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Nutritional strategies to influence adaptations to training

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This article highlights new nutritional concerns or practices that may influence the adaptation to training. The discussion is based on the assumption that the adaptation to repeated bouts of training occurs during recovery periods and that if one can train harder, the adaptation will be greater. The goal is to maximize with nutrition the recovery/adaptation that occurs in all rest periods, such that recovery before the next training session is complete. Four issues have been identified where recent scientific information will force sports nutritionists to embrace new issues and reassess old issues and, ultimately, alter the nutritional recommendations they give to athletes. These are: (1) caffeine ingestion; (2) creatine ingestion; (3) the use of intramuscular triacylglycerol (IMTG) as a fuel during exercise and the nutritional effects on IMTG repletion following exercise; and (4) the role nutrition may play in regulating the expression of genes during and after exercise training sessions. Recent findings suggest that low doses of caffeine exert significant ergogenic effects by directly affecting the central nervous system during exercise. Caffeine can cross the blood–brain barrier and antagonize the effects of adenosine, resulting in higher concentrations of stimulatory neurotransmitters. These new data strengthen the case for using low doses of caffeine during training. On the other hand, the data on the role that supplemental creatine ingestion plays in augmenting the increase in skeletal muscle mass and strength during resistance training remain equivocal. Some studies are able to demonstrate increases in muscle fibre size with creatine ingestion and some are not. The final two nutritional topics are new and have not progressed to the point that we can specifically identify strategies to enhance the adaptation to training. However, it is likely that nutritional strategies will be needed to replenish the IMTG that is used during endurance exercise. It is not presently clear whether the IMTG store is chronically reduced when engaging in daily sessions of endurance training or if this impacts negatively on the ability to train. It is also likely that the increased interest in gene and protein expression measurements will lead to nutritional strategies to optimize the adaptations that occur in skeletal muscle during and after exercise training sessions. Research in these areas in the coming years will lead to strategies designed to improve the adaptive response to training.

Keywords: caffeine, creatine, gene expression, muscle triacylglycerol, nutrition, training.

Introduction

Here, we highlight new nutritional concerns or practices that may influence the adaptation to training. We have identified four areas where recent scientific information will force sports nutritionists to embrace new issues and reassess old issues and, potentially, alter some nutritional recommendations to athletes. These are: (1) caffeine ingestion; (2) creatine ingestion; (3) the use of intramuscular triacylglycerol (IMTG) as a fuel during exercise and the nutritional effects on IMTG repletion following exercise; and (4) the role that nutrition may play in determining the expression of genes during and after exercise training sessions. Recent experiments examining the effects of caffeine on the central nervous system during exercise strengthen the case for using low doses of caffeine during training. On the other hand, equivocal data on the role that creatine plays in augmenting the increase in skeletal muscle mass and strength during resistance training may do the opposite. Will the recent surge in information examining IMTG use during exercise and recovery after exercise alter our post-exercise nutritional advice to include a greater emphasis on fat? Lastly, based on recent measurements of gene expression, are there nutritional strategies that we should adopt to max-

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imize the adaptation that occurs in skeletal muscle following training sessions?

This article works on the assumption that the adaptation to repeated bouts of training occurs during the recovery periods and that if one can train harder, the adaptation will be greater. This can only be achieved if recovery before the next training session is complete. The goal is to use nutrition to maximize the recovery/adaptation that occurs in the rest periods between training sessions. It is also clear that characteristics of each training session (e.g. duration, intensity and nutritional intake) will also influence the rest period recovery/adaptation processes.

Caffeine and the central nervous system

Traditionally, it was believed that the ergogenic effect of caffeine during endurance exercise was due to a peripheral mechanism. The finding that caffeine in high doses of 5–9 $mg \cdot kg^{-1}$ BM (where BM = body mass) spared the use of muscle glycogen early in exercise supported this contention (Essig *et al.*, 1980; Spriet *et al.*, 1992). However, recent studies using 9 $mg \cdot kg^{-1}$ BM reported a variable glycogen sparing response (Chesley *et al.*, 1998) and other studies have reported no effect on glycogen use when consuming 6 $mg \cdot kg^{-1}$ BM (Graham *et al.*, 2000; Laurent *et al.*, 2000). Of course, there was always the realization that caffeine may also have a central effect during endurance exercise, but separating the peripheral and central effects of caffeine in studies with humans is difficult, as caffeine has the potential to affect many tissues at once. In addition, the recent finding that caffeine is also ergogenic during exercise of varying duration and intensities at doses as low as 3 $mg \cdot kg^{-1}$ BM (Graham and Spriet, 1995; Pasman *et al.*, 1995) suggests that its main effect is on the central nervous system (CNS). At these low doses, the risk of adverse side-effects is greatly diminished. Additional peripheral effects of caffeine acting directly on skeletal muscle (inhibition of enzymes or alterations of ion handling) also appear unlikely given the low plasma caffeine concentrations reported at these low doses. This suggests that the CNS is sensitive to lower caffeine doses and responsible for improved performance during endurance exercise and exercise of shorter duration.

Caffeine is known to be a CNS stimulant, causing increased arousal, wakefulness, alertness and vigilance as well as elevations of mood (Nehlig *et al.*, 1992; Daly, 1993). Caffeine increases brain neurotransmitter concentrations, leading to increased locomotor activity and neuronal firing in animals (Nehlig *et al.*, 1992). It is generally believed that the effects of caffeine are exerted via adenosine receptor antagonism. The brain has high levels of adenosine receptors (Fernstrom and Fernstrom, 1984; Daly, 1993; Fredholm, 1995) and adenosine generally decreases the concentration of the major neurotransmitters, including serotonin, dopamine, acetylcholine, norepinephrine and glutamate. This leads to lower motor activity, wakefulness and vigilance. Caffeine is an adenosine receptor antagonist and increases the concentration of these major neurotransmitters. However, the exact consequences of these changes for exercise performance are currently unclear.

Two recent studies have re-examined the role the CNS may play in the ability of caffeine to enhance exercise performance. Davis *et al.* (2003) examined the effects of direct intracerebroventricular injections of caffeine on the ability of rats to run to exhaustion on a treadmill. Rats were injected 30 min before running with either vehicle (placebo), caffeine, an adenosine receptor agonist (5-*N*-ethylcarboxamidoadenosine, NECA), or caffeine and NECA together. Rats were able to run ~80 min in the placebo trial, 120 min after caffeine injection and only 25 min with NECA (Fig. 1). When caffeine and NECA were given together, run time was not different from placebo. Rats were encouraged to run when needed by gentle hand prodding and mild electric shock. Fatigue was defined as the time when the rats would no longer run and chose to rest on the electric wires despite continual hand prodding and mild electric shocks for 30 s. When the study was repeated with peripheral intraperitoneal injections instead of brain injections, there was no effect on run performance. The authors concluded that

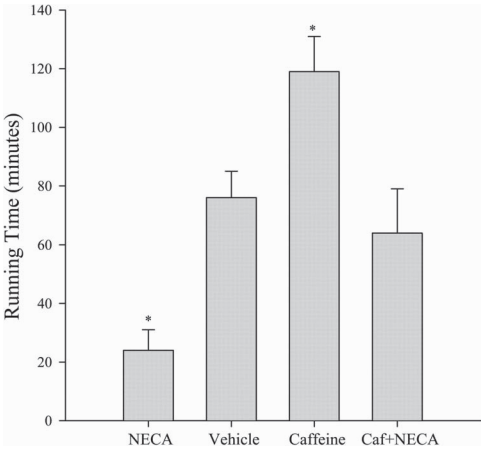

Fig. 1. Running time for rats running on a treadmill 30 min after direct intracerebroventricular injections of (1) vehicle (placebo), (2) caffeine, (3) an adenosine receptor agonist (5-*N*-ethylcarboxamidoadenosine, NECA) or (4) caffeine and NECA together. *Significantly different than vehicle. Adapted from Davis *et al.* (2003).

caffeine delayed fatigue through CNS effects in part by blocking adenosine receptors (Davis *et al.*, 2003).

A second study examined the effects of ingesting flat cola late in a simulated cycle race. This practice is common among endurance cyclists and was undertaken to determine if either the ingestion of extra carbohydrate and/or caffeine late in exercise could increase performance. Eight well-trained cyclists ($\dot{V}O_{2max}$, $\sim 71\ ml \cdot kg^{-1} \cdot min^{-1}$) rode for 2 h at 70% maximal oxygen uptake ($\dot{V}O_{2max}$) and then completed a time-trial in which each cyclist was asked to complete 7 kJ of work per kilogram of body mass as quickly as possible ($\sim$30 min) on four separate occasions (Cox *et al.*, 2002). They consumed 5 ml of a 6% sports drink every 20 min for the first hour and then switched to the same volume of flat cola at 80 and 100 min (and 120 min if desired). The cola beverage was varied in the four conditions to include: (a) no caffeine and 6% carbohydrate (Control); (b) caffeine (90 mg +) and 11% carbohydrate (Cola); (c) no caffeine and 11% carbohydrate (Extra carbohydrate); and (d) caffeine (90 mg +) and 6% carbohydrate (Caffeine). Performance times (min: s) to complete the time-trials were 27:05 $\pm$ 0:42 (Control), 26:15 $\pm$ 0:43 (Cola), 26:55 $\pm$ 0:43 (Extra Cola) and 26:36 $\pm$ 0:42 (Caffeine) (Fig. 2). The flat cola, with the extra carbohydrate and caffeine, significantly improved performance time by 50 s. Caffeine appeared to be the most important ingredient, as caffeine alone improved performance by 29 s (significant effect for caffeine compared with no caffeine), whereas the extra carbohydrate alone improved performance by a non-significant 10 s. In the trials with caffeine ingestion, plasma caffeine increased to low levels, suggesting that the performance-enhancing effects were unlikely to be peripheral and more likely to be centrally mediated. It appeared that caffeine reduced the normal progression of fatigue late in endurance exercise.

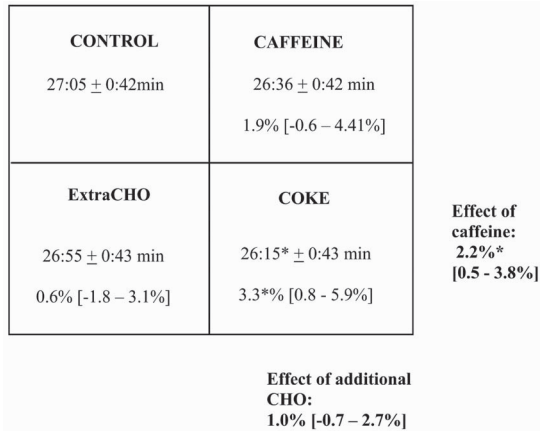

Fig. 2. Time taken for eight well-trained cyclists to complete a time-trial. Each cyclist was asked to complete 7 kJ of work per kilogram of body mass as quickly as possible ($\sim$30 min) after cycling for 2 h at 70% $\dot{V}O_{2max}$. The participants completed the protocol on four separate occasions and consumed 5 ml of a 6% sports drink every 20 min for the first hour and then switched to the same volume of flat cola at 80 and 100 min (and 120 min if desired). The cola beverage was varied in the four conditions to include: (1) no caffeine and 6% carbohydrate (Control); (2) caffeine (90 mg +) and 11% carbohydrate (Cola); (3) no caffeine and 11% carbohydrate (Extra CHO); or (4) caffeine (90 mg +) and 6% carbohydrate (Caffeine). There was a significant main effect for caffeine compared with no caffeine. Adapted from Cox *et al.* (2002).

These recent studies add strong support to the body of evidence suggesting that caffeine can improve performance by directly affecting the CNS. Clearly, this implies that caffeine in low doses should enhance the ability to perform in both training and competition. During training, the ability to train harder should lead to greater training adaptations.

Does creatine supplementation enhance gains in muscle size in response to resistance training?

Over the past decade, no other nutritional supplement has received as much attention from athletes and coaches as creatine, an amino acid derivative formed naturally in the body but also present in relatively small quantities in the diet. The topic of creatine supplementation and high-intensity exercise performance is reviewed by Maughan *et al.* (2004) and will not be reviewed again here. Rather, our purpose is to review evidence for and against a potential role for creatine in enhancing resistance training-induced gains in skeletal muscle size. It has been consistently reported that creatine ingestion during heavy resistance training augments gains in body mass, fat-free mass and muscular strength (e.g. Earnest *et al.*, 1995; Vandenberghe *et al.*, 1997; for a recent review, see Krieder, 2003). These adaptations have often been attributed to an accelerated rate of muscle protein accretion, but only recently have investigators tested this hypothesis directly by examining changes within skeletal muscle.

The first study to directly examine creatine supplementation in conjunction with heavy resistance training on skeletal muscle hypertrophy in humans was published by Volek and colleagues in 1999. Nineteen resistance-trained men were randomly assigned in a double-blind fashion to either a creatine or placebo (cellulose) group, and then performed whole-body, periodized heavy resistance training for 12 weeks. The supplementation consisted of 25 $g \cdot day^{-1}$ of creatine/placebo for the first 7 days after baseline testing,

followed by 5 $g \cdot day^{-1}$ until the end of the study. After training, the creatine-supplemented group displayed significantly greater gains in bench press and squat strength, as well as large increases in body mass and fat-free mass. The most notable finding, however, was a significantly greater increase in Type I, IIa and IIb muscle fibre cross-sectional area (CSA) in the creatine-supplemented group compared with placebo. When expressed as a relative change, the mean increase in CSA for the fibre types examined was ~34% in the creatine group versus ~10% in the placebo group. A potential limitation to the study, however, was the unexpected finding that the participants assigned to the creatine group had ~20% smaller muscle fibre areas at baseline. Thus, while the relative increase in CSA was larger in the creatine group, there were no differences between conditions in absolute CSA for any fibre type after training (Fig. 3).

Since the first report by Volek *et al.* (1999), four other studies have described the effects of creatine supplementation on resistive-training-induced changes in skeletal muscle (Hespel *et al.*, 2001; Stevenson and Dudley, 2001; Tarnopolsky *et al.*, 2001; Willoughby and Rosene, 2001). These latter investigations have produced equivocal findings, with two studies supporting the initial work of Volek *et al.* (1999) (Hespel *et al.*, 2001; Willoughby and Rosene, 2001) and two studies concluding that creatine supplementation does not augment training-induced gains in muscle size (Stevenson and Dudley, 2001; Tarnopolsky *et al.*, 2001).

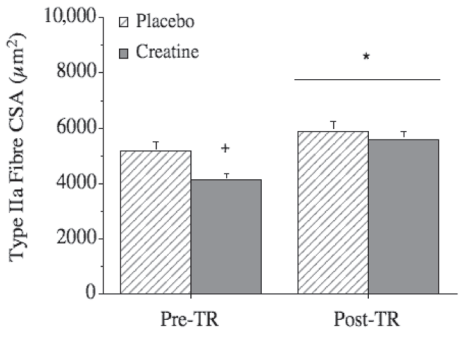

Fig. 3. Cross-sectional area (CSA) of type IIa muscle fibres in the vastus lateralis of resistance-trained volunteers before (Pre-TR) and after (Post-TR) a 12-week periodized leg resistance training programme, during which the participants were supplemented with either creatine or placebo (cellulose). The relative increases in CSA for Type I, IIa, IIab and IIb fibres were higher for the creatine-supplemented group than the placebo group (mean change of 36 and 10%, respectively). However, the absolute muscle fibre CSAs were not different between groups after training, due to the unexpected finding that the creatine group had 20% smaller muscle CSA than the placebo group before training. Adapted from Volek *et al.* (1999).

The lack of congruent results may be related in part to differences in study methodology, including: the study population (i.e. resistance-trained or untrained); mode, frequency and duration of the training intervention; technique used to assess muscle adaptation; and, possibly, the type of 'placebo' intervention employed.

Hespel and colleagues (2001) examined the effect of creatine ingestion on the contractile, biochemical and histochemical properties of human skeletal muscle during immobilization and rehabilitation. Participants had their right leg immobilized with a polyester cast for 2 weeks before participating in a 10-week rehabilitation programme that consisted of 4–6 sets of unilateral knee extension exercises, with 12 repetitions per set at an intensity of 60% one-repetition maximum (1-RM), three times a week. The participants were divided into two groups and throughout the entire period of immobilization and rehabilitation received either creatine monohydrate (initial loading dose of 20 $g \cdot day^{-1}$, followed by 5 $g \cdot day^{-1}$) or a placebo (not stated). After immobilization, quadriceps muscle CSA and isometric knee extension torque were decreased to the same extent in both conditions when compared with baseline. However, muscle CSA and peak torque recovered faster in the creatine-supplemented group than the placebo group, when assessed after 3 and 10 weeks of the rehabilitation period. Biopsies obtained from the vastus lateralis revealed that type I, IIa and IIb muscle fibre cross-sectional areas after 10 weeks of rehabilitation when compared with baseline were only elevated in the creatine-supplemented group. A unique aspect of that study was that biopsy samples were also examined to assess, for the first time in humans, the expression of the myogenic transcription factors MyoD, myogenin, Myf5 and MRF4. There is substantial evidence from experiments with rats to suggest that these factors are involved in regulating processes intrinsic to muscle cell catabolism and anabolism (e.g. Marsh *et al.*, 1997; Adams *et al.*, 1999). After 10 weeks of rehabilitation, MRF4 protein content was higher in the creatine-supplemented group than in the placebo group, whereas myogenin protein showed the opposite effect, and protein expression of Myf5 and MyoD remained unchanged. The authors suggested that changes in specific myogenic transcription factors induced by oral creatine supplementation might influence the muscle hypertrophy response during rehabilitative strength training.

In support of the findings of Volek *et al.* (1999) and Hespel *et al.* (2001), Willoughby and Rosene (2001) concluded that creatine supplementation during chronic resistance training increased muscle strength and size, possibly as a result of increased myosin heavy chain (MHC) synthesis. Untrained young men were randomly assigned to a control, placebo (6 $g \cdot$ dextrose $\cdot day^{-1}$) or creatine-supplemented group (6 $g \cdot day^{-1}$)

in a double-blind fashion. They then performed 12 weeks of lower-body heavy resistance training (leg press, leg extension, leg curl; three sets of 6–8 repetitions at 85–90% 1-RM, 3 times per week). Needle biopsy samples from the vastus lateralis revealed that, following training, myofibrillar protein increased by 58% in the creatine-supplemented group, and this was significantly greater compared with the relative increases in the placebo and controls groups (12% and 3%, respectively). The relative changes in MHC isoform mRNA for Type I, IIa and IIx were also higher in the creatine-supplemented condition than in the placebo and control conditions. A subsequent report by the same authors (Willoughby and Rosene, 2003), based on additional analyses of muscle samples collected during their original study, concluded that creatine supplementation combined with heavy resistance training increased the mRNA and protein expression of MRF4 and myogenin. This latter finding differs from the results of Hespel *et al.* (2001), although direct comparisons between studies are hampered by differences in study design.

In contrast to the studies described above, which concluded creatine supplementation augments muscle protein accretion during resistance training in humans, two well-controlled studies from separate laboratories have failed to support this hypothesis (Stevenson and Dudley, 2001; Tarnopolsky *et al.*, 2001). Stevenson and Dudley (2001) studied 18 resistance-trained individuals who ingested either creatine or table sugar (initial loading dose of 20 $g \cdot day^{-1}$ for 7 days followed by 5 $g \cdot day^{-1}$ thereafter) and then performed an 8-week electrostimulation resistive-training programme. The authors' laboratory previously showed that electrostimulation can induce marked hypertrophy in a few months without requiring voluntary effort or the participants to alter their resistance training (Ruther *et al.*, 1995). The specific electrostimulation protocol consisted of 3–5 sets of coupled concentric and eccentric actions that were applied to the left quadriceps femoris twice weekly while the participants continued voluntary resistance training on both lower limbs unsupervised. Quadriceps femoris CSA, assessed using magnetic resonance imaging, increased after electrostimulation by 10% and 12% in the placebo and creatine-supplemented groups, respectively, with no significant differences between conditions. A notable observation was that the CSA of the right quadriceps, which did not receive electrostimulation but continued to experience a chronic, unsupervised training stimulus, increased by 5% in the creatine-supplemented group, whereas the placebo group showed no change. Although speculative, one potential explanation for this finding was that the participants in the creatine-supplemented group may have performed a larger overall volume of unsupervised leg training and this provided a greater cumulative stimulus to the right (non-electrostimulated) leg compared with the placebo condition.

Finally, Tarnopolsky and colleagues (2001) provided a unique perspective on this issue by highlighting that virtually all studies have compared a creatine-supplemented group with a group who received either a non-isoenergetic or non-isonitrogenous placebo (e.g. cellulose or dextrose). In addition, these authors noted that few creatine supplementation studies controlled the timing of supplement ingestion after exercise. This is an important consideration, given that the composition and timing of nutrient delivery can profoundly alter post-exercise protein metabolism and thus potentially impact gains in mass, strength and muscle protein balance (Tipton and Wolfe, 2004). Tarnopolsky *et al.* (2001) specifically tested the hypothesis that a post-exercise creatine–carbohydrate supplement would result in similar gains in strength and muscle fibre area as an isoenergetic and isonitrogenous protein–carbohydrate supplement. In a double-blind fashion, young male participants were randomized to receive either 10 g of creatine + 75 g glucose or 10 g of casein + 75 g glucose, immediately after each exercise bout during an 8-week whole-body progressive resistance training programme. Training increased Type I and II muscle fibre CSA by ~20 and ~25%, respectively, but there was no significant difference between groups, and gains in 1-RM strength for each of 16 tested exercises were similar (Fig. 4). The only difference between conditions was that the body mass gains were higher in the creatine-supplemented group. From a practical standpoint, therefore, the authors concluded that athletes

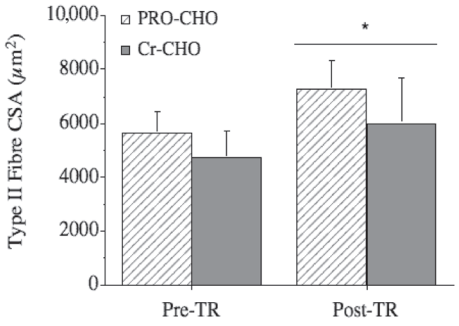

Fig. 4. Cross-sectional area (CSA) of type II muscle fibres in the vastus lateralis of untrained volunteers before (Pre-TR) and after (Post-TR) an 8-week progressive leg resistance training programme, during which the participants were provided with a creatine–carbohydrate supplement (Cr-CHO) or isonitrogenous and isoenergetic protein (casein)–carbohydrate supplement (PRO-CHO). The relative increases in CSA for Type I and II fibres were not different between groups after training. Adapted from Tarnopolsky *et al.* (2001).

who are engaged in sports where a high strength:lean mass ratio is important may wish to consider a protein–carbohydrate supplement, whereas athletes who desire a high absolute mass might consider a creatine–carbohydrate supplement.

In summary, there are equivocal data to suggest that creatine supplementation may enhance resistance training-induced gains in muscle size, but the potential mechanisms involved and the potential confounding influence of nutrient composition/timing remain unclear. From a theoretical standpoint, the increase in body water retention that typically accompanies creatine supplementation could alter protein turnover through changes in cellular hydration status (Haussinger *et al.*, 1993), but there is no direct evidence for this in human muscle following either acute or chronic creatine ingestion. There are *in vitro* data which suggest that creatine administration may stimulate muscle protein synthesis (Ingwall *et al.*, 1972, 1974), although this is not a universal finding (Fry and Morales, 1980). Vierck *et al.* (2003) recently reported that treatment with creatine induced modest differentiation of myogenic satellite cells. This is a noteworthy observation given that adult myofibres are terminally differentiated and muscle regeneration after injury (e.g. myotrauma induced by resistance exercise training) appears to be dependent upon satellite cell activation and proliferation (Hawke and Garry, 2001). Nonetheless, the human studies that have been conducted to date have largely failed to detect any specific effect of creatine ingestion on skeletal muscle protein turnover. Parise *et al.* (2001) reported no effect of short-term creatine loading ($20\ g \cdot day^{-1}$ for 7 days) on whole-body or mixed-muscle protein fractional synthetic rate in humans, although the breakdown and oxidation of some proteins were lower in men, suggesting a possible anticatabolic effect of creatine. However, a recent comprehensive study by Louis *et al.* (2003) concluded that creatine supplementation ($21\ g \cdot day^{-1}$ for 5 days) had no effect on human muscle protein turnover (synthesis or degradation) at rest in both the post-absorptive and fed states. Similarly, Phillips *et al.* (2002) examined the combined effects of creatine supplementation and chronic resistance training on skeletal muscle protein turnover in men, and reported no significant difference compared with a group that received an isoenergetic and isonitrogenous placebo. Given that creatine supplementation facilitates muscle recovery during repeated bouts of high-intensity exercise, it has been suggested that strength athletes who supplement with creatine may be able to tolerate higher training volumes and chronically this leads to greater cumulative overload on the muscles (Krieder, 2003). While this theory appears plausible, at present there is insufficient evidence to directly evaluate it. Moreover, one of the few relevant studies that accurately quantified training volumes (Volek *et al.*, 1999) reported greater gains in leg muscle hypertrophy in a creatine-supplemented group, even though the volume of leg training performed was not different from that performed by the placebo group. Clearly, additional work is warranted to clarify our understanding of the potential interactive effect of creatine supplementation and chronic resistance exercise training on skeletal muscle hypertrophy and the potential regulatory mechanisms involved.

Post-exercise nutrition: is fat intake a concern?

The importance of ingesting carbohydrates in the minutes and hours after a training bout to maximize the resynthesis of muscle glycogen is examined by Burke *et al.* (2004). Essentially, as soon as one exercise training session ends, the athlete is in recovery mode and preparing for the next training session. It seems fair to conclude that carbohydrate repletion is of paramount importance, as most training sessions for most sports will require muscle glycogen as a fuel for exercise.

Of late, concern has been raised about post-exercise nutrition by a series of experiments examining the use and replenishment of intramuscular triacylglycerol (IMTG) during and after exercise. It is interesting to note that skeletal muscle stores a significant amount of IMTG, enough that the energy equivalent represents 67–100% of the energy stored as muscle glycogen in both untrained and trained individuals. Until recently, there had been considerable controversy about whether IMTG contributes a significant amount of fuel during exercise where fat makes a major contribution. However, recent studies using new techniques for measuring IMTG and examining the variability of direct assessments of IMTG have largely resolved this controversy. In short, there appears to be general consensus that IMTG is an important fuel during prolonged moderate-intensity exercise (and up to $\sim 85\%\ \dot{V}O_{2max}$ in well-trained athletes) and that the fat content of the post-exercise diet (and, therefore, carbohydrate) influences the rate that IMTG recovers (van Loon *et al.*, 2003; Watt *et al.*, 2002b).

Many laboratories have measured net IMTG use with direct biochemical analysis of needle biopsy samples taken from the vastus lateralis muscles of men and reported that IMTG was not an important fuel during exercise lasting 90–120 min at a power output of 50–65% $\dot{V}O_{2max}$. Others have reported net IMTG use during exercise in men and women (see Watt *et al.*, 2002b, for a review). A major criticism of this work was that the needle muscle biopsy samples were contaminated by the presence of adipose tissue triacylglycerol,

making any estimation of IMTG inaccurate. The fact that the *between-biopsy* (3 biopsies) variability of this measurement was about ~20–26% in a group of untrained and active individuals supported this contention (Wendling *et al.*, 1996). Interestingly, the *within-biopsy* variability was low (~6%) and in the same range reported for other fuels and metabolites measured in human muscle biopsies. At the same time, almost all of the studies that have estimated the use of IMTG during exercise by measuring whole-body respiratory exchange ratio (RER) and exogenous free fatty acids reported a significant use of IMTG during prolonged exercise. In addition, many studies employing histochemical IMTG staining techniques reported that IMTG was reduced after endurance exercise (Watt *et al.*, 2002b).

Recently, a new technique that uses ^{1}H-magnetic resonance spectroscopy (MRS) to distinguish between intra- and extramuscular triacylglycerol has been used to examine this issue (Szczepaniak *et al.*, 1999). Again, the studies using this technique have for the most part reported a net IMTG use during endurance exercise in a variety of upper and lower leg muscles (see Watt *et al.*, 2002b). In addition, Watt *et al.* (2002a) re-investigated the issue of IMTG use during exercise when measured biochemically by taking double biopsy samples throughout exercise in a group of well-trained cyclists. They reported that the between-biopsy variability was lower in trained (~12%) than untrained (~24%) cyclists and that this allowed for the detection of significant decreases in IMTG content during 2 h of cycling at 57% $\dot{V}O_{2max}$. Therefore, the authors argued in a recent review (Watt *et al.*, 2002b) that much of the controversy regarding IMTG use during exercise in the studies employing biochemical analyses of muscle biopsy samples is a function of two things: (1) there is significant variability between muscle biopsy samples in human skeletal muscle, although this is less in trained individuals; (2) because of the high energy density of fat, the amount of IMTG used during 90–120 min of cycling at 50–65% $\dot{V}O_{2max}$ is not large and can be less than the between-biopsy variability in untrained/active individuals. Another point that is apparent from these recent studies is that adipose tissue contamination of the biochemical estimates of IMTG is not present or is minimal, as the measured values are in the same range as or lower than the IMTG values reported using the ^{1}H-MRS technique. A final point is that the few studies that have examined this issue in well-trained females all suggest that IMTG is a significant fuel source during endurance exercise. Interestingly, this includes one study that used muscle biopsies and biochemical IMTG determination (Steffensen *et al.*, 2002), one that deduced IMTG use from RER and plasma free fatty acid oxidation estimates (Romijn *et al.*, 2000) and one that used the ^{1}H-MRS technique (Larson-Meyer *et al.*, 2002). In summary, it would appear that there is general consensus that IMTG is a significant source of fuel during moderate aerobic exercise in active and trained individuals. This may extend to intense aerobic power outputs (~85% $\dot{V}O_{2max}$) in well-trained individuals. However, there still exists controversy over the accuracy of the various methods used to estimate IMTG use during exercise and, therefore, the magnitude of the IMTG contribution to total fuel use.

The practical reality of using IMTG during exercise training sessions is that it will need to be replenished during the recovery period between workouts. Although it is unclear at present whether beginning an exercise session with an IMTG store that is less than normal will actually limit the ability to exercise or train, it is clear that an inability to replenish this store over repeated training sessions could lead to such a situation. The remainder of this section will highlight the recent work that has examined the issue of IMTG repletion after exercise.

Starling *et al.* (1997) reported that the vastus lateralis IMTG content (biochemical measurement) was higher 1 day after exercise when participants ingested a high fat diet (68% of energy) rather than a very low fat diet (5%). The participants first completed 120 min of exercise at 65% $\dot{V}O_{2max}$, then consumed one of the diets for 12 h and fasted another 12 h. The IMTG concentration increased in the 24 h after exercise on the high fat diet (from 32.8 to 44.7 $mmol \cdot kg^{-1}$ dry mass) but did not recover when on the low fat diet (from 30.9 to 27.5 $mmol \cdot kg^{-1}$ dry mass). However, the high fat diet was also a low carbohydrate diet and the repletion of glycogen was impaired and performance during a subsequent self-paced cycling time-trial decreased (time to complete 1600 kJ; high fat = 139 min, low fat = 117 min). Two other studies measured recovery IMTG after exhaustive cycling using muscle biopsies and biochemical techniques. In both cases, the well-trained athletes consumed a diet high in carbohydrate (65–70% carbohydrate, 20% fat, 10–15% protein) in the first 18–30 h after a prolonged and exhaustive exercise bout. Neither study found evidence of an increase in IMTG after exercise. Kiens and Richter (1998) reported significantly lower IMTG concentrations at 3, 6, 18 and 30 h with a low point of 20% below the immediate post-exercise value 18 h after exercise. On the other hand, Kimber *et al.* (2003) reported no significant change in IMTG after 3, 6 and 18 h of recovery compared with the immediate post-exercise value. Additional studies have specifically examined the effects of two or more diets on IMTG use and recovery.

Coyle *et al.* (2001) examined the effect of 7-day diets that contained 32, 22 or 2% of energy intake from fat on IMTG and glycogen stores and subsequent fuel utilization during 2 h of exercise at 67% $\dot{V}O_{2max}$ in

well-trained cyclists. The changes in carbohydrate consumption were reciprocal with fat and energy intake from protein constant at 10%, such that all diets were eucaloric. Participants exercised for 2 h at 67% $\dot{V}O_{2max}$ in the morning on all days of the 7-day diets except the first day. The pre-exercise IMTG store was unaffected by a reduction in fat intake from 32 to 22%, but was decreased by ~20% following the 2% fat diet. Muscle glycogen increased from a low of ~530 $mmol \cdot kg^{-1}$ dry mass after consuming 32% fat to ~700 and 825 $mmol \cdot kg^{-1}$ dry mass after the 22 and 2% fat diets, respectively. During exercise, there were no differences in estimated fuel use between the 32 and 22% fat diets, but the 2% fat diet decreased fat and increased carbohydrate oxidation rates compared with the 22% fat diet (RER, 0.908 *vs* 0.876). The amount of plasma free fatty acid oxidation was not decreased in the 2% fat condition, implying that the reduction in whole-body fat oxidation was solely due to a reduction in free fatty acid oxidation from IMTG. The finding that IMTG was well maintained even during the very low fat intake diet led the authors to suggest that fat may be synthesized from carbohydrate in such a low fat/high carbohydrate condition.

Surprisingly, the small decrement in IMTG and increase in muscle glycogen contents following the low fat diet led to increased glycogen oxidation and reduced IMTG oxidation while not affecting the amount of extramuscular fuel that was oxidized. It is not known how the changes in initial fuel stores predisposed the muscle to these changes in fuel use. It could be argued that, at an intensity of 67% $\dot{V}O_{2max}$, it doesn't matter whether the athlete consumes a diet of 2–22% fat, 10% protein and the balance carbohydrate, as fuel reliance simply follows dietary intake. However, what happens to the IMTG store over time with repeated bouts of training and a low fat diet?

Subsequent studies have specifically examined the time-course of IMTG repletion after a single bout of prolonged exercise. Decombaz *et al*. (2001) examined the effect of post-exercise diets containing either 14.5 or 55.5% of the total energy intake from fat on IMTG and glycogen repletion in the tibialis anterior of six untrained men and six trained male runners at 9 and 30 h post-exercise. The protein contribution was constant at 14% and carbohydrate was reciprocal with fat such that diets were eucaloric. The exercise consisted of 2 h of running or walking uphill on a treadmill at ~50% $\dot{V}O_{2max}$. The resting IMTG and glycogen contents were higher in the muscles of trained participants. The IMTG content decreased by about 22–26% in both groups but the absolute decrease was almost double in the trained group (Fig. 5). Whole-body RER averaged ~0.89 and ~0.93 in the trained and untrained groups, respectively, and IMTG was estimated to contribute ~15% and ~17% of whole-body fat metabolism, respectively. Repletion of IMTG on the high fat diet was apparent at 9 h of recovery and reached values ~30% higher than pre-exercise at 30 h of recovery in both groups. Recovery on the low fat diet produced no recovery in IMTG during the initial 9 h, with values reaching ~80% and ~95% of that pre-exercise at 30 h in the trained and untrained groups (Fig. 5). Glycogen use during exercise was 35–43% of the resting store in both groups. Glycogen repletion was faster on the low fat (high carbohydrate, ~70%) diet, reaching pre-exercise values by 9 h. No further changes occurred in the trained group but the untrained participants supercompensated glycogen stores to 155% of pre-exercise by 30 h. In contrast, on the high fat (low carbohydrate, ~30%) diet, muscle glycogen was not replenished to pre-exercise values until 30 h. The authors concluded that, given the dynamic nature of the IMTG store and the potential for it to spare muscle glycogen, 'it would be wise to try and optimize [IMTG] storage before competition, at the same time ensuring that glycogen storage is not compromised' (Decombaz *et al*., 2001). However, this statement seems premature given that there is no evidence to demonstrate that IMTG is ever limiting for an athlete during training or competition. Also, there is no information determining the importance of IMTG during the higher exercise intensities that are employed during training and competitions. Exercise at power outputs of ~50% would have little relevance to a population of trained athletes.

Larson-Meyer *et al*. (2002) also examined this issue by measuring the influence of recovery diet on soleus muscle IMTG repletion after a 2-h treadmill run at ~67% $\dot{V}O_{2max}$ in seven well-trained recreational female runners. Post-exercise diets contained either 10 or 35% of the total energy intake from fat, 15% from protein and, therefore, 75 or 50% from carbohydrate. Soleus IMTG was measured before, immediately after and 22 h (~1 day) and 70 h (~3 days) after the exercise bout. Participants were allowed to run for 45 min after the IMTG measurement on the first recovery day and again on the second recovery day. Exercise decreased IMTG content by ~25%, and the higher fat diet repleted IMTG by 22 h and to a value ~22% higher than pre-exercise by 70 h. In contrast, the low fat diet did not allow complete repletion of IMTG, reaching ~88% of pre-exercise levels, even after 70 h. Absolute IMTG numbers were not reported, only changes relative to the bone marrow peak ^{1}H-MRS signal. The authors noted that the consequences of compromised IMTG stores on the low fat recovery diet need to be investigated during heavy training and performance (Larson-Meyer *et al*., 2002). They also stated that, 'the current study provides

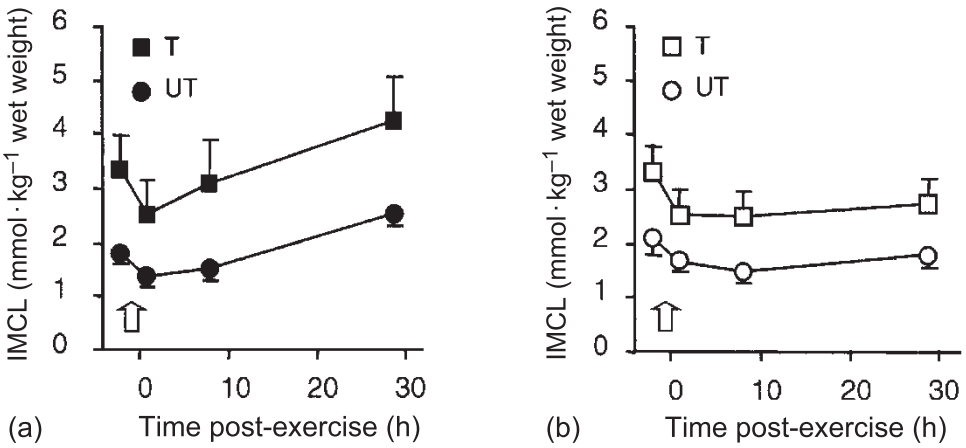

Fig. 5. Effects of recovery diet on intramuscular triacylglycerol (IMCL) content in the tibialis anterior of six trained male runners (T) and six untrained men (UT). Arrows indicate exercise that consisted of 2 h of running/walking uphill on a treadmill at ~50% $\dot{V}O_{2max}$. The participants then consumed eucaloric diets containing either 55.5% (a) or 14.5% (b) of the total caloric intake from fat (14% protein, balance carbohydrate) for the next 30 h.

evidence that diets too low in fat are probably not ideal for endurance athletes'.

Johnson *et al.* (2003) recently examined the effects of a strenuous exercise bout, followed by a recovery diet with varying fat contents for 2 days, on vastus lateralis IMTG concentrations and the ability to complete a subsequent cycling time-trial (~3 h) in six highly trained male cyclists. The high fat/low carbohydrate diet contained 56% fat, 6% carbohydrate and 37% of total energy from protein; the low fat/high carbohydrate diet contained 19% fat, 63% carbohydrate and 17% protein. Unfortunately, the diets were not isoenergetic and the participants consumed ~33% more energy on the low fat/high carbohydrate diet. Calculated IMTG was ~10.7 and 7.0 $mmol \cdot kg^{-1}$ after the high and low fat recovery diets, respectively. Muscle glycogen was very low after the high fat/low carbohydrate diet and high after the low fat/high carbohydrate diet. During the subsequent time-trial rides, performance time was worse (~200 *vs* 178 min), exercise intensity was lower (66 *vs* 71% $\dot{V}O_{2max}$) and RER was lower (~0.80 *vs* 0.90) after the high fat compared with the low fat diet. Plasma free fatty acids were higher throughout exercise after the high fat diet and the participants received a carbohydrate drink at timed intervals during exercise in both trials. The IMTG concentration decreased by about 55–65% in both time-trial rides, but the absolute amount of IMTG used was much higher in the high than in the low fat trial (6.63 *vs* 3.63 $mmol \cdot kg^{-1}$ wet weight). The authors concluded that near-depletion of IMTG was evident in some of the athletes during prolonged strenuous cycling, regardless of the pre-diet. If the depletion of IMTG was prevalent in the Type I fibres, it remains to be seen whether this could have a negative effect on training or competition performance.

Lastly, van Loon *et al.* (2003) had nine endurance-trained athletes cycle for 3 h at 55% $\dot{V}O_{2max}$ on two occasions followed by either a diet high in fat (39% fat, 49% carbohydrate, 14% protein) or low in fat (24% fat, 62% carbohydrate and 14% protein) for 3 days. Vastus lateralis IMTG decreased by ~21% after the 3-h cycle and had repleted towards normal in the high fat diet at 24 h and reached pre-exercise values at 48 h. In the low fat trial, IMTG was not significantly repleted even after 48 h. In addition, quantitative fluorescence microscopy of the muscle following 48 h of recovery and ingestion of the high fat diet revealed higher IMTG content in Type 1 muscle fibres than with the low fat diet (2.1 *vs* 1.4% area staining). These results are surprising given that the so-called 'low fat diet' contained 24% of the total energy intake as fat.

In summary, it is clear that high fat intakes (35–57% of energy) in the recovery periods following a prolonged exercise bout will replete IMTG stores quicker than low fat intakes (10–24%). The amount of ingested fat required for IMTG repletion has been estimated to be ~2 $g \cdot kg^{-1}$ $BM \cdot day^{-1}$ (Decombaz, 2003). However, a high fat intake may compromise the ability to replete muscle glycogen and impair performance. One might expect that diets with moderate fat intake (about 20–30% fat) would be adequate for IMTG replenishment, but the findings with intakes of 19 and 24% do not support this contention. However, the 19% fat diet in the study of Johnson *et al.* (2003) was confounded by a low energy intake and although van Loon *et al.* (2003) reported no repletion in 48 h with a 24% fat recovery

diet, the exercise-induced decrease in IMTG was only of the order of ~20%. It must be borne in mind that there is considerable discussion in these papers regarding the best way to express the IMTG data – against a muscle water or creatine reference or a bone marrow reference (for discussion, see Larson-Meyer *et al.*, 2002). Given the possibility for water shifts during exercise and the variability in the creatine measurement in some studies (Johnson *et al.*, 2003), it is possible that 20% changes in IMTG content are near the detectable limit of the ^{1}H-MRS technique. However, van Loon *et al.* (2003) reported IMTG repeatability of the order of 5%. Recovery of IMTG was complete after only 22 h when a 35% fat diet was consumed after exercise. Additional studies examining fat intake after exercise in the range of 20–30% are needed to clarify this issue. It may be that when 2 days are available for recovery, a diet that provides sufficient energy and includes about 2 g fat $\cdot$ kg^{-1} and 6–10 g carbohydrate $\cdot$ kg^{-1} will be optimal. If prolonged exercise occurs nearly every day, is a chronically low IMTG content simply a reality? This would leave the endurance athlete in a carbohydrate dependent state where their post-exercise diet would concentrate on adequate carbohydrate and protein intake. Decombaz (2003) has suggested that carbohydrate content should be high and fat content low in the initial 6–8 h of recovery and then fat can be added in the form of regular meals.

Nutrient–gene interactions

Exercise physiologists have been interested in the ability of skeletal muscle to adapt to repeated bouts of exercise since this field of study began. Understanding of the basic mechanisms that regulate these changes has progressed tremendously in the last 10 years with the increasing use of powerful molecular and cellular tools (Booth, 1998; Hargreaves and Cameron-Smith, 2002). Numerous investigations have now reported that exercise upregulates the expression of several genes that encode for skeletal muscle proteins that play a role in meeting the demands of exercise (Kraniou *et al.*, 2000; Pilegaard *et al.*, 2000; Tunstall *et al.*, 2002; Nordsborg *et al.*, 2003; see Hargreaves and Cameron-Smith, 2002, for a review). It has been suggested that the cellular adaptations to exercise training may be due to the cumulative effects of the transient increases in gene transcription that occur during and after repeated exercise bouts (Williams and Neufer, 1996; Hargreaves and Cameron-Smith, 2002). In this section, we are interested in particular in the recent work that has been done that links nutrition to the adaptations that occur in human skeletal muscle during training. Selected examples of the consequences of short-term dietary manipulations on gene expression in human skeletal muscle are discussed below.

Peters *et al.* (2001) examined the effects of consuming an isoenergetic high fat diet (73% fat, 5% carbohydrate, 22% protein) following a standardized pre-diet (30% fat, 50% carbohydrate, 21% protein) on pyruvate dehydrogenase kinase (PDK) isoform mRNA and protein content and PDK activity in human skeletal muscle. Six active males had muscle biopsies taken from the vastus lateralis before the high fat diet (day 0) and in the morning 1, 2 and 3 days after being on the high fat diet. One confounding factor in this study was that the participants were not allowed to exercise as they normally would have during the 3 days of the high fat diet. The resting RER decreased progressively from ~0.8 on day 0 to ~0.7 on day 3. Ketone body and free fatty acid (0.38–0.83 mmol $\cdot$ l^{-1}) concentrations increased progressively during the high fat diet while fasting insulin decreased by 50%. The PDK activity increased after only 1 day on the high fat diet and continued to increase in a linear fashion while on this diet (Fig. 6). Concentrations of both mRNA and protein PDK 4 isoform increased dramatically after 1 day on the high fat diet and remained high with no further increases on days 2 or 3, while PDK 2 concentrations were unchanged (Fig. 7). The linear increase in PDK activity in the face of a constant PDK 4 protein concentration during high fat ingestion, suggested that either another PDK isoform (i.e. PDK 1 or 3 – there are only four) contributed to the increase in PDK activity or there was an increase in PDK-specific activity. Given that it has been shown that the

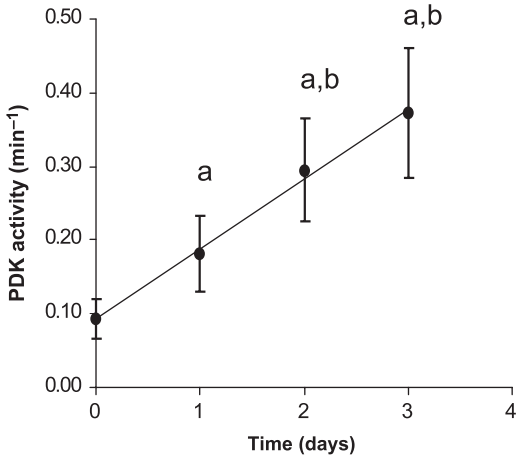

Fig. 6. Pyruvate dehydrogenase kinase (PDK) activity in the vastus lateralis of men following a normal diet (time 0) and following 1, 2 and 3 days on a high fat/low carbohydrate diet (73% fat, 5% carbohydrate, 22% protein). [a]Significantly different from time 0. [b]Significantly different from 'a' alone.

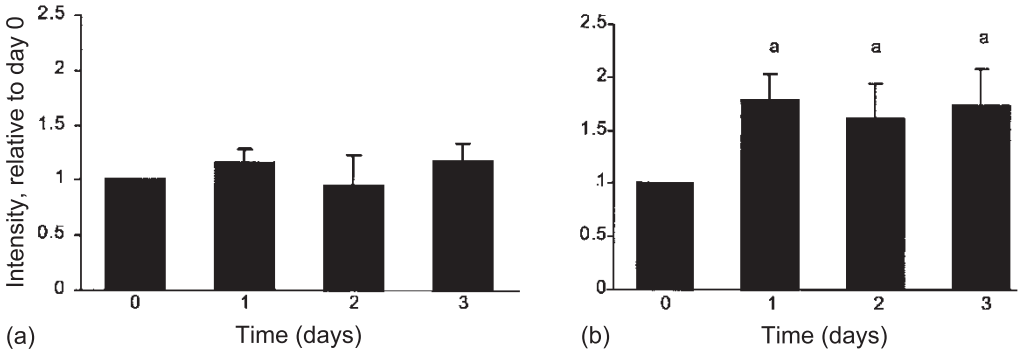

Fig. 7. Pyruvate dehydrogenase kinase 2 (a) and 4 (b) isoform protein content in the vastus lateralis of men following a normal diet (time 0) and following 1, 2 and 3 days on a high fat/low carbohydrate diet (73% fat, 5% carbohydrate, 22% protein). [a]Significantly different from time 0.

abundance of PDK 1 and 3 are very low in human skeletal muscle and do not respond to 40 h of fasting (Spriet *et al.*, 2002), it is likely that the latter explanation is correct. There are existing data for rodent skeletal muscle that demonstrate the ability of the PDH complex to increase the binding of newly formed PDK 4 protein or existing PDK 2 protein, thereby accounting for the increased PDK-specific activity. This is an impressive adaptive quality for an enzyme that plays such an important role in the oxidation of carbohydrate both at rest and during exercise.

The PDH complex quickly responds to a lack of carbohydrate availability and/or increased fat oxidation by upregulating PDK activity, which drives more of the PDH enzyme into the inactive form and ultimately decreases skeletal muscle carbohydrate oxidation and spares the small store of carbohydrate in the body. Experiments that have artificially elevated plasma free fatty acid for 4–5 h have reported very large increases in PDK 4 mRNA levels, underscoring how rapidly fuel availability can upregulate PDK gene expression and decrease carbohydrate oxidation in skeletal muscle (R. J. Tunstall, unpublished observations). We also recently examined the response of 4 h of exercise at $\sim$55% $\dot{V}O_{2max}$ on PDK activity, as it has been shown that carbohydrate oxidation and PDH activity decrease as exercise is prolonged beyond 2–3 h (Watt *et al.*, 2002a). Muscle biopsies were taken at rest, and at 10 min and 4 h of exercise. Carbohydrate oxidation and PDH activity (at 4 h) decreased and plasma free fatty acid concentration and fat oxidation increased during exercise as expected. The PDK activity was unchanged at 10 min but doubled at 4 h with no changes in PDK 2 and 4 protein levels (Peters *et al.*, 2003). Again, but this time in an exercise context, PDK increased and appeared to account for the decreasing PDH activity without an increase in total PDK protein. The participants consumed only water during this trial, so it is not known whether supplemental carbohydrate ingestion could reverse these changes both at the gene expression and enzyme activity levels.

Cameron-Smith *et al.* (2003) examined the effects of either a high carbohydrate diet (70–75% carbohydrate, $<$15% fat) or an isoenergetic high fat diet ($>$65% fat, $<$29% carbohydrate) for 5 days on the expression of genes encoding proteins for fatty acid transport and beta-oxidation in human skeletal muscle. The participants were 14 well-trained cyclists and triathletes ($\dot{V}O_{2max} = 67\ ml \cdot kg^{-1} \cdot min^{-1}$) who continued to train daily during the 5-day diet interventions. Plasma free fatty acids were significantly higher after 5 days on the high fat diet compared with baseline and after the high carbohydrate diet ($\sim$0.85 *vs* 0.39–0.41 $mmol \cdot l^{-1}$). Carbohydrate oxidation was reduced and fat oxidation was increased during 20 min of cycling at $\sim$70% $\dot{V}O_{2max}$ following the high fat diet compared with the high carbohydrate diet. The mRNA concentrations of carnitine palmitoyltransferase I, uncoupling protein 3 and the plasma membrane fatty acid binding protein ($FABP_{pm}$) measured in the muscles of six of the participants were unaffected by either diet, although there was large variability in the data. However, there were significant increases in the fatty acid transporter (FAT/CD36) and beta-hydroxyacyl-CoA dehydrogenase mRNA values following the high fat diet compared with the high carbohydrate diet and baseline values. FAT/CD36 protein content was also increased in the muscles of eight participants following the high fat diet, although $FABP_{pm}$ was unchanged. These data provide strong support for the idea that increased dietary fat intake can increase the mRNA content of genes that are necessary for the uptake and oxidation of free fatty acids. This muscle adaptation seems entirely appropriate in the face of reduced availability of carbohydrate and increased availability of plasma free fatty acids. It is also noteworthy that these changes occurred in very well-trained athletes, who presumably have maximized the ability of their muscles to oxidize fat through years

of endurance training. It is also important to again stress that increases in mRNA are not always predictive of increases in protein or measures of functional activity (as discussed above). There are many additional steps and points of regulation between increased mRNA contents and increased protein synthesis rates. This fact points to the need for simultaneous measurements of mRNA and proteins and functional measures including substrate transport and enzyme activities.

The mechanisms by which the adaptations to a high fat/low carbohydrate diet are mediated appear to be related to free fatty acid activation of the family of peroxisome proliferator activator receptors (PPARs) (Duplus *et al*., 2000; Jump and Clarke, 1999) and/or an insulin effect consequent to the decreased carbohydrate availability. However, it has been pointed out that it is unlikely that PPAR-mediated processes are the only mechanism by which free fatty acids can induce gene expression (Duplus and Forest, 2002). Pilegaard *et al*. (2002) have also recently suggested that low concentrations of muscle glycogen may enhance the mRNA content of some genes involved in exercise metabolism. They manipulated pre-exercise muscle glycogen with a combination of exercise and diet and found that the PDK 4 and UCP 3 genes were upregulated to a greater extent in response to exercise. It is not known whether these exaggerated responses translate into greater protein contents or higher functional activities. One would predict that signals responsive both to increased fat availability and decreased carbohydrate availability would work in concert to determine the exact responses in gene expression in skeletal muscle.

In summary, it is clear that nutrition can alter gene expression both at rest and in combination with exercise. However, it is important to note that the dietary manipulations discussed above were for the most part drastic and unlikely to be used by athletes actively engaging in training and competition. It remains to be seen whether smaller changes in dietary carbohydrate and fat content will have effects on gene expression independent of, or in combination with, the training-induced changes.

Summary

In this review, we have attempted to highlight four areas where recent scientific information has forced us to reassess old issues and embrace new ones that may lead to altered nutritional recommendations for athletes. We revisited the issues of supplementing with caffeine and creatine, supplements that are already commonly used by athletes. Recent findings suggest that low doses of caffeine exert a major ergogenic effect on the central nervous system during exercise and strengthen the case for using low doses of caffeine during training. On the other hand, the data on the role that creatine plays in augmenting the increase in skeletal muscle mass and strength during resistance training remain equivocal and further study is required. The final two topics were nutritional issues that are new and have not progressed to the point that we can specifically identify strategies to enhance the adaptation to training. However, it is likely that nutritional strategies will be needed to replenish intramuscular fat stores in individuals engaging in chronic endurance training. It is also likely that the increased interest in gene and protein expression measurements will lead to nutritional strategies that will optimize the adaptations that occur in skeletal muscle during and after exercise training sessions.

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