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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 baseline values 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) to affect many of the adaptive responses to training.

Keywords: *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 & 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 adenosine triphosphate (ATP) production from both aerobic and oxygen-independent pathways, maintain tighter metabolic control (i.e. match ATP production with ATP hydrolysis), minimize cellular disturbances, and improve fatigue resistance during exercise (for a 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 baseline values within 24 h after exercise (Hildebrandt, Pilegaard, & Neufer, 2003; Pilegaard, Ordway, Saltin, & Neufer, 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, Ryder, & Zierath, 2001; Williams & 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, Tsintzas, Boobis, & Williams, 1999) typical of the patterns of soccer match-play (Hargreaves, 1994), and in the replenishment of energy reserves for subsequent training sessions (Burke, Kiens, & Ivy, 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

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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 that take 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 & 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 & 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 sequence of events produces mitochondrial biogenesis (Hood, 2001), muscle hypertrophy (Glass, 2003), and concomitant alterations in muscle phenotype that serve to improve cellular function and thereby enhance exercise capacity.

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 oxygen flux. With an increase in exercise duration, endogenous muscle substrates (principally glycogen) become depleted. These contraction-induced metabolic disturbances in muscle, together 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 kinases (AMPK), several of the mitogen-activated

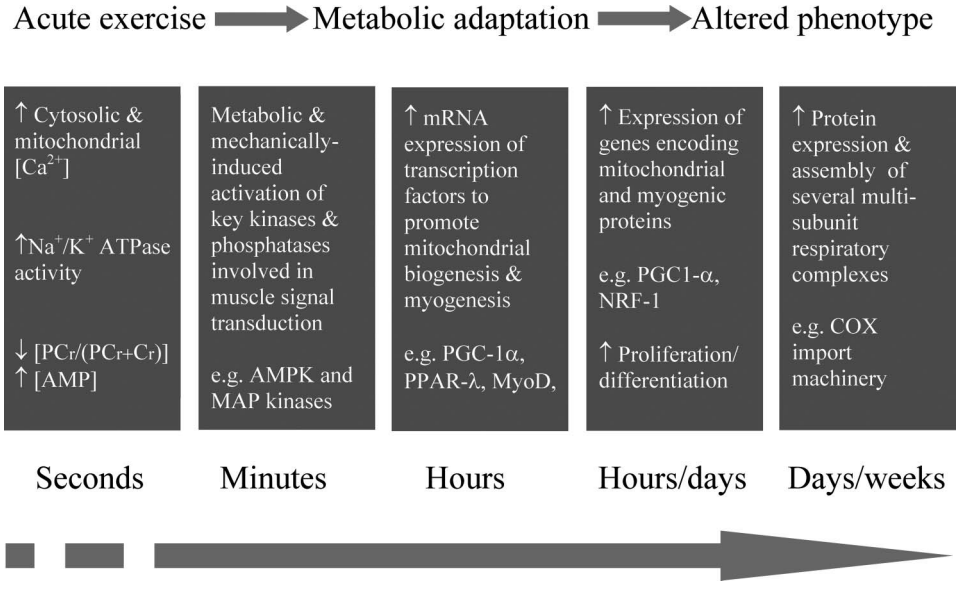

Figure 1. Schematic representation of the time-course of selected contraction-induced physiological, biochemical, and molecular responses in skeletal muscle that lead to the training adaptation. Adapted and redrawn from Hood (2001).

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 reviews, see Ashenbach, Sakamoto, & Goodyear, 2004; Winder, 2001). 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 & Hawley, 2001). Activation of AMPK is rapid (<30 s) and occurs in an intensity-dependent and isoform-specific fashion (Chen *et al.*, 2003; Fujii *et al.*, 2000; Wojtaszewski, Nielsen, Hansen, Richter, & Kiens, 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 (Aschenbach *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 have revealed that muscle from endurance-trained athletes shows increased AMPK protein levels (Nielsen *et al.*, 2003), while AMPK activation during exercise is blunted in highly trained individuals compared with untrained individuals when exercising at the same relative intensity (Frosig *et al.*, 2004; 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 with normal glycogen stores (Wojtaszewski *et al.*, 2003). AMPK is also likely to mediate the contraction-induced increase in glucose uptake (Hayashi, Hirshman, Kurth, Winder, & Goodyear, 1998) and thus may play a role in promoting post-exercise glycogen accumulation in skeletal muscle (Barnes *et al.*, 2005; Carling & 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 & 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.*, 1997; 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-twitch 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, Gallagher, Harber, Hollon, & Trappe, 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 a review, see Bolster, Jefferson, & Kimball, 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 & 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 reviews, see Deldicque, Theisen, & Francaux, 2005; Kimball, Farrell, & Jefferson, 2002). Activation of AMPK inhibits mTOR, either directly or indirectly (Bolster, Crozier, Kimball, & Jefferson, 2002; Cheng, Fryer, Carling, & Shepherd, 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, Arnall, Loy, Silverster, & Conlee, 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, McGee, Murphy, & Hargreaves, 2005; Kraniou, Cameron-Smith, Misso, Collier, & Hargreaves, 2000; Neufer & 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, Derave, & Wojtaszewski, 2001). As gene expression and its associated phenotypic/functional manifestations do not occur 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.*, 2002). 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 & 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 (especially carbohydrate 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 utilization during exercise, but also regulatory processes underlying gene expression (Arkinstall, Tunstall, Cameron-Smith, & Hawley, 2004; Hargreaves & Cameron-Smith, 2002; Tunstall & 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 & Clarke, 1999) and thus has the potential to modulate many of the adaptive responses to training.

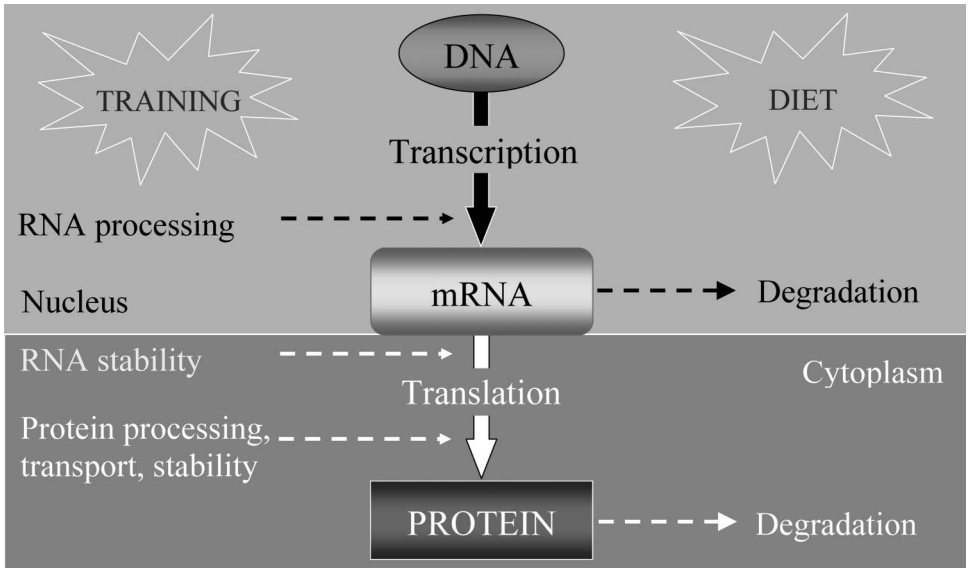

Figure 2. Steps at which gene expression can be controlled/regulated. The effect of diet/training interactions on these processes is largely unknown. Adapted and redrawn from Williams and Neufer (1996).

Dietary interventions that modify the training adaptation

Carbohydrate availability

It has long been recognized that there is a close association between dietary carbohydrate intake, muscle glycogen concentration, and endurance capacity (Bergstrom, Hermansen, Hultman, & Saltin, 1967). For this reason, it is recommended that individuals training for sports in which carbohydrate is the most heavily metabolized fuel (including football) should consume a diet rich in carbohydrate (Balsom, Wood, Olsson, & Ekblom, 1999; Clark, 1994; Hargreaves, 1994; Hawley, Dennis, & Noakes, 1994; Kirkendall 1993; Rico-Sanz *et al.*, 1998; Rico-Sanz, Zehnder, Buchli, Dambach, & Boutellier, 1999). However, it should be noted that only a few researchers have chronically manipulated dietary carbohydrate intake in well-trained individuals and examined the effect on subsequent training responses/adaptations and performance (for a review, see Hawley, Dennis, Lindsay, & Noakes, 1995).

Sherman, Doyle, Lamb and Strauss (1993) compared the effects of 7 days of two diets (5 or 10 g carbohydrate per kilogram of body mass [BM] per day) 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 on the high-carbohydrate diet maintained basal muscle glycogen concentrations over the training period, but those on the moderate-carbohydrate 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, Rinehardt, Bartels, Sherman and Snook (1990) determined the effects of a ''moderate'' ($6.5\,g \cdot kg\ BM^{-1} \cdot day^{-1}$) or high ($12\,g \cdot kg\ BM^{-1} \cdot day^{-1}$) carbohydrate diet during 9 days of intense interval training. Although muscle glycogen was not measured in that study, the high-carbohydrate diet did not permit the athletes to maintain a higher intensity of training compared with the ''moderate''-carbohydrate diet. These workers concluded that ''there may be an upper limit of carbohydrate intake (perhaps $500-600\,g \cdot day^{-1}$) beyond which additional carbohydrate does not contribute significantly to muscle glycogen storage and athletic performance'' (Lamb *et al.*, 1990), a hypothesis originally proposed by Costill and coworkers (1981).

In contrast, the results of other studies demonstrate improved performance following increased dietary carbohydrate during training. Achten and colleagues (2004) reported that consumption of a high- ($8.5\,g \cdot kg\ BM^{-1} \cdot day^{-1}$) versus a moderate-carbohydrate ($5.4\,g \cdot kg\ BM^{-1} \cdot day^{-1}$) diet sustained higher rates of carbohydrate oxidation during exercise and that this was associated with a better maintenance of physical performance and mood state during 11 days of intensified training in competitive athletes. Increasing the *ad libitum* daily intake of carbohydrate from 6.5 to $9\,g \cdot kg\ BM^{-1} \cdot day^{-1}$ during a week of training improved run time to exhaustion at 90% maximal oxygen uptake ($\dot{V}O_{2max}$) following a 90 min pre-load in trained athletes (Millard-Stafford, Cureton, & Ray, 1988). Balsom *et al.* (1999) observed that soccer players performed more high-intensity movement during a simulated 90 min four-a-side game when fed a high versus a low (65% or 30% of energy intake) carbohydrate diet, presumably because the high-carbohydrate 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 ($5\,g \cdot kg\ BM^{-1} \cdot day^{-1}$) carbohydrate diet maintained muscle glycogen concentrations ($\sim 120\,mmol \cdot kg\ wet\ weight^{-1}$) over 4 weeks of twice-daily workouts in rowers. However, athletes consuming the high-carbohydrate diet ($10\,g \cdot kg\ BM^{-1} \cdot day^{-1}$) had a progressive (65%) increase in glycogen stores by the end of the fourth week (to $\sim 155\,mmol \cdot kg\ wet\ weight^{-1}$). While all participants were able to successfully complete the prescribed training sessions, athletes consuming the high-carbohydrate diet showed greater improvements (11%) in power output in time-trials performed three times weekly than those consuming the moderate-carbohydrate diet (2%). This study provides evidence that while a moderate-carbohydrate diet may not reduce the ability of trained athletes to complete rigorous training sessions for up to a month, consumption of a high-carbohydrate diet optimizes improvements in performance of these individuals. Taken collectively, the results of these studies (Achten *et al.*, 2004; Balsom *et al.*, 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 carbohydrate intake during periods of intensified training, probably due to the maintenance (or an increase) in muscle glycogen stores and an ability to sustain higher rates of carbohydrate oxidation sustained during exercise. Certainly, there are no reports in the literature of impairments in training capacity and performance when athletes ingest a high-carbohydrate diet. Soccer players engaged in strenuous training and competition should be

encouraged to consume a diet that provides a minimum of $7\,g \cdot kg\ BM^{-1} \cdot day^{-1}$.

While the available evidence suggests that a high-carbohydrate intake during training allows athletes to train faster/harder and for longer 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 & Booth, 2004). Indeed, Hansen *et al.* (2005) recently reported that *untrained* participants 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 with when participants commenced training sessions with normal glycogen levels. These results suggest that under certain conditions, a lack of substrate (i.e. carbohydrate) 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 several 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, Brady, & Saltiel, 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 with 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, McGee, Watt, Hargreaves, & Febbraio, 2004; Wojtaszewski *et al.*, 2003). Well-trained individuals have been studied under conditions of low- and high-glycogen content (160 vs. $900\,mmol \cdot kg$ dry $weight^{-1}$), at rest, and subsequently during 1 h of endurance exercise (Wojtaszewski *et al.*, 2003). At rest, AMPK activity was approximately 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 carbohydrate 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 individuals performed a bout of moderate-intensity resistance exercise (similar to that likely to be undertaken by soccer players) with low ($\sim 175\,mmol \cdot kg$ dry $weight^{-1}$) muscle glycogen content, phosphorylation of Akt, a critical signalling mediator of cell growth and metabolism (Glass 2003), was diminished compared with when they undertook the same workout with normal ($\sim 600\,mmol \cdot kg$ dry $weight^{-1}$) glycogen stores.

Glucose availability has been shown to modulate metabolic regulation within skeletal muscle (Arkinstall, Bruce, Nikolopoulos, Garnham, & Hawley, 2001; Coyle, Coggan, Hemmert, & Ivy, 1986) and to exert effects on gene expression (Cheng *et al.*, 2005; Civitarese, Hesselink, Russell, Ravussin, & Schrauwen, 2005; Cluberton *et al.*, 2005; Febbraio *et al.*, 2003). In this regard, it has been proposed that carbohydrate ingestion during and after exercise could inhibit long-term adaptation to training (Åkerstrom, Wojtaszewski, Plomgaard, & Pedersen, 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 per day, 5 days per week). 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 appear prudent to recommend that athletes maximize carbohydrate availability during and after training sessions, in line with current sports nutrition guidelines (Burke, 2003). Clearly, the role of carbohydrate 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 utilize an alternative fuel source to carbohydrate and/or to slow its normal rate of utilization 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 & 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-carbohydrate diet for 5–7 days, 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 carbohydrate availability is increased following ''fat adaptation'', by the restoration of muscle glycogen stores and provision of exogenous carbohydrate during exercise, the enhanced capacity for muscle fat oxidation persists (Burke *et al.*, 2002).

In terms of 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% $\dot{V}O_{2max}$) interval training during short-term (<5 days) 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. $>60\,\mu mol \cdot kg^{-1} \cdot min^{-1}$). However, compared with a high-carbohydrate diet, training sessions were associated with increased ratings of perceived exertion. Recently, Stellingwerff *et al.* (2006) examined the effects of 5 days of a high-fat diet while training, followed by 1 day of carbohydrate restoration (and rest), on the regulation of key regulatory enzymes in the pathways of skeletal muscle fat and carbohydrate metabolism during sprint exercise. Resting pyruvate dehydrogenase (PDH) activity was lower at rest and estimated rates of glycogenolysis were reduced upon the completion of a standardized 1 min sprint after fat-adaptation compared with control (high carbohydrate). 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 optimizing 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 & 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 $\sim 2.0\,g \cdot kg\ BM^{-1} \cdot day^{-1}$ (Lemon, 2000), others maintain that athletes should consume the same amount recommended for the general population (i.e. $\sim 1.0\,g \cdot kg\ BM^{-1} \cdot day^{-1}$) (Rennie & Tipton, 2000; Tipton & Wolfe, 2004). The discrepancy is probably 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 with resting conditions, the net (negative) balance is attenuated because the increase in protein synthesis is greater than the increase in protein breakdown (Biolo, Maggi, Williams, Tipton, & Wolfe, 1995; Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997). Ingesting a mixture of carbohydrate 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, Tipton, Klein, & Wolfe, 1997). In this situation, protein synthesis is increased (Biolo *et al.*, 1997; Borsheim, Tipton, Wolf, & Wolfe, 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). It is noteworthy that the effect of post-exercise amino acid supplementation on protein balance is enhanced by co-ingestion of carbohydrate (Miller, Tipton, Chinkes, Wolf, & Wolfe, 2003), possibly via the elevated insulin concentrations. After resistance exercise, a mixture of whey protein, amino acids, and carbohydrate stimulated muscle protein synthesis to a greater extent and for a longer duration than isoenergetic carbohydrate alone (Borsheim, Aarsland, & Wolfe, 2004). This has been demonstrated for both casein and whey protein added to carbohydrate (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, Ferrando, Phillips, Doyle, & Wolfe, 1999; Tipton *et al.*, 2001). Furthermore, net muscle protein synthesis may be greater when a carbohydrate–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 carbohydrate) 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 carbohydrate may improve net protein balance in the early post-exercise period (Koopman *et al.*, 2004) and possibly enhance glycogen re-synthesis (Ivy *et al.*, 2002, Williams, Raven, Fogt, & Ivy, 2003; Zawadzki, Yaspelkis, & Ivy, 1992). Marked improvements (>40%) in exercise capacity during a subsequent bout of exercise have been demonstrated when protein was added to carbohydrate (Saunders, Kane, & Todd, 2004, Williams *et al.*, 2003), but neither of these studies used an isoenergetic carbohydrate comparison treatment. When an isocaloric carbohydrate recovery drink is compared with carbohydrate + protein, subsequent running performance is not improved (Millard-Stafford *et al.*, 2005) and rates of muscle glycogen synthesis are similar (Carrithers *et al.*, 2000; Jentjens, 2001; Van Hall, Shirreffs, & Calbet, 2000, Van Loon, Saris, Kruijshoop, & Wagenmakers, 2000). Therefore, improved performance and/or muscle glycogen observed after the co-ingestion of protein and carbohydrate may be attributed to the greater energy intake *per se* rather than any proven physiological effect. It has also been reported that the co-ingestion of protein with carbohydrate immediately after endurance exercise attenuates muscle soreness (Saunders *et al.*, 2004) and plasma creatine kinase responses to high-intensity exercise (Millard-Stafford *et al.*, 2005; Saunders *et al.*, 2004).

Two recent reports offer evidence that habitual daily protein intake may influence muscle protein metabolism and thus the adaptations to training. Harber, Schenk, Barkham and Horowitz (2005) reported that muscle protein synthesis in the basal state (i.e. resting, post-absorptive) was increased following 7 days 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 by Harber *et al.* (2005). Since increased muscle protein breakdown is usually associated with increased synthesis (Tipton & 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). After treadmill running, rates of muscle protein synthesis were higher in athletes who consumed 0.8 and 1.8 g protein · kg BM^{-1} · day^{-1} for 2 weeks (Bolster *et al.*, 2005) than in athletes who consumed ~3.6 g protein · kg BM^{-1} · day^{-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) participants (Volpi, Sheffield-Moore, Rasmussen, & Wolfe, 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 to suggest 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 most 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 carbohydrate 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 carbohydrate. With advances in molecular biology, several techniques are now 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 greatest 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 & Zierath, 2004). Nevertheless, several lines of inquiry may yield useful practical information concerning the interaction between nutrient intake and training adaptation. It is currently unclear 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 after training, it is not presently known if carbohydrate 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 carbohydrate 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:

- daily CHO intake during intense training should approach $7\,g \cdot kg\ BM^{-1} \cdot day^{-1}$;
- nutrient timing before, during, and after training can affect many of the adaptive responses to training;
- the provision of calories (in the form of carbohydrate and/or protein) before and within the hour after training are recommended.

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