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Nutritional modulation of training-induced skeletal muscle adaptations

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Hawley JA, Burke LM, Phillips SM, Spriet LL. Nutritional modulation of training-induced skeletal muscle adaptations. J Appl Physiol 110: 834–845, 2011. First published October 28, 2010; doi:10.1152/japplphysiol.00949.2010.—Skeletal muscle displays remarkable plasticity, enabling substantial adaptive modifications in its metabolic potential and functional characteristics in response to external stimuli such as mechanical loading and nutrient availability. Contraction-induced adaptations are determined largely by the mode of exercise and the volume, intensity, and frequency of the training stimulus. However, evidence is accumulating that nutrient availability serves as a potent modulator of many acute responses and chronic adaptations to both endurance and resistance exercise. Changes in macronutrient intake rapidly alter the concentration of blood-borne substrates and hormones, causing marked perturbations in the storage profile of skeletal muscle and other insulin-sensitive tissues. In turn, muscle energy status exerts profound effects on resting fuel metabolism and patterns of fuel utilization during exercise as well as acute regulatory processes underlying gene expression and cell signaling. As such, these nutrient-exercise interactions have the potential to activate or inhibit many biochemical pathways with putative roles in training adaptation. This review provides a contemporary perspective of our understanding of the molecular and cellular events that take place in skeletal muscle in response to both endurance and resistance exercise commenced after acute and/or chronic alterations in nutrient availability (carbohydrate, fat, protein, and several antioxidants). Emphasis is on the results of human studies and how nutrient provision (or lack thereof) interacts with specific contractile stimulus to modulate many of the acute responses to exercise, thereby potentially promoting or inhibiting subsequent training adaptation.

SKELETAL MUSCLE IS A MALLEABLE TISSUE with the capacity to alter its phenotype in response to external stimuli such as contractile activity and nutrient availability (22). The process of exercise-induced adaptation in muscle can be simplistically viewed as the consequence of the accumulation of the type and amount of specific proteins, with the gene expression promoting an increase in protein concentration pivotal to the training-induced response (42). Although the responsiveness of individual messenger RNA (mRNA) species to contractile activity is variable (59), ultimately any increase in steady-state mRNA levels is the result of synthesis (transcription) and degradation (mRNA stability), with the relative contribution of these processes playing an important role in phenotypic adaptation (22). The functional consequences of contraction-induced adaptations are determined largely by the mode of training (i.e., endurance vs. resistance based) and the volume, intensity, and frequency of this stimulus (44). Ultimately, however, the ability of a given muscle cell to alter the type and quantity of protein is a function of its half-life; proteins that turn over rapidly and have high rates of synthesis are capable of attaining a new steady-state level faster than those that turn over slowly during adaptation to contractile and other stimuli.

The interaction between exercise training-induced responses and nutrient availability has long been recognized. Indeed, the extent to which acutely altering substrate supply can modify the “training impulse” has been a key research area among exercise scientists for several decades (5, 46–49). Skeletal...
The metabolic signals that are believed to play major roles in activating the various exercise/nutrient pathways can be broadly classified into three categories: calcium release, metabolites related to the cytoplasmic phosphorylation potential ([ATP]/[ADP] [Pi]), and the mitochondrial reduction/oxidation (redox) state of nicotinamide adenine dinucleotide (NAD/NADH). In addition, the prevailing hormonal milieu, elevated production of O₂ free radicals, electrolyte imbalances across cell membranes, and disturbances in pH all help “fine-tune” the metabolic pathways that match the rate of ATP synthesis with ATP demand (Fig. 1). In response to these perturbations, the postexercise period is characterized by a “general homeostatic recovery phase” that includes the resynthesis of fuel stores, free radical quenching, repair of free radical-mediated damage, and the restoration of intracellular electrolyte concentrations and pH (60). This phase is facilitated through the activation of multiple stress-activated protein kinases and upregulation of gene expression (50, 60) and may ultimately be the stimulus for the chronic intracellular adaptation (42, 68, 104).

The major disruptions to cellular homeostasis occur during exercise, being largely dependent on the relative exercise intensity and prevailing substrate availability within the active muscles. The metabolic signals that are believed to play major roles in activating the various exercise/nutrient pathways can be broadly classified into three categories: calcium release, metabolites related to the cytoplasmic phosphorylation potential ([ATP]/[ADP] [Pi]), and the mitochondrial reduction/oxidation (redox) state of nicotinamide adenine dinucleotide (NAD/NADH). In addition, the prevailing hormonal milieu, elevated production of O₂ free radicals, electrolyte imbalances across cell membranes, and disturbances in pH all help “fine-tune” the metabolic pathways that match the rate of ATP synthesis with ATP demand (Fig. 1). In response to these perturbations, the postexercise period is characterized by a “general homeostatic recovery phase” that includes the resynthesis of fuel stores, free radical quenching, repair of free radical-mediated damage, and the restoration of intracellular electrolyte concentrations and pH (60). This phase is facilitated through the activation of multiple stress-activated protein kinases and upregulation of gene expression (50, 60) and may ultimately be the stimulus for the chronic intracellular adaptations that occur over months and years of repeated contractile activity (97). This premise is attractive and currently forms the rationale on which the majority of exercise/nutrient studies are based (i.e., acute interventions coupled with serial measurements of muscle-signaling proteins and substrate fluxes during several hours of postexercise recovery). This review provides a contemporary perspective of our understanding of the molecular and cellular events that occur in skeletal muscle in response to endurance and resistance exercise undertaken after acute and chronic manipulations of substrate availability. Emphasis is on the results of human studies and how nutrient provision (or lack thereof) interacts with specific contractile stimuli to modulate many of the acute responses to exercise, thereby potentially promoting or inhibiting subsequent training adaptation.

### Carbohydrate Availability and the Training Response/Adaptation

Skeletal muscle glycogen concentration exerts a regulatory effect on many cellular processes. For example, contraction-induced as well as insulin-stimulated glucose transport and glucose transporter 4 (GLUT4) translocation are inhibited by high muscle glycogen levels (84). Carbohydrate availability also modulates the expression of many exercise-induced genes; the rate of translation of postexercise skeletal muscle interleukin-6 (IL-6) mRNA is reduced by glucose feeding during exercise, whereas lowering glycogen content prior to exercise amplifies the induction of IL-6 transcription and mRNA in skeletal muscle. Commencing endurance exercise with low muscle glycogen stores also results in a greater transcriptional activation of enzymes involved in carbohydrate metabolism, including the adenosine 5’-monophosphate-activated protein kinase (AMPK), GLUT4, hexokinase, and the pyruvate dehydrogenase (PDH) complex, compared with when glycogen

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**Systemic factors (substrate availability, hormones, blood flow)**

- **Glucose**
- **Insulin**
- **Lipids**
- **FAT/CD36**
- **PPAR**
- **ROS**
- **ERK/INK**
- **Temperature**

**Local release of cytokines and growth factors**

- **Hypoxia**
- **Amino acids**
- **Adiponectin**
- **mTOR**
- **SIRT1**
- **PGC-1**
- **MAPK**
- **AMPK**
- **MAPK**
- **PPAR**
- **coactivator-1**
- **PPAR, peroxisome proliferator-activated receptor.**

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content is normal (for review, see Ref. 46). Because the time course of transcriptional activation for many exercise-induced genes occurs during the first few hours of recovery (60), returning to basal values within 24 h (101), such events may be linked by common signaling and/or regulatory mechanisms, such as the restoration of muscle energy stores, predominantly glycogen (84).

Against this “molecular background,” Hansen et al. (42) proposed that training commenced with low muscle glycogen availability would promote adaptation to a greater extent than when the same sessions were commenced with normal glycogen concentration. To test this hypothesis, these workers used a 10-wk training block and a study design in which the right and left legs of the same (untrained) subject performed the same amount of total work during the intervention period with different preexercise muscle glycogen content. Resting muscle glycogen content, the maximal activity of citrate synthase, and exercise time to exhaustion were all enhanced to a greater extent in the leg that commenced training sessions with low compared with normal muscle glycogen content. The term “train-low, compete-high” was promulgated to describe this novel training approach (42). Subsequently, we investigated the train-low paradigm during a 3-wk intervention in well-trained individuals (104). We reasoned that athletes would have maximized their training adaptation and that further gains would be negligible, irrespective of whether they trained with low or normal levels of muscle glycogen. Training frequency and intensity was manipulated so that intense interval training sessions were commenced at a time when glycogen stores were lowered by ∼50% through prior exercise or were replete (104). Maximal self-selected power output was lower when athletes commenced intervals with low compared with normal glycogen availability. Yet despite a lower training impulse resting muscle glycogen concentration, the maximal activities of citrate synthase and β-hydroxyacyl-CoA-dehydrogenase and the total protein content of cytochrome c oxidase subunit IV were higher (compared to pretraining values) only in subjects who commenced interval training with low muscle glycogen content. Hulston et al. (53) also reported that self-selected power output was compromised when trained cyclists started high-intensity interval training sessions with low glycogen stores and that tracer-derived measures of fat oxidation during submaximal cycling were increased after train-low. Specifically, muscle-derived triacylglycerol oxidation increased after training with low glycogen availability (53).

In addition to altering endogenous carbohydrate stores, other exercise/diet protocols have been utilized to manipulate exogenous glucose availability, including training after an overnight fast, prolonged training with or without an overnight fast, and withholding carbohydrate intake during the session and/or withholding carbohydrate during the first hours of recovery (reviewed in Ref. 46). In contrast to the robust effects of commencing endurance training sessions with low muscle glycogen stores, the results of studies that have manipulated glucose availability before, during, and/or after endurance exercise on selected muscle adaptations are equivocal. Several investigations report that, after 6- to 10-wk intervention periods, a range of training-responsive metabolic markers (including succinate dehydrogenase activity, GLUT4, and hexokinase II content) are increased by a similar extent with or without carbohydrate supplementation (1, 32). However, others have reported subtle differences in muscle adaptation after reducing carbohydrate availability during training (23, 73). To date, only one study has examined the interactive effects of endogenous muscle glycogen and exogenous glucose availability on training adaptation. Morton et al. (68) employed a variety of training protocols (twice/day, 2 days/wk, or once/day, 4 days/wk) and dietary manipulations (with or without carbohydrate support before and during exercise) in recreational subjects for 6 wk. All protocols were associated with an increase in the protein concentrations of cytochrome c oxidase IV and the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), with no differences in the magnitude of change between groups who trained with low vs. high carbohydrate availability. Only the maximal activity of succinate dehydrogenase was greater in subjects who commenced training with lowered muscle glycogen stores and who did not receive carbohydrate support during/after training.

Taken collectively, the results of these studies (1, 23, 32, 42, 53, 73, 104) demonstrate that, independent of prior training status, short-term (3–10 wk) training programs in which a portion of workouts are commenced with either low muscle glycogen and/or low exogenous glucose availability augment training adaptation (i.e., they increase the maximal activities of selected enzymes involved in carbohydrate and/or lipid metabolism and promote mitochondrial biogenesis) to a greater extent than when all workouts are undertaken with normal or elevated glycogen stores. However, carbohydrate availability is not the only variable manipulated in these investigations. The studies employed different modes of training and a range in the number of training sessions (both in total number and those undertaken under conditions of low carbohydrate availability), along with variable intervention periods. It is quite possible that some of the results are not directly attributable to differences in carbohydrate availability per se but rather to the effects of the exercise training protocol itself (i.e., differences in recovery time between workouts, training once/day vs. twice every 2nd day). Notwithstanding this possibility, there is no evidence of impaired adaptation (or a decrement to performance outcomes) after short-term training with low carbohydrate availability. Although the potential mechanisms underlying these training-nutrient interactions have not been elucidated, they may involve roles for two exercise-sensitive signaling molecules that promote many of the contractile-induced adaptations in skeletal muscle, the AMPK and the mitogen-activated protein kinase (MAPK). Recent studies (discussed below) demonstrate that the activation of these two proteins is certainly modified by nutrient availability (or lack thereof), but at present it is not clear how altering AMPK and/or p38 activity might lead to an improved training adaptation.

One molecule with a central role in monitoring cellular energy status is the AMPK. In humans, seven genes encode AMPK subunits (α1, α2, β1, β2, γ1, γ2, and γ3), giving the possibility of at least 12 α, β, γ heterotrimers. The recent discovery of glycogen-binding sites on the AMPK β-subunits (61) has led to the hypothesis that this regulatory domain may also allow AMPK to act as a sensor of endogenous glycogen stores (62). In this scenario, the glycogen-binding domains act as sensors enabling AMPK to gauge the state of cellular glycogen, increasing AMPK activity when stores are low and decreasing the signal when stores are replete or elevated.
Experimental evidence from human studies provides indirect support for this hypothesis. Wojtaszewski et al. (100) first demonstrated that lowering muscle glycogen stores in trained individuals increased α1 and α2 activity and acetyl-CoA carboxylase Ser21 phosphorylation at rest and during subsequent exercise to a greater extent than when exercise was commenced with elevated glycogen levels. We also found that the magnitude of increase in AMPK phosphorylation in well-trained individuals who performed a bout of high-intensity interval training was greater when the session was commenced with low compared with normal glycogen stores (103). The AMPK has been reported to phosphorylate and inactivate histone deacetylase 5 (HDAC5), leading to the removal of HDAC5 from the nucleus and allowing the myocyte enhancer factor 2 to bind and activate PGC-1α. PGC-1α has a cAMP response element in its promoter region, whereas cAMP response element-binding protein (CREB) has also been identified as a downstream target of AMPK. To determine whether the increase in AMPK activation after an acute bout of intense training commenced with low glycogen availability might explain our previously observed training-induced differences in muscle adaptation (104), we analyzed several downstream protein targets of AMPK with putative roles in mitochondrial biogenesis. We found no differences in the localization of HDAC5 and the phosphorylation state of CREB when subjects commenced intense exercise with either low or normal muscle glycogen content (103), suggesting that the exercise/nutrient-induced increase in AMPK phosphorylation was insufficient to specifically increase its activity toward HDAC5 and CREB or that there are other mechanisms by which chronic activation of AMPK increases mitochondrial enzyme activity.

MAPK pathways have also been implicated as possible signaling mechanisms involved in the regulation of exercise/nutrient adaptations (97). Signal transduction through one of these kinases, the p38 MAPK, is mediated by four isoforms (α, β, δ, and γ), with the existence of these isoforms giving specificity in mediating downstream biological responses induced by exercise and/or nutrient availability (97). Chan et al. (19) first showed that alterations in macronutrient intake that reduced muscle glycogen content lead to an increased phosphorylation of nuclear p38 MAPK in human skeletal muscle in response to moderate exercise. However, we recently found little change in either the phosphorylation state of this kinase or one of its downstream targets (activating transcription factor 2) after a bout of intense cycling commenced with either low or normal muscle glycogen content (103). Cochran et al. (21) investigated the effects of altering glucose availability during recovery from exercise on metabolic and signaling responses to a subsequent bout of exercise undertaken several hours later (i.e., twice/day training). Two identical sessions of high-intensity interval exercise separated by a 3-h recovery period, during which subjects consumed carbohydrate (HI-HI) or placebo (HI-LO), were performed. There was a fourfold increase in phosphorylated p38 MAPK after the first session that had returned to baseline levels prior to the second session regardless of carbohydrate availability. However, after the second session, p38 MAPK phosphorylation was ~50% higher in HI-LO vs. HI-HI. Since resting glycogen content and glycogen utilization during exercise were similar between treatments, these workers (21) concluded that “carbohydrate ingestion, and not necessarily changes in muscle glycogen content per se, alters the metabolic response to repeated sessions of high-intensity interval exercise.” Although it is tempting to presume that there is an amplified training adaptation when training sessions are commenced with low muscle stores and/or altered glucose availability before, during, and/or after endurance-based exercise, it is important to note that carbohydrate restriction has reciprocal and pronounced effects on lipid availability. The responses to altering fat availability on training adaptation are now discussed.

**Fat Availability and the Training Response/Adaptation**

The oxidation of fat makes an important contribution to energy production during exercise at low to moderate intensities, with the absolute amount of fat oxidized being largely a function of the mitochondrial volume of the contracting muscle. Although muscle and liver glycogen stores are paramount to the successful performance of intense aerobic exercise, the oxidation of fat plays an important role in “sparing” or providing an alternate substrate to carbohydrate at exercise intensities of up to ~60–65 of maximal O2 uptake (VO2max) in untrained and 75–85% of VO2max in trained individuals (89). Accordingly, consuming a high-fat diet while undertaking daily training could augment the normal training-induced increases in fat oxidation, reduce carbohydrate utilization, and delay the onset of fatigue during prolonged exercise. Helge et al. (49) were the first modern-day researchers to study the interaction of training and a high-fat diet on metabolism and training capacity. They studied 20 untrained males who ingested either a high-fat (n = 10) or high-carbohydrate (n = 10) diet while performing endurance training 3–4 times/wk for 7 wk. During the 8th wk, both groups ingested a high-carbohydrate diet. Endurance performance undertaken at the completion of the 7th wk of the training program revealed a significantly greater improvement after a carbohydrate-rich diet had been consumed during training compared with a fat-rich diet (56%). Even when the high-fat diet was replaced by a high-carbohydrate diet for the 8th wk of training, endurance performance was still significantly lower than in subjects who had trained for the complete duration on a high-carbohydrate diet. Helge et al. (49) concluded that “ingesting a fat-rich diet during an endurance training programme is detrimental to endurance performance...due to suboptimal adaptations that are not remedied by the short-term increase in carbohydrate availability.”

Given that the greatest stimulus to any exercise-induced skeletal muscle adaptation is repeated training bouts, diet alterations must be tolerable and robust enough to ultimately cause an augmented adaptation that serves some functional purpose. With these issues in mind, a more practical training diet approach was formulated in which already well-trained athletes were exposed to a short-term (4–7 days) high-fat diet (“fat adaptation”) while they maintained their normal strenuous training regimens (15–17, 88, 89). This period of fat adaptation is immediately followed by a “carbohydrate restoration” phase, during which a high-carbohydrate diet is consumed for 24–72 h. Compared with an isoenergetic carbohydrate diet, such “dietary periodization” increases rates of whole body fat oxidation and attenuates the rate of muscle glycogenolysis during submaximal exercise (15) without concomitant changes in mitochondrial content. Remarkably, these metabolic perturbations favoring the oxidation of fat persist even in the face...
of increased carbohydrate availability, namely replenished muscle and liver glycogen stores and exogenous glucose ingestion (16).

Although consumption of high-fat diets in individuals who maintain their normal training regimen elicits higher rates of fat oxidation during submaximal exercise compared with high-carbohydrate diets (45), such efforts require greater physiological and mental effort (89). Indeed, it seems unlikely that fat oxidation can sustain the demands of high-intensity training typically undertaken by competitive endurance athletes. To test this hypothesis, Stepto et al. (89) determined the effect of consuming a high-fat diet for 4 days on the training responses of competitive ultraendurance cyclists/triathletes. During the intervention period, subjects performed two high-intensity cycle interval training sessions consisting of a 20-min warm up at 65% of $\dot{V}O_{2\text{max}}$ followed by 8 × 5-min work bouts at 86% of $\dot{V}O_{2\text{max}}$ with 1-min recovery. Rates of fat oxidation during the 20-min warmup were 69 ± 25 μmol·kg$^{-1}$·min$^{-1}$, and despite an increase in both the relative and absolute power output (from 232 to 323 W), rates of fat oxidation were not significantly different (89). This indicates that after 4 days of a high-fat diet, well-trained athletes were better able to oxidise fat during high-intensity exercise to compensate for their low muscle glycogen stores. Although the subjects in that study (89) could complete a short-term diet-training regimen, it seems likely that consumption of a fat-rich diet for longer periods would limit the ability of well-trained athletes to undertake strenuous training.

What mechanisms explain how fat adaptation strategies increase fat oxidation during exercise and reduce the reliance on carbohydrate in the absence of mitochondrial volume changes in athletes who already have the capacity for high absolute rates of fat oxidation? Potential signals for upregulating the proteins that metabolize fat (while also possibly downregulating proteins that metabolize carbohydrate) are the chronic decreases in insulin concentration and reciprocal increases in plasma fatty acid (FA) levels that occur during the fat adaptation phase of the intervention. For example, FAs are ligands for the family of peroxisome proliferator-activated receptors (PPARs), transcription factors that upregulate fat-metabolizing proteins (36). To date, however, few cellular studies have been undertaken in an attempt to unravel the mechanisms underpinning the carbohydrate sparing reported after fat adaptation.

The movement of long-chain FAs into contracting skeletal muscle cells is mainly via the FA transporters fatty acid binding protein (FABPpm) (40, 70). FAs also require transport into mitochondria, where they are ultimately oxidized, a process involving the carnitine palmitoyl transferase complex (52). Five days of fat adaptation did not alter the total muscle protein abundance of carnitine palmitoyl transferase I or FABPpm but did increase total FAT/CD36 protein content in skeletal muscle (17). Carbohydrate restoration restores FAT/CD36 protein content to those levels measured prior to fat adaptation (102), suggesting that FAT/CD36 is the transport protein most sensitive to alterations in dietary fat intake. However, FA transport proteins are present in the cytoplasm and on both the sarcolemmal and mitochondrial membranes. Previous studies have been limited to measurements of changes in total muscle protein and therefore provide no information regarding the location where such perturbations occurred. Recent human experiments reveal that, although exercise training increases total muscle FABPpm and FAT/CD36 content, the changes at the sarcolemma were confined to FABPpm, with the increase in FAT/CD36 occurring on the mitochondrial membranes (90). In addition, exercise (2 h of moderate-intensity cycling) resulted in a translocation of FAT/CD36 to the muscle membrane (Bradley NS, Snoek LA, Jain SS, Heigenhauser GJF, Bonen A, and Spriet LL, unpublished observations) and the mitochondrial membranes (51). Future work is required to determine whether fat adaptation and carbohydrate restoration alters the presence of FA transporters on the muscle and mitochondrial membranes both at rest and during subsequent exercise.

Fat adaptation increases intramyocellular triglyceride (IMTG) levels (102) and also increases the activity of hormone-sensitive lipase (HSL) (88), suggesting that IMTGs contribute more fuel to exercise following fat adaptation. However, such a hypothesis has not been verified experimentally. The overall triglyceride (TG) content within muscle following a training session or prior to the next session is dependent on the balance between the rates of FA uptake, oxidation, and storage and TG hydrolysis. The esterification of TG requires acylation by acyl-CoA synthetase and the sequential addition of FAs to a glycerol backbone in a series of reactions with the activities of glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT) believed to be regulatory. On the breakdown side, adipose triacylglycerol lipase (ATGL) and HSL appear to work hierarchically to regulate complete TG hydrolysis. ATGL initiates lipolysis by removing the first FA from TG to produce diacylglycerol, which is then hydrolyzed by HSL to generate a second FA and monoglycerol (MG), which is converted to FA and glycerol by MG lipase (96). Although all the key enzymes may be upregulated after fat adaptation, the activities of DGAT and GPAT may be dominant at rest (resulting in higher IMTG levels), whereas ATGL and HSL may be more active during exercise (resulting in higher IMTG utilization), but this hypothesis has not been tested.

Fat adaptation also has profound effects on the regulation of PDH, the key enzyme regulating muscle carbohydrate oxidation. High-fat diets rapidly downregulate the active form of PDH protein at rest (74), accomplished by rapid upregulation of PDH kinase (PDK) activity, mainly through an increased content of the diet-sensitive PDK4 isozyme. Increased PDK activity moves most of the PDH to the inactive form and decreases carbohydrate oxidation. It is likely that the high-fat diet-induced reduction in circulating insulin concentration and the increased FA levels rapidly affect these changes (75). Following fat adaptation (81) and carbohydrate restoration (88), PDH activation is reduced at rest and over a range of exercise intensities. Accordingly, carbohydrate oxidation is reduced and contributes to a sparing of muscle glycogen (81, 88). Refeeding with carbohydrate following a high-fat diet quickly decreases PDK activity, but the suppression of PDH activation and impaired carbohydrate oxidation remain for several hours at rest (9). Carbohydrate restoration for 24 h partially restores rates of carbohydrate oxidation to normal, but PDH activation remains blunted during moderate and all-out exercise (88). These studies are consistent with a persistent effect of fat adaptation on muscle fuel choice, but clearly,
additional work is needed to examine the cellular regulation of these changes following this dietary protocol.

**Protein Availability and the Training Response/Adaptation**

Protein synthesis is the end point in the complex process of contraction-induced adaptation that promotes phenotype-specific characteristics in skeletal muscle. From this perspective, it is clear that both resistance exercise and the feeding of protein or amino acids (AAs) have a marked stimulatory effect on rates of muscle protein synthesis (MPS) (6, 12, 78). AA feeding is synergistically stimulatory with resistance-type exercise for MPS, and when combined the resultant increase in net protein balance [defined as MPS minus muscle protein breakdown (MPB)] is greater than either alone (7, 77). Although the time course of stimulation of MPS with feeding is rather transient, being in the range of hours at most (66), contractile activity can stimulate MPS for 48–72 h (64, 79). Although the duration for which MPS is elevated postexercise is reduced in trained individuals (92), the stimulation of MPS is still much longer than after feeding alone. Undertaking chronic resistance exercise also elevates basal rates of MPS (77, 79) and results in a latent stimulatory effect on the feeding-induced rise in MPS as long as 24 h postexercise (13). In contrast, it is not currently known whether protein supplementation has similar benefits for athletes undertaking repeated endurance training sessions. However, mitochondrial protein synthesis is stimulated following acute and chronic endurance exercise (98), and one might speculate that this capacity would be amplified with increased exogenous protein availability. Our current view of the adaptive response to exercise is that acute changes in net protein balance are relevant and meaningful since they have been shown to be, at least qualitatively, predictive of a long-term phenotypic change (93). In this model, protein is more than merely substrate; it is an input into the system that affects phenotype due to the influence it exerts over the regulation of rates of MPS.

There are three easily defined time periods for increasing nutrient (i.e., protein/AA) availability to augment the acute contraction-induced adaptive response in skeletal muscle: pre-exercise (within 1 h before the start of a bout of exercise), during the exercise bout itself, and postexercise (<3 h after the completion of the exercise bout). Tipton et al. (95) investigated whether consumption of an oral essential AA-carbohydrate supplement before a bout of resistance exercise results in a greater anabolic response than supplementation after exercise. Blood and muscle phenylalanine concentrations were increased by ~130% after drink consumption in both trials. However, delivery of AA ([AA] × blood flow) was greater with pre- vs. postexercise feeding during the exercise bout and in the 1st hour after exercise (95). These results show that the response of net muscle protein synthesis to consumption of protein immediately before resistance exercise is greater than when the solution is consumed after exercise and suggest a preexercise feeding enhancement of blood flow and subsequent amino acid delivery as being primary determining factors in protein accrretion. However, the results from that study (95) could not be reproduced subsequently by the same group (94) or others (38). Thus, preexercise protein feeding is unlikely to confer benefit for promotion of increases in MPS and long-term gains in muscle mass.

The provision of protein during exercise appears to be an effective strategy for promoting MPS since the AA substrate may allow earlier rises in the blood AA profile and “kick-start” MPS and/or trigger local or systemic signaling responses that promote increased MPS. However, during exercise, MPS is blunted (18, 33). Although the precise mechanisms underlying this phenomenon are not well understood, Rose et al. (86) have demonstrated that Ca2+-mediated signaling is involved in suppressing MPS during contraction, at least in fast-twitch muscles of rats undergoing in situ stimulation. Using the phosphorylation of eukaryotic elongation factor-2 as a proxy marker for MPS, Rose et al. (87) also showed that endurance exercise induced a rapid increase in the phosphorylation state of this protein, suggesting that the elongation phase of MPS is suppressed. However, it is unlikely that the same situation occurs in humans during resistance exercise since the continual rest-recovery cycles that are an integral part of resistance-based training bouts are likely to permit a degree of recovery in muscle energetic and/or calcium ion homeostasis not observed in animal models. In fact, when protein is consumed during resistance exercise, a rise in MPS does occur (4), indicating either that the perturbations in energy status and/or to Ca2+ signaling were not sufficient to suppress synthesis or that protein feeding overcomes any suppression of MPS. Thus, exercise has already started to activate signaling mechanisms during resistance exercise, and AA delivery to the muscle begins to stimulate a significant rise in MPS.

Postexercise nutrient provision has a significant, positive impact on MPS, being markedly greater than exercise alone (7, 77). Nutrient provision (i.e., increased AA availability) or the associated rise in insulin (8) also suppresses the resistance exercise-induced MPB that occurs in the absence of nutrition (6, 78). The resultant positive net protein balance has been postulated to be additive and result in protein accumulation and skeletal muscle hypertrophy over time. A number of training studies have utilized immediate postexercise protein provision vs. delayed nutrition or provision of energy alone (usually as carbohydrate) and have reported that early postexercise protein provision is more beneficial in promoting hypertrophy (25, 43). In summary, there is robust evidence to support the hypothesis that postexercise protein consumption promotes a pronounced rise in the rate of MPS while concomitantly suppressing rates of MPB, leading to increases in net muscle protein balance and protein accretion. Long-term studies that have manipulated the timing of protein provision report enhanced gains in the anabolic profile with early postexercise ingestion, although this is not always the case. A number of factors may explain the variable results between training studies, including the protein dose (66), the type (i.e., source) of protein (43, 91, 99), and the age and training status of the participants. Finally, several training studies have used a combination of pre- and postexercise feeding to augment gains in muscle mass (14, 26), preventing a clear conclusion regarding protein timing being made.

Recent work has provided insight into the identity of the molecular pathways involved in the nutrition- and resistance training-induced increase in MPS. A comprehensive evaluation of this topic is beyond the scope of the present article, and readers are referred to recent reviews of this area (34, 55). Many groups have identified a central role for the mammalian target of rapamycin cascade in anabolic processes following
Recent recognition that vitamins and other food chemicals interact with the signaling events that underpin training adaptation has stimulated several new areas of research. Such interest has been driven largely by the discovery of a broad range of bioactive but nonnutritive substances in plant sources, including fruits, vegetables, grains, herbs, and spices. Collectively, these compounds are known as phytochemicals, and although many are well recognized for their antioxidant properties, their bioactivity may be promoted via a multitude of mechanisms.

An acute bout of exercise results in an increased generation of reactive nitrogen and oxygen species (known collectively as RNOS) within skeletal muscle and other tissues (28). Increased levels of RNOS are associated with acute impairment of muscle force production as well as excessive inflammation and damage to the cell (56). Although increasing cellular antioxidant capacity via supplementary intake of antioxidants seems a logical approach to counteract the negative effects of RNOS on muscle force capability, studies of supplementation with antioxidant vitamins in association with acute or chronic exercise models have produced equivocal outcomes with respect to restoration of performance capacity and attenuation of muscle damage (37). The theory of “hormesis,” which states that biological systems respond to some stresses in a bell-shaped, curve-like manner, has been applied to RNOS to explain such findings (82). In this model (Fig. 2), large changes in oxidative stress impair cellular function, whereas small and confined changes confer positive benefits. For example, small redox changes play a role in signal transduction of key events underlying mitochondrial biogenesis, with RNOS recently being shown to induce PGC-1α promoter activity and mRNA expression via the activation of AMPK (54). In addition, small amounts of oxidative stress promote upregulation of the endogenous oxidative defense system, which consists of complex interactions of enzymatic and nonenzymatic antioxidants compartmentalized throughout the cytoplasm and within various structures such as the mitochondria (80).

The hormetic curve predicts several potential interactions between exercise, RNOS, and dietary compounds with antioxidant properties that warrant further investigation. For instance, since “antioxidants” are redox agents that can act as antioxidants in some circumstances and pro-oxidants in others, depending on the dosage and site of activity, as such, can antioxidant supplementation mimic the oxidative stimulus achieved by contractile stimuli? Does provision of antioxidants confer an additive stimulus when combined with exercise or suppress the signaling activities underpinning the acute re-
responses to exercise, leading to subsequent training adaptation? Adding complexity to these issues is the type and dosage of antioxidant compounds, the training status of individuals, and the other bioactive properties of these compounds. In reviewing the evidence for these interactions, it would be ideal to consider only human data; however, because of the recent nature of this work, we are forced to rely on investigations with in vitro or animal models. We note that particular caution should be taken in extrapolating these data to trained humans because of the potential for differences in the bioavailability or bioactivity of vitamins and phytochemicals.

The results of investigations that have determined the effect of antioxidant vitamin supplementation on mitochondrial biogenesis and the training response are mixed. Ristow et al. (85) studied the effects of 4 wk of aerobic training and antioxidant supplementation (1,000 mg/day vitamin C and 400 IU/day vitamin E) in groups of trained and previously untrained males. Matched groups treated with placebos exhibited an increase in oxidative stress as a result of exercise (increased thiobarbituric acid-reactive substances) as well as increased expression of PPARγ and its coactivators PGC-1α and PGC-1β. These outcomes were not observed in antioxidant-treated groups, regardless of background training status. Molecular mediators of endogenous antioxidant defense (superoxide dismutases 1 and 2; glutathione peroxidase) and an increase in insulin sensitivity were also induced by exercise but blocked by antioxidant supplementation (85).

A similar blunting of exercise benefits was noted when previously untrained men undertook 8 wk of aerobic training supplemented by a daily dose of 1 g of vitamin C; a placebo-matched group improved their $\dot{V}O_2_{\text{max}}$ by 22%, whereas the supplemented group experienced only an 11% increase (41). A parallel set of experiments detailed in the same study reported impaired aerobic capacity and endurance in exercised rats supplemented with vitamin C compared with a placebo-treated group. Nonsupplemented rats displayed an increase in mitochondrial cytochrome $c$ concentrations and the transcription factors PGC-1, nuclear respiratory factor-1, and mitochondrial transcription factor A, whereas these measurements were inhibited in animals supplemented with vitamin C. A blunting of the increase in antioxidant enzymes superoxide dismutase and glutathione peroxidase was also observed. In contrast, others have failed to find evidence of an impaired response to training while exposed to antioxidant supplements. Yfanti et al. (105) studied healthy but previously sedentary men who trained strenuously for 12 wk and were supplemented with a daily intake of 500 mg of vitamin C and 400 IU of vitamin E or a placebo treatment. Independent of treatment condition, all subjects had improved exercise capacity and increased muscle superoxide dismutase, glycogen concentration, and maximal enzyme activities of citrate synthase and β-hydroxacyl-CoA dehydrogenase (105). It is important to undertake further investigation of the effect of supplementation with antioxidant vitamins on training adaptations because of the prevalence of vitamin supplementation among athletes and the potential for negative outcomes.

The literature pertaining to the interaction of exercise and supplementation with phytochemicals is also equivocal. Many of the polyphenolic compounds exert a variety of biological activities, making it hard to determine direct cause-effect relationships. For example, quercetin is known to be an antioxidant, an anti-inflammatory and adenosine receptor antagonist (30). In vitro and rodent models show that some phytochemicals can also act as so-called “exercise mimetics,” promoting a direct enhancement of mitochondrial biogenesis. Table 1 summarizes studies involving sedentary models and supplementation with compounds such as resveratrol, quercetin, nootkatone, and epigallocatechin-3-gallate (EGCG), agents that induce metabolic responses that mimic the effects of aerobic training in the absence of an exercise stimulus through the activation of PGC-1α, sirtuin 1, and other exercise-inducible pathways (30, 58, 69). Human studies involving short-term supplementation in sedentary subjects have focused on functional outcomes such as enhancement of insulin sensitivity and exercise capacity, with mixed findings (27, 29, 39, 83). The lack of direct measurements of muscle responses in human studies makes it difficult to ascertain whether the divergent findings of the various studies reflect differences in the efficacy of supplementation compounds in different circumstances (i.e., background fitness level of subjects, type and dosing regimen of phytochemicals, etc.) or differences in the sensitivity of various research protocols to detect changes in the functional outcome variables. Whether dietary sources of phytochemicals can attain the optimal dose of any products found to be

Table 1. Examples of phytochemicals with exercise mimetic effects in sedentary models (supplementation in the absence of exercise or training stimulus)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sources</th>
<th>Evidence of Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG</td>
<td>Green tea</td>
<td>Forty-eight hours of EGCG supplementation (3 × 135 mg/day) increased $\dot{V}O_2_{\text{max}}$ in healthy subjects (83).</td>
</tr>
<tr>
<td>Nootkatone</td>
<td>Grapefruit</td>
<td>Mice fed with nootkatone showed increases in muscle AMPK activity and β-oxidation, protection against weight gain with a high-fat diet, and enhanced swimming endurance (64). Cell culture studies showed increased activity of PGC-1α with nootkatone (69).</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Apples, onions, berries</td>
<td>Seven days supplementation with quercetin (1 g/day) increased $\dot{V}O_2_{\text{max}}$ and cycling endurance in sedentary men; although no mechanisms were measured, authors proposed an increase in mitochondrial biogenesis (29). Fourteen days of supplementation (1 g/day) increased cycling performance and was associated with a trend toward increased mRNA content for SIRT1, PGC-1α, cytochrome $c$ oxidase, and citrate synthase (72). In contrast, other studies of untrained subjects have failed to find improvements in exercise capacity or metabolism with 1 g/day quercetin supplementation for 7–16 (27) or 5 days (39).</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Grapes</td>
<td>Mice fed with resveratrol were protected against weight gain with high-fat diet and showed increases in muscle mitochondrial size and density, oxidative enzymes, and endurance capacity. Effects were achieved via activation of SIRT1 and PGC-1α (58).</td>
</tr>
</tbody>
</table>

EGCG, epigallocatechin-3-gallate; $\dot{V}O_2_{\text{max}}$, maximal oxygen uptake; AMPK, AMP-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; SIRT1, sirtuin 1.
beneficial will be important to determine. In the case of substances like quercetin, this seems unlikely, since human studies have typically provided doses of ~1 g/day (see Table 1), whereas dietary intakes are typically <40 mg/day (27).

The additive effect of phytochemical intake and exercise training is also of interest, but again, the present literature is not sufficiently robust to allow firm guidelines and recommendations to be made. Among human studies of trained individuals, supplementation with quercetin (1 g/day) for 3 wk did not alter O2 consumption or patterns of substrate utilization (35), whereas EGCG intake (270 mg/day) for 6 days had little effect on cycling performance or fuel oxidation (31). Two weeks of supplementation with quercetin (1 g/day) with or without the addition of EGCG (120 mg/day), isoquercetin, and ω-3 fatty acids had no effect on muscle mRNA expression for PGC-1α, cytochrome c oxidase, and citrate synthase; there was also no effect on cycling performance during a 3-day period of intensified training, although the combined supplement appeared to achieve a reduction in the inflammatory response to the increased training stimulus (71). However, 6 wk of supplementation with quercetin (600 mg/day) added to a multicomponent antioxidant supplement enhanced cycling performance over baseline measurements and the effect of the antioxidant supplement alone (63).

Summary and Directions for Future Research

Acute alterations in substrate availability modify the immediate exercise response and, when repeated over days and weeks, modulate many adaptive processes in skeletal muscle that ultimately underpin the phenotype-specific characteristics observed in trained individuals. Investigations that have manipulated carbohydrate availability demonstrate that, independent of prior training status, short-term training programs in which some workouts are commenced with either low muscle glycogen levels and/or low exogenous carbohydrate availability augment training adaptation to the same or to a greater extent than when similar workouts are undertaken with normal glycogen stores. From a molecular/cellular perspective, unraveling the precise mechanism(s) underlying the benefit to training adaptation with reduced carbohydrate availability is difficult because carbohydrate restriction has reciprocal and marked effects on lipid availability and utilization. Indeed, future work involving alterations in carbohydrate and fat availability should determine whether such exercise-diet interventions alter the localization (i.e., sarcomemal and/or mitochondrial) and activity of putative carbohydrate and fat transporters both at rest and during exercise; this may help explain the persistent effects of low-carbohydrate and/or high-fat diets on muscle fuel choice. From a practical perspective, low-carbohydrate, high-fat diets have been associated with the development of insulin resistance and associated health risks, so studies are urgently needed to identify the minimal dietary intervention period required to “trigger” the cascade of positive adaptive responses as well as the optimal volume and/or intensity of training required to augment training adaptation.

Both resistance exercise and the feeding of protein and AAs have a marked stimulatory effect on rates of MPS. Although MPS is blunted during contractile activity, the provision of protein after a resistance-based exercise bout is an effective strategy for promoting MPS, since the AA substrate may allow earlier rises in the blood AA profile and kick-start MPS and/or trigger local or systemic signaling responses that promote increased MPS. It is clear that the increase in MPS in response to resistance exercise is critically dependent on the timing and pattern of protein intake. As such, investigations to define the time course of the signaling events and MPS responses during prolonged (12–24 h) recovery from resistance exercise with different protein feeding protocols are needed. Such evidence will provide the framework to design effective and practical population-specific interventions to increase muscle mass in athletes and prevent sarcopenia in the elderly.

At present there would appear to be merit in further investigating the effects of antioxidants and phytochemicals on mitochondrial biogenesis and adaptations to exercise. Currently, much of the evidence linking antioxidants and phytochemicals can be measured in trained humans along with the mechanism(s) underpinning such an enhancement. Clearly, many future studies are required to enhance our understanding of how nutrient availability acts at a cellular level to modulate training-induced adaptation in skeletal muscle.

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DISCLOSURES

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