr]/[PCr+Cr] ratios) in trained muscle. Muscle glycogen content also modulates the AMPK response to exercise. Low muscle glycogen stores elevate resting AMPK activity compared to normal glycogen levels (Wojtasezewski et al. 2003). AMPK is also likely to mediate the contraction-induced increase in glucose uptake (Hayashi et al. 1998) and thus may play a role in promoting post-exercise glycogen accumulation in skeletal muscle (Barnes et al. 2005; Carling and Hardie 1989; Sakoda et al. 2005).

The MAPK signal transduction cascade has been identified as a candidate system that converts contraction-induced biochemical perturbations into appropriate intracellular responses (for reviews see Hawley and Zierath 2004; Widegren et al. 2001). Exercise is a powerful and rapid activator of several MAP kinases and numerous downstream enzymes (Widegren et al. 1998; Wretman et al. 2001). Both local and systemic factors mediate phosphorylation of the MAPK signalling cascades (Aronson et al. 1977; Widegren et al. 1998), which have been implicated in transcriptional regulation of important genes in skeletal muscle in response to exercise (Widegren et al. 2001). In this regard, exercise-induced activation of the MAPK pathway has recently been demonstrated to play a role in aerobic muscle adaptation by promoting specific co-activators involved in mitochondrial biogenesis and slow muscle fibre formation (Akimoto et al. 2005). Crucially, MAPK activation can result not only in the production of transcription factors mediating gene expression, but can also stimulate the activity of the translational stage of protein synthesis. Muscle hypertrophy through increased protein synthesis may also require activation of the MAPK signalling cascades (Williamson et al. 2003).

The specific cascades linking growth stimuli to the activation of protein synthesis in skeletal muscle are not fully resolved. However, they involve phosphorylation of mTOR and sequential activation of S6 protein kinase ($p70^{S6k}$) (Glass 2003; Proud 2002). Both insulin and amino acids are potent activators of mTOR. While the mechanisms of action of insulin on mTOR are well documented (for review see Bolster et al. 2004), the precise pathways by which amino acids act are presently unclear. In rodents, exercise-induced $p70^{S6k}$ activation correlates with increased skeletal muscle mass after 6 weeks of resistance training (Baar and Esser 1999). Thus, changes in $p70^{S6k}$ phosphorylation in skeletal muscle after exercise may partially account for increases in protein synthesis during the early recovery phase. Exercise and amino acid supplementation recruit different signalling pathways upstream of mTOR: exercise seems to activate partially the same pathways as insulin, whereas amino acids may act directly on the mTOR complex itself (for review see Kimball et al. 2002; Deldicque et al. 2005). Activation of AMPK inhibits mTOR, either directly or indirectly (Bolster et al. 2002; Cheng et al. 2004), making mTOR less active in promoting protein synthesis. The practical implication of this observation is obvious when planning the order of training sessions that include both endurance and strength/resistance components. There is some evidence to suggest that simultaneous endurance and strength training inhibits the normal adaptation to either training regimen when performed alone (Nelson et al. 1990).

With regard to the effects of contraction on gene expression, many studies have reported that mRNA abundance for several metabolic and stress-related genes is acutely and transiently elevated in muscle

after a single bout of exercise (Cluberton et al. 2005; Kraniou et al. 2000; Neufer and Dohm 1993; Pilegaard et al. 2000). Indeed, it appears that for many exercise-related genes, the time-course of transcriptional activation occurs during the first few hours of recovery (Pilegaard et al. 2000) and may be linked by common signalling and/or regulatory mechanisms to the restoration of muscle energy stores, predominantly glycogen (Richter et al. 2001). As gene expression and its associated phenotypic/functional manifestations do not take place until there is an increase in the concentration of the protein encoded by the gene, the extent to which a protein will increase in response to an adaptive stimulus cannot be predicted from the increase in mRNA. This makes the measurement of protein concentrations critical when studying the adaptive responses to exercise training or other stimuli (Baar et al. 2001). Physical preparation for soccer requires several divergent yet interdependent types of training incorporating sprint, endurance and resistance training (Bangsbo 1994). Under conditions in which the training inputs (intensity, duration and frequency) are held constant, any training programme must be of sufficient length for the cellular proteins to reach their new "steady-state" concentration and the biochemical/metabolic adaptations to develop fully (Hildebrandt et al. 2003; Terjung and Hood 1986).

Modification of the training response/adaptation via dietary interventions

Changes in dietary intake that alter the concentration of blood-borne nutrients and hormones can regulate the short-term macronutrient oxidative and storage profile of skeletal muscle. Perturbations in muscle and blood substrates (particularly CHO and fat) alter the uptake and flux of these fuel-specific intermediates within related metabolic pathways (i.e. skeletal muscle). This response serves to redirect enzymatic processes involved in substrate metabolism and the subsequent concentration of particular proteins critical for metabolic pathway function. Altering substrate availability affects not only resting energy metabolism and subsequent fuel utilisation during exercise but also regulatory processes underlying gene expression (Arkinstall et al. 2004; Hargreaves and Cameron-Smith 2002; Tunstall and Cameron-Smith 2005). To bring about such modifications, a number of highly coordinated processes occur, including gene transcription, RNA transport from the nucleus, protein synthesis and, in some cases, post-translational modification of the protein (Figure 2). However, the initiation of gene transcription is strongly related to both acute and chronic changes in dietary intake and composition (Jump and Clarke 1999) and thus has the potential to modulate many of the adaptive responses to training.

FIGURE 2 HERE

Dietary interventions that modify the training adaptation

Carbohydrate availability

It has long been recognised that there is a close association between dietary CHO intake, muscle glycogen concentration and endurance capacity (Bergstrom et al. 1967). For this reason it is recommended that anyone when training for sports in which CHO is the most heavily metabolised fuel (including football), should consume a diet rich in CHO (Balsom et al. 1999; Clark 1994; Hargreaves 1994; Hawley et al. 1994; Kirkendall 1993; Rico-Sanz et al. 1998, 1999). However, it should be noted that only a few researchers have chronically manipulated dietary CHO intake in well-trained individuals and examined the effect on subsequent training responses/adaptations and performance (for review see Hawley et al. 1995).

Sherman et al. (1993) compared the effects of 7 d of either a 5 or 10 g CHO·kg BM·d^{-1} diet on training capacity and performance in trained endurance athletes. Training incorporated both sprint and endurance workouts typical of those that might be encountered during soccer training. Athletes consuming the high-CHO diet maintained basal muscle glycogen concentrations over the training period, but those on the moderate CHO regimen had a 33% reduction by day 5. Despite this decline in glycogen stores, all athletes were able to successfully complete the prescribed training sessions and had a similar (endurance) exercise performance on day 7. Lamb et al. (1990) determined the effects of a "moderate" (6.5 g CHO·kg BM·d^{-1}) or high (12 g CHO·kg BM·d^{-1}) CHO diet during 9 d of intense interval training. Although muscle glycogen was not measured in that study, the high CHO diet did not permit the athletes to maintain a higher intensity of training compared to the "moderate" CHO-diet. These workers concluded "there may be an upper limit of CHO intake (perhaps 500-600 g/d) beyond which additional CHO does not contribute significantly to muscle glycogen storage and athletic performance" (Lamb et al. 1990), a hypothesis originally proposed by Costill and co-workers (1981).

In contrast, the results of other studies demonstrate improved performance following increased dietary CHO during training. Achten and colleagues (2004) reported that consumption of a high (8.5 g CHO·kg BM·d^{-1}) versus a moderate (5.4 g CHO·kg BM·d^{-1}) CHO diet sustained higher rates of CHO oxidation during exercise and that this was associated with a better maintenance of physical performance and mood state during 11 d of intensified training in competitive athletes. Increasing the *ad libitum* daily intake of CHO from 6.5 to 9 g·kg·d^{-1} during 1 wk of training improved run time to exhaustion at 90% VO_{2max} following a 90-min pre-load in trained athletes (Millard-Stafford et al 1988). Balsom (1999) observed that soccer players performed more high-intensity movement during a simulated 90 min 4-a-side game when fed a high versus low (65% or 30% of energy intake) CHO diet, presumably because the high-CHO intake resulted in higher pre-game muscle glycogen content. Of note was that other technical measures of the game were not impacted by the dietary regimen.

To date, the longest study to examine the interaction of daily diet and training in athletes was undertaken by Simonsen et al. (1991). In contrast to the results of Sherman et al. (1993), consuming a moderate (5 g CHO·kg BM·d^{-1}) CHO diet maintained muscle glycogen concentrations (~120 mmol·kg w.w.$^{-1}$) over 4 weeks of twice-daily workouts in rowers. However, athletes consuming the high-CHO diet (10 g CHO·kg BM·d^{-1}) had a progressive (65%) increase in glycogen stores by the end of the fourth week (to ~155 mmol·kg w.w.$^{-1}$). While all subjects were able to successfully complete the prescribed training sessions, athletes consuming the high-CHO diet showed greater improvements (11%) in power output in time-trials performed three times weekly than those consuming the moderate-CHO diet (2%). This study provides evidence that while a moderate-CHO diet may not reduce the ability of trained athletes to complete rigorous training sessions for up to one month, consumption of a high-CHO diet optimises improvements in performance of these individuals. Taken collectively, the results from these investigations (Achten et al. 2004; Balsom, 1999, Lamb et al. 1990; Millard-Stafford et al. 1988, Sherman et al. 1993; Simonsen et al. 1991) demonstrate that trained athletes benefit from a high-CHO intake during periods of intensified training, probably due to the maintenance (or an increase) in muscle glycogen stores and ability to sustain higher rates of CHO oxidation sustained during exercise. Certainly there are no reports in the literature of impairments in training capacity and performance when athletes ingest a high-CHO diet. Soccer players engaged in strenuous training and competition should be encouraged to consume a diet that provides a minimum of 7 g CHO·kg BM·d^{-1}.

While the available evidence suggests that a high-CHO intake during training allows athletes to train faster/harder and for longer duration to achieve a superior training response, it has recently been proposed that a "cycling" of muscle glycogen stores may be desirable to further promote the training response/adaptation (Chakravarthy and Booth 2004). Indeed, Hansen et al. (2005) recently reported that *untrained* subjects who completed 10 weeks of training with low muscle glycogen levels had a more pronounced increase in resting glycogen content and citrate synthase activity compared to when the same volume of training was undertaken with normal glycogen concentrations. Remarkably, this "train-low, compete-high" approach also resulted in a two-fold increase in exercise time to fatigue compared to when subjects commenced training sessions with normal glycogen levels. These results suggest that under certain conditions, a lack of substrate (i.e. CHO) might trigger selected training adaptations that would be viewed as beneficial for performance. Certainly there is accumulating evidence to demonstrate that commencing endurance exercise with low muscle glycogen content enhances the transcription rate of a number of genes involved in the training adaptation (Febbraio et al. 2003; Keller et al. 2001; Pilegaard et al. 2002). This is probably because several transcription factors include glycogen-binding domains, and when muscle glycogen is low, these factors are released and become free to associate with different targeting proteins (Printen et al. 1997). Coaches and athletes should be careful not to draw practical consequences of these studies with regard to training regimens. In the real world, training on a high muscle glycogen content may allow the athlete to train for longer periods and thereby obtain better results.

With regard to intracellular signalling, muscle glycogen content is a potent modulator of both resting and contraction-induced AMPK and MAPK responses (Chan et al. 2004; Wojtasezewski et al. 2003). Well-trained subjects have been studied under conditions of low- and high-glycogen content (160 vs. 900 mmol·g d.w.$^{-1}$), at rest and subsequently during 1 h of endurance exercise (Wojtasezewski et al. 2003). At rest, AMPK activity was ~2.5-fold higher in the low- versus the high-glycogen states. Low pre-exercise glycogen content also increased AMPKα-2 activity during subsequent submaximal exercise. Altering dietary CHO intake to reduce muscle glycogen content also leads to an increased MAPK signalling response (Chan et al. 2004). In contrast to the up-regulation of signalling cascades

when endurance exercise is commenced with low muscle glycogen stores, resistance exercise undertaken in a glycogen-depleted state may disrupt mechanisms involved in protein translation and blunt the normal adaptive response. Creer et al. (2005) recently reported that when endurance-trained subjects performed a bout of moderate-intensity resistance exercise (similar to that likely to be undertaken by soccer players) with low (~175 mmol.kg d.w.$^{-1}$) muscle glycogen content, phosphorylation of Akt, a critical signalling mediator of cell growth and metabolism (Glass 2003), was diminished compared to when subjects undertook the same workout with normal (~600 mmol/kg d.w.) glycogen levels.

Glucose availability has been shown to modulate metabolic regulation within skeletal muscle (Arkinstall et al. 2001; Coyle et al. 1986) and also exert effects on gene expression (Cheng et al. 2005; Civitarese at al. 2005; Cluberton et al. 2005; Febbraio et al. 2003). In this regard it has been proposed that CHO ingestion during and after exercise could inhibit long-term adaptation to training (Åkerstrom et al. 2005; Febbraio et al. 2003). To test this hypothesis, Åkerstrom et al. (2005) determined the effects of chronic oral glucose supplementation (or placebo) in *untrained individuals* on substrate metabolism, training responses, and performance during 10 weeks of endurance-training (2 h·d^{-1}, 5 d·week^{-1}). Training induced large improvements in performance for both experimental conditions. However, glucose ingestion during training did not alter patterns of substrate metabolism or alter a variety of muscle markers of training adaptation (i.e. metabolic enzymes, glycogen content and GLUT4 protein). Accordingly, it would seem prudent to recommend that athletes maximise CHO availability during and after training sessions, as per current sports nutrition guidelines (Burke 2003). Clearly the role of CHO availability in modifying the activation of transcription factors and signalling responses to contraction requires further research. Whether chronic perturbations in glycogen and/or glucose availability can translate into improved training adaptations in *well-trained individuals* is currently not known.

Fat availability

Another nutritional strategy that might enhance the training adaptation, presumably by allowing athletes to train for longer, would be to utilise an alternative fuel source to CHO and/or to slow its normal rate of utilisation during exercise. Such a fuel is fat, and there has been recent interest in the effects of both acute and chronic fat supplementation on metabolism and exercise performance (for reviews see Burke and Hawley 2002; Hawley 2002b). Of interest here is whether such dietary modification can enhance the adaptive response to training. Certainly when well-trained individuals consume a high-fat, low-CHO diet for 5-7 d, there is a rapid and marked capacity for these changes in macronutrient availability to modulate the expression of mRNA-encoding proteins that are necessary for fatty acid transport and oxidative metabolism (Cameron-Smith et al. 2003). Accompanying these changes are large shifts in substrate metabolism in favour of fat, and a sparing of muscle glycogen (Burke et al. 2000). Even when CHO availability is increased following "fat adaptation", by the restoration of muscle glycogen stores and provision of exogenous CHO during exercise, the enhanced capacity for muscle fat oxidation persists (Burke et al. 2002).

With regard to the effect of such metabolic perturbations on the training response, Stepto et al. (2002) reported that competitive endurance athletes are able to perform intense (40 min at 86% of VO_{2max}) interval training during short-term (<5 d) exposure to a high-fat diet. Such training was associated with rates of fat oxidation that are among the highest reported in the literature (i.e. >60 µmol·kg·min^{-1}). However, compared with a high-CHO diet, training sessions were associated with increased ratings of perceived exertion. Recently, Stellingwerff et al. (2005) investigated the effects of 5 d of a high fat diet while training, followed by 1 day of CHO restoration (and rest) on the regulation of key regulatory enzymes in the pathways of skeletal muscle fat and CHO metabolism during sprint exercise. Resting pyruvate dehydrogenase (PDH) activity was lower at rest and estimated rates of glycogenolysis were reduced at the completion of a standardised 1 min sprint after fat-adaptation compared to control (high-CHO). These results suggest that the muscle glycogen "sparing" observed in previous studies of fat-adaptation may actually be an impairment of glycogenolysis (due to a down-regulation of PDH). Such an adaptation would not be favourable to athletes in a sport such as soccer that requires repeated bouts of maximal sprint activity.

Protein availability

Although insulin, amino acids and exercise individually activate multiple signal transduction pathways in skeletal muscle, one pathway, the phosphatidylinositol 3-kinase (PI3K)- mTOR signalling pathway, is a common target of all three. Activation of the PI3K-mTOR signal pathways results in both acute (i.e. minutes to hours) and long-term (i.e. hours to days) up-regulation of protein

synthesis through modulation of multiple steps involved in mediating the initiation of mRNA translation and ribosome biogenesis respectively. In addition, changes in gene expression through altered patterns of mRNA translation promote cell growth, which in turn promotes muscle hypertrophy.

Protein availability is critical for optimising many of the adaptations that take place in muscle in response to both endurance and resistance training. The main determinants of an athlete's protein needs are their training regimen and habitual nutrient intake (Tipton and Wolfe 2004). However, the optimal amount of protein required by athletes to enhance the training adaptation is unclear. While some researchers suggest that during periods of intense training, protein requirements should be increased to ~2.0 $g \cdot kg\ BM \cdot d^{-1}$, (Lemon 2000), others maintain that athletes should consume the same amount recommended for the general population (i.e. ~1.0 $g \cdot kg\ BM \cdot d^{-1}$ (Rennie and Tipton 2000; Tipton and Wolfe 2004). The discrepancy is likely due to the difficulty in determining true protein requirements for athletes, and the disparate methods used for such determination. Of note is that the scientific evidence is probably immaterial for the vast majority of athletes, because most individuals, including soccer players (Rico Sanz et al. 1998), consume sufficient protein to accommodate even the highest estimates of protein needs.

Increased muscle protein results from a positive net muscle protein balance (i.e. when protein synthesis is greater than protein breakdown). At rest and in the fasted state, net protein balance is negative because protein breakdown exceeds the rate of synthesis. Following exercise in the fasted state, the rates of both protein synthesis and breakdown are increased but compared to resting conditions, the net (negative) balance is attenuated because the increase in protein synthesis is greater than the increase in protein breakdown (Biolo et al. 1995; Phillips et al. 1997). Ingesting a mixture of CHO and amino acids before or immediately after completion of a training session (Tipton et al. 2001) counteracts this catabolic state by increasing amino acid availability and transport into muscle (Biolo et al. 1997). In this situation protein synthesis is increased (Biolo et al. 1997; Boersheim et al. 2002), while the increase in protein breakdown is attenuated (Biolo et al. 1997) resulting in a net positive protein balance.

Acute protein ingestion near the time of exercise appears to have the greatest potential impact on training adaptation. Recently, Karlsson et al. (2004) examined the effect of resistance exercise alone or in combination with oral intake of branch-chain amino acids (BCAA) on the signalling pathways responsible for translational control of protein synthesis. In that study a single bout of resistance training led to a robust and persistent (2-3 h) increase in $p70^{S6k}$ phosphorylation that was further enhanced by BCAA ingestion. These workers speculated that BCAA supplementation enhances protein synthesis during recovery from resistance training through a $p70^{S6k}$ –dependent signalling cascade (Karlsson et al. 2004). Of note is that the effect of post-exercise amino acid supplementation on protein balance is enhanced by co-ingestion of CHO (Miller et al. 2003), possibly via the elevated insulin concentrations. After resistance exercise, a mixture of whey protein, amino acids and CHO stimulated muscle protein synthesis to a greater extent and for a longer duration than isoenergetic CHO alone (Borsheim 2004). This has been demonstrated for both casein and whey protein added to CHO (Tipton et al 2004). The amount of protein necessary for ingestion immediately after exercise to elicit this effect appears to be quite modest (~6 g) (Tipton et al. 1999; Tipton et al. 2001). Furthermore, net muscle protein synthesis may be greater when a CHO-amino-acid solution is consumed immediately before resistance exercise than when the same solution is consumed after exercise, primarily because of an increase in muscle protein synthesis as a result of increased delivery of amino acids to the leg (Tipton et al. 2001).

While the impact of protein ingestion (alone or co-ingested with CHO) before or after resistance training appears to enhance net muscle protein balance, the effects on endurance exercise responses are not as clear. When consumed immediately after prolonged, glycogen-depleting exercise, protein co-ingested with CHO may improve net protein balance in the early post-exercise period (Koopman et al. 2004) and possibly enhance glycogen re-synthesis (Ivy 2002, Williams et al. 2003; Zawadzki et al. 1992). Marked improvements (>40%) in exercise capacity during a subsequent bout of exercise have been demonstrated when protein was added to CHO (Saunders 2004, Williams 2003), but neither of these studies used an isoenergetic CHO comparison treatment. When an isocaloric CHO recovery drink is compared to CHO + protein, subsequent running performance is not improved (Millard-Stafford 2005) and rates of muscle glycogen synthesis are similar (Carrithers 2000, Jentjens 2001, Van Hall, 2000, Van Loon 2000). Therefore, improved performance and/or muscle glycogen seen after the co-ingestion of protein and CHO may be attributed to the greater energy intake *per se* rather

than any proven physiological effect. There are also reports indicating that the co-ingestion of protein with CHO immediately after endurance exercise attenuates muscle soreness (Saunders 2004) and plasma creatine kinase responses to high-intensity exercise (Millard-Stafford 2005; Saunders 2004).

Two recent reports offer evidence that habitual daily protein intake may influence muscle protein metabolism and thus the adaptations to training. Harber et al. (2005) reported that muscle protein synthesis in the basal state (i.e. resting, post-absorptive) was increased following 7 d of high (35% of total energy intake) protein intake. Presumably, such increased protein synthesis would lead to gains in muscle protein. However, no measurements of muscle protein turnover were made in this study (Harber et al. 2005). Since increased muscle protein breakdown is usually associated with increased synthesis (Tipton and Wolfe 1998), the actual accretion of muscle protein is unlikely to be as high as the increased rate of synthesis would suggest (Harber et al. 2005). Presumably, the increased protein synthesis was mediated by increased signalling of the translation initiation pathways. However, increased muscle protein synthesis occurred without increased phosphorylation of two proteins downstream of mTOR (ribosomal protein S6 and eIF4G). This finding suggests that muscle protein synthesis is enhanced by high protein intake, but may not be associated with a chronic alteration in components of the mTOR signalling pathway. Accordingly, any acute up-regulation of selected signalling pathways after protein feeding may simply be a transient change in phosphorylation state and would not necessarily be evident at a time when increased muscle protein synthesis takes place.

Following exercise, the response of muscle protein synthesis to high protein intake seems to be different than at rest (Bolster et al. 2005). Following treadmill running, rates of muscle protein synthesis were higher in athletes that consumed 0.8 and 1.8 g for 2 weeks (Bolster et al. 2005) than in athletes who consumed ~3.6 g protein·kg BM·d^{-1}. In fact, rates of muscle protein synthesis following exercise in athletes who consumed the chronic high protein diet were similar to those generally measured in resting (untrained) subjects (Volpi et al. 2001). These data suggest that a high protein diet may actually inhibit the response of muscle protein synthesis to exercise. Accordingly, such high levels of protein intake would not be recommended for individuals during training. There is preliminary evidence that the decreased level of protein synthesis after high protein intake is accompanied by decreased muscle protein breakdown, thus further reducing the effect on net muscle protein balance (Bolster et al. 2005). Taken collectively, there does not seem to be any reason to suggest that soccer players need to consume greater daily protein than currently recommended for the majority of athletes. While the signalling cascades that stimulate muscle protein synthesis are undoubtedly complex, an understanding of how these pathways respond to exercise and specific nutritional interventions could provide sports scientists and coaches with information that may lead to modification of training/recovery processes and maximize training adaptations.

Summary and directions for future research

It is clear from the preceding discussion that nutrient supplementation can serve as a potent modulator of many of the acute responses to both endurance and resistance training. In this regard, recent scientific enquiry has focused on the role of specific nutrition strategies in promoting optimal biological adaptations to training. Research has focussed on the role of CHO availability before, during and after exercise to amplify the training response, while there has been an emerging interest in the role of protein intake to enhance muscle hypertrophy after resistance exercise and possibly facilitate recovery from endurance exercise when co-ingested with CHO. With advances in molecular biology, several techniques have become available that allow for the investigation of the interactive effects of exercise and diet on skeletal muscle gene expression and the early signalling responses to these different interventions. The biggest challenge for the exercise physiologist and sport nutritionist in the forthcoming years will be to link early gene and signalling responses in skeletal muscle that occur after exercise to chronic training-induced adaptations in already highly-trained athletes. This task is complicated because many of these pathways are not linear, but rather constitute a complex network, with a high degree of cross talk, feedback regulation, and transient activation (Hawley and Zierath 2004). Nevertheless several lines of inquiry may yield useful practical information concerning the interaction between nutrient intake and training adaptation. It is not currently known whether periods of endurance training in the face of low glycogen stores can further drive the training adaptation in already well-trained athletes (the so-called “train-low, compete-high” approach). However, the muscle glycogen “sparing” observed in early studies of fat-adaptation may actually be an impairment of glycogenolysis, and such a nutritional strategy is not recommended for athletes involved in high-intensity activities such as soccer. While protein synthesis in strength-trained athletes may be increased by protein ingestion before or following training, it is not presently known if CHO

supplementation *alone* during recovery from resistance or endurance exercise can enhance gene, protein, and signalling responses to a greater/lesser degree than protein, or a combination of the two macronutrients. Furthermore, the efficacy of protein and/or protein plus CHO ingestion following intense, intermittent exercise in promoting recovery (e.g. increasing muscle protein synthesis and muscle glycogen storage) and attenuating muscle damage and soreness during days of multiple training sessions and/or tournament play requires additional investigation. At present the following recommendations are made: 1) daily CHO intake during intense training should approach > 7 g/kg $BM.d^{-1}$; 2) nutrient timing before, during and following training can affect many of the adaptive response to training, and 3) the provision of calories (in the form of CHO and/or protein) prior to and within the hour after training are recommended.

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Figure legends

Figure 1. Schema of the time-course of selected contraction-induced physiological, biochemical, and molecular responses in skeletal muscle that lead to the training adaptation. Adapted and redrawn from Hood (2001).

Figure 2. Steps at which gene expression can be controlled/regulated. The effect of diet/training interactions on these processes is largely unknown. Adapted and redrawn from Williams and Neufer (1996).

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