Influence of Nutrition on Responses to Resistance Training

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ABSTRACT


A variety of dietary practices designed to enhance acute responses and chronic adaptations to resistance training have been examined with little consensus on the optimal nutritional approach for maximizing muscle and strength gains. From a scientific and practical perspective, the quantity, quality, and timing of nutrient ingestion around a workout are important factors to consider. Manipulation of exercise and nutritional variables can alter events that impact adaptations to training by a variety of mechanisms related to nutrient availability and uptake into tissues, hormonal secretion and interactions with receptors on target tissues, and gene transcription and translation of proteins that eventually impact protein, carbohydrate, and lipid metabolism. If the nutrition-mediated postresistance exercise change in any of these processes is of sufficient magnitude and duration, then over time an effect of muscle size, strength, and body composition is possible. To date, the majority of research has concentrated on providing carbohydrate alone or combined with protein before or after resistance exercise. Carbohydrate and protein intake significantly alters circulating metabolites and the hormonal milieu (i.e., insulin, testosterone, growth hormone, and cortisol), as well as the response of muscle protein and glycogen balance. The pathway of adaptation is proposed as a model to assist in integrating research findings from the current body of literature and future studies examining various diet and resistance exercise configurations. Key Words: WEIGHT TRAINING, HORMONES, DIET, CARBOHYDRATE, PROTEIN

Resistance training is an integral part of nearly every athletic training regimen and has become a popular recreational mode of exercise for many physically active individuals. Several experimental approaches have been used to study the effects of nutrition on acute and chronic responses to resistance exercise. Recently, there has been a greater focus on specific nutritional strategies to enhance acute responses to resistance exercise (e.g., protein balance, glycogen breakdown, and resynthesis), but little work has been done linking these acute physiological perturbations to chronic adaptations to resistance training (e.g., muscle size and strength) (65). This review will overview research that has examined interactions among diet and resistance exercise. The role of nutrient intake on postprandial and exercise-induced endocrine responses is emphasized because hormones play significant roles in regulating metabolic balance. A model, the pathway of adaptation, is proposed to assist in assimilating research findings from diverse studies and provide a framework to build upon for future studies that examine physiological responses to exercise and nutritional interventions.

From a practical standpoint, three important concepts need to be considered when examining any diet plan. First, is the quantity of nutrient intake. In other words, how much of a particular nutrient should be consumed? Second, is the quality of nutrient intake; that is, what kind of nutrients should be consumed (i.e., high vs low glycemic carbohydrates, saturated vs unsaturated fat, animal vs plant-based protein). Third, is the timing of nutrient intake. Should certain nutrients be consumed at precise times during the day? These decisions will ultimately impact the pathway of adaptation and, depending on the magnitude, have some observable or measurable effect on adaptations to training.

PATHWAY OF ADAPTATION

The pathway of adaptation represents a model describing fundamental steps involved in mediating acute responses to resistance exercise with nutrient intake and chronic adaptations to resistance training. The sequence of events starts with an acute resistance exercise stimuli and ends with resistance training outcome variables (e.g., increased muscle strength and size) (Fig. 1). The acute exercise stimuli alone (i.e., without nutrient intake) create a specific hormonal response pattern reflecting the physiological demands of the workout (36). Several factors, collectively referred to as the acute program variables (i.e., load, numbers of sets and repetitions, rest periods), impact the hormonal response to resistance exercise (36) and the specific adaptations to training (reviewed in 16). Thus, the seemingly simple decision to employ a resistance exercise stimuli is in fact a very com-
plex choice that will impact the entire chain of events in the pathway of adaptation.

Quantity, quality, and timing of dietary intake around the workout also influence nutrient and hormone availability at specific receptors on target tissues (i.e., skeletal muscle). Nutrient intake before and after resistance exercise alters the hormonal response and nutrient delivery and availability in skeletal muscle. The combination of nutrients and hormones interact to initiate cell signaling that influences enzyme activity and transcription and translation of proteins. The balance of anabolic/catabolic hormones and the availability of nutrients (i.e., glucose and amino acids) will impact the regulation of carbohydrate, protein, and lipid balance. Over time, if this acute response to resistance exercise is of sufficient magnitude and duration, then protein accretion and muscle fiber hypertrophy will occur. The greater muscle mass will in turn increase expression of strength in subsequent workouts and affect the acute resistance exercise response.

ACUTE EFFECTS OF PROTEIN AND CARBOHYDRATE INTAKE ON PROTEIN METABOLISM

Resistance exercise stimulates protein synthesis and protein breakdown, the balance of which determines the anabolic response of muscle to resistance exercise. Feeding has been shown to be a simple and effective method to alter rates of protein synthesis (55). Infusion of amino acids or exogenous administration of amino acids with or without carbohydrate stimulates protein synthesis after exercise (2,4,45,59). Compared with placebo, carbohydrate intake (1 g glucose·kg\(^{-1}\) body mass) immediately and 1 h after a bout of resistance exercise resulted in higher plasma glucose and insulin, decreased myofibrillar protein breakdown and urea nitrogen excretion, and slightly increased fractional muscle protein synthetic rate (50). These favorable effects of increased synchronization of the motor units, increased activation of the contractile apparatus, and inhibition of golgi tendon organs can contribute significantly to strength gains, especially during the early phases of a training program (16).

This review will focus on studies that have examined how diet and resistance exercise impact the pathway of adaptation. No studies have documented a complete view of the longitudinal adaptations in the pathway of adaptation but several studies have provided important information related to the interaction of diet and resistance exercise on segments of the pathway. Many of the studies reviewed have concentrated on providing protein and/or carbohydrate before or after exercise.

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carbohydrate intake on protein balance were achieved from a simple redistribution of the timing of the subject’s habitual dietary energy intake. Consumption of both protein and carbohydrate results in even greater effects on protein balance. Protein synthesis was stimulated ~400% above pre-exercise values when a protein and carbohydrate supplement (6 g essential amino acids and 35 g sucrose) was consumed 1 or 3 h after a bout of resistance exercise (45). Consumption of this same protein and carbohydrate supplement immediately before exercise resulted in increased amino acid delivery to muscle and greater net muscle protein synthesis compared with consumption of the supplement at various times after exercise (58). These effects were evident in both men and women. In summary, there appears to be an interaction between increased availability of amino acids and increased insulin after exercise and the timing of supplement ingestion (i.e., immediately before exercise) may be important to maximize the anabolic response (58). Consumption of a protein-carbohydrate supplement at times around exercise (i.e., immediately before and immediately after exercise) may provide the ideal anabolic situation for muscle growth.

The amino acid composition is an important consideration when examining the effects of protein feeding. Essential amino acids have been shown to be primary regulators of muscle protein synthesis with little contribution from nonessential amino acids (54,59,60). The branched-chain amino acids, particularly leucine, appear to be the most important stimulators of skeletal muscle protein synthesis (31). Recent work indicates that it is the extracellular levels of essential amino acids in the blood that regulate muscle protein synthesis as opposed to intramuscular amino acids (8). Certain amino acids may also regulate protein breakdown (30); however, these effects appear to be less important in magnitude than those controlling protein synthesis at physiological concentrations of amino acids.

ACUTE EFFECTS OF PROTEIN AND CARBOHYDRATE INTAKE ON CARBOHYDRATE METABOLISM

Several studies have shown muscle glycogen to be depleted by ~30–40% after resistance exercise (43,49,51), especially in Type II muscle fibers (57). Specific Type II muscle fiber glycogen depletion may limit performance during high volume and/or intensity workouts or when multiple workouts are performed in a single day. Glycogen resynthesis is slow unless carbohydrate is provided after exercise. Subjects who received a carbohydrate supplement (1.5 g·kg⁻¹·body mass) immediately and 1 h after a bout of resistance exercise had significantly greater rates of glycogen resynthesis during the initial 2 h of recovery compared with placebo (water). After 6 h, muscle glycogen was restored to 91% and 75% of preexercise with carbohydrate and water trials, respectively (43). Further, the rate of glycogen resynthesis following resistance exercise was similar when carbohydrate or a mixed drink (carbohydrate, protein, and fat) was consumed (51). Thus, protein and fat do not appear to interfere with glycogen resynthesis and may help in other processes such as supplying needed amino acids for incorporation into muscle proteins or enhancing the hormonal environment. Compared with placebo (water), carbohydrate intake (1 g·kg⁻¹·body mass) before and every 10 min during resistance exercise attenuated the decrease in muscle glycogen (22). Haff et al. (23) examined the effects of carbohydrate supplementation (0.3 g·kg⁻¹·body mass) or placebo on the ability to perform multiple sets of squat exercise during a second training session of a single day. A glycogen-depleting resistance exercise session (15 sets of various squat exercises) was performed in the morning and then 4 h later subjects were required to squat to exhaustion (sets of 10 repetitions at 55% of one-repetition maximum with 3-min recovery between sets). Compared with placebo, carbohydrate supplementation significantly improved the (mean ± SEM) number of sets (18.7 ± 4.8 vs 11.3 ± 2.7), number of repetitions (199 ± 47 vs 131 ± 27) and duration (78 ± 19 vs 46 ± 9 min). In summary, carbohydrate supplementation (before, during, and after a bout of resistance exercise) can attenuate the rate of muscle glycogen depletion during exercise (22) and speed the rate of glycogen resynthesis after exercise (43,51), which may enhance performance (23). This affect on glycogen is most likely achieved by increasing glucose availability and by affecting hormones such as insulin, which may activate important steps involved in glycogen resynthesis (i.e., glycogen synthase).

CHRONIC EFFECTS OF PROTEIN AND CARBOHYDRATE INTAKE ON ADAPTATIONS TO TRAINING

The finding that protein and carbohydrate intake can alter the acute phase response to resistance exercise in favor of anabolism is intriguing; however, a more pertinent question is whether these repeated metabolic alterations are of sufficient magnitude to alter long term adaptations to resistance training. Although a strong theoretical basis exists for expecting a beneficial effect of protein and carbohydrate supplementation during resistance training, no studies have systematically addressed the timing of supplementation and linked acute physiological responses to chronic adaptations in the same study.

Burke et al. (9) recently reported that men supplemented with whey protein (1.2 g·kg⁻¹·body mass) had greater increases in lean body mass compared with a placebo group after 6 wk of resistance training. Total protein was nearly two-fold higher in the whey protein vs the placebo group (2.1 vs 1.2 g·kg⁻¹·body mass, respectively), indicating that additional protein promotes anabolism during resistance training. Amino acid supplementation also favorably affects muscle performance. Our laboratory recently examined the effect of an amino acid supplement (rich in branched-chain amino acids) on muscular strength during short-term resistance training overreaching (each muscle group trained 5 d-wk⁻¹) (46). Resistance-trained men were randomly assigned to supplement with 0.4 × g·kg⁻¹·d⁻¹ of amino acids...
or placebo. As expected, maximal squat and bench press strength significantly decreased in the placebo group after 1 wk (−5.2 and −3.4%, respectively), whereas performance with amino acid supplementation was statistically unchanged (−0.4 and −1.2%, respectively, P < 0.05), indicating that provision of additional essential amino acids can prevent the acute stress response associated with short-term resistance training overreaching.

Supplying additional protein or amino acids may augment adaptations to training but precise timing of protein intake may enhance the response further. A recent study in elderly men investigated the effect of timing of protein-carbohydrate supplementation on muscle size and strength responses to 12 wk of resistance training (14). The supplement (10 g protein, 7 g carbohydrate) was consumed immediately or 2 h after each training session. The group who ingested the supplement immediately after exercise had significantly greater increases in (mean ± SEM) lean body mass (1.8 ± 0.7% vs −1.5 ± 0.7%), muscle fiber area (22 ± 6% vs −5 ± 6%), and quadriceps femoris area (7 ± 1% vs no change). These data indicate that altering the timing of calories, without altering the amount consumed, can impact chronic adaptation to training. Specifically, early intake of protein and carbohydrate after a workout is more effective at increasing skeletal muscle hypertrophy and lean body mass than a supplement consumed later. These findings are in conflict with a study that showed no differences in acute measures of protein balance when protein was ingested 1 or 3 h after exercise in healthy young subjects (45). This apparent discrepancy related to timing of protein ingestion highlights the importance of linking acute studies that measure protein kinetics to long-term training studies that assess outcome measures related to muscle size.

**NUTRITION AND HORMONAL RESPONSES**

Hormones regulate nearly every physiological process in the body. Metabolically, hormones regulate the synthesis and breakdown of proteins, carbohydrates, and lipids. Thus, hormones play an integral role in regulating genes, coordinating fuel selection and partitioning nutrients, which over time impacts lean and adipose tissue. The impact of hormones in mediating the effects of nutrition on acute and chronic responses to resistance exercise is poorly understood and requires further investigation at the cellular level where the signal at the receptor and gene level initiates the cascade of events leading to protein accretion. A first point of understanding is how nutritional intake affects postprandial and particularly resistance exercise-induced hormones such as insulin, growth hormone (GH), insulin-like growth factor-I (IGF-I), testosterone, and cortisol because these hormones have major regulatory roles in protein, carbohydrate, and lipid metabolism.

**Insulin.** In terms of carbohydrate and protein metabolism, insulin promotes glucose uptake, glycogen formation, and protein synthesis in the presence of sufficient amino acids. Carbohydrate ingestion results in elevated insulin levels that depend to some extent on the glycemic effect of the food. Certain amino acids can increase insulin and thus there has been some interest in combining protein with carbohydrate to maximize insulin secretion in the hopes of enhancing postexercise glycogen resynthesis (63,64,68) and protein anabolism (45,58). Although the effects of carbohydrate combined with protein on glycogen resynthesis after resistance exercise is unknown, there does appear to be a beneficial effect of ingesting some protein and/or amino acids in combination with carbohydrate on glycogen resynthesis after submaximal cycling exercise compared with the same amount of carbohydrate only (63,68). This effect is likely due to greater insulin secretion after combined carbohydrate and protein intake although it is pointed out that when carbohydrate intake is very high (1.2 g·kg⁻¹·h⁻¹), additional protein does not further enhance the rate of glycogen resynthesis (28). Enhanced insulin levels resulting from carbohydrate combined with protein could be expected to have a favorable effect on net protein balance because insulin is generally accepted as a stimulator of protein synthesis only when adequate amino acids are available (32).

**Growth hormone (GH).** GH increases muscle and skeletal growth and metabolically increases protein synthesis, lipolysis, and glucose conservation. Studies have shown that carbohydrate, protein, and fat intake each have independent effects on regulation of GH secretion. Glucose or carbohydrate-rich meals that increase blood glucose generally decrease GH levels that may be followed by a rebound hypoglycemia-induced rise in GH (19,24,42,62). Although infusion and oral ingestion of large doses of certain amino acids (e.g., arginine, lysine, and ornithine) can increase GH levels, the effect is quite variable and reduced by physical training and high-protein diets (11). Increased circulating fatty acids inhibit GH secretion (6,15). Our laboratory reported that a protein and carbohydrate supplement consumed before and immediately after exercise exercise enhanced the acute GH response from 0 to 30 min postexercise compared with a noncaloric placebo despite similar glucose levels between trials (37). Compared with placebo, a protein and carbohydrate supplement consumed immediately and 120 min after resistance exercise increased GH during late recovery when glucose levels were lower (10). In contrast, a recent study reported no significant effect of protein and carbohydrate intake immediately after resistance exercise on GH responses (67).

**Insulin-like growth factor-I (IGF-I).** IGF-I is an anabolic hormone that stimulates growth in almost all tissues and is responsible for many of the anabolic effects of GH. IGF-I is primarily produced in the liver under regulation by GH but is also found in other tissues including skeletal muscle. Single nutrient and mixed meals do not appear to affect total IGF-I levels (3,18,19,55,62), but feeding does impact IGF binding protein I (IGFBP-I), which is one of six high-affinity IGFBP that circulate bound to the vast majority of IGF-I. IGFBP-I shows the most rapid, dynamic regulation in plasma after meals and
may contribute to glucose regulation by countering the insulin-like, hypoglycemic effects of free IGF-I (40). After glucose or mixed meals, IGFBP-I levels gradually decrease followed by an increase 3–4 h later (3,18,19). Although there is a significant amount of evidence supporting the role of the GH-IGF-I axis in glucose metabolism (25), there has been debate whether the IGF-I system is important in glucose regulation in response to metabolic stressors (e.g., feeding and/or exercise). This is because free IGF-I levels are generally not affected during the early postprandial period when IGFBP-I, glucose, and insulin are elevated, but free IGF-I levels are reduced during the late postprandial period and during the night when glucose and insulin are normalized (18,19). Thus, the majority of evidence indicates that the IGF-I system does not participate in meal-related glucose regulation but does contribute to glucose disposal during the fasted state. Because IGF-I is produced in many tissues including skeletal muscle, it is possible that the IGF-I system is contributing to meal-related glucose regulation or the anabolic effect of feeding through an autocrine/paracrine fashion. However, this hypothesis is unlikely based on findings from a recent study showing that feeding does not affect postprandial skeletal muscle IGF-I mRNA expression in humans (55).

The interactions of feeding and resistance exercise on the IGF-I system are unclear, but there has been work done with endurance exercise. Similar to the situation after feeding, IGFBP-I may have an important role in glucose regulation during and after exercise (35). Compared with placebo, exercise-induced IGFBP-I levels were reduced in response to feeding of carbohydrate (26). In this study, IGFBP-I and glucose levels were correlated in the control condition but not during the carbohydrate feeding trial, suggesting that other factors other than glucose and insulin regulate IGFBP-1 responses to prolonged exercise (26). Provision of a nutritionally complete meal immediately after exercise did not alter the exercise-induced increase in hepatic IGFBP-I mRNA expression or circulating IGFBP-I levels despite higher glucose and insulin levels (1). Again, this is consistent with the hypothesis that exercise-induced increases in IGFBP-I levels are not directly regulated by glucose levels and do not assist in preventing IGF-I induced hypoglycemia by binding free IGF-I in plasma. Findings from a recent study showed high correlations between liver glycogen and IGFBP-1 responses to exercise, suggesting that the magnitude of liver glycogen depletion may mediate IGFBP-1 responses to exercise (38). Although speculative, it is possible that IGFBP-1 responses to exercise are modulating anabolic (growth), as opposed to the metabolic (glucose-lowering), effects of IGF-I.

**Testosterone.** Testosterone has potent anabolic effects on muscle tissue. Total and free testosterone were not affected after a low-fat meal (1% fat, 26% protein, 73% carbohydrate) but were significantly reduced by ~30% after an isocaloric high-fat meal (57% fat, 9% protein, 34% carbohydrate) in healthy men (41). The decrease was not related to changes in other steroids (i.e., estrone, estradiol, dihydrotestosterone, lutenizing hormone, percent free testosterone, or sex hormone binding globulin binding capacity). Our laboratory has also observed a significant reduction in total and free testosterone (−23%) after a fat-rich meal in healthy men (66). Another study compared testosterone responses with meals with different sources of protein (soy vs meat), amounts of fat (lean vs fatty meat), and sources of fat (animal vs vegetable) (21). The decrease in testosterone was greater after a low-fat meal consisting of lean meat (−22%) compared to tofu (−15%), a meal consisting of lean meat (−22%) compared with lean meat cooked with animal fat meal (−9%), and a meal consisting of lean meat cooked with vegetable fat (−17%) compared with vegetable oil (−9%). Total testosterone and estimated free testosterone were significantly decreased during a 2-h oral glucose tolerance test (24). Collectively, these studies indicate that feeding decreases circulating testosterone and the composition of meals, particularly the amount and type of fat, influence postprandial testosterone levels. Insulin and testosterone tend to exhibit an inverse relation in the above studies; however, findings from studies using the euglycemic hyperinsulinemic clamp procedure have shown that acute elevations in insulin do not affect total or free testosterone levels in healthy normal-weight men (13,44) or women (12).

Similar to feeding studies, pre- and postexercise meals decrease postresistance exercise testosterone responses. Our laboratory showed that a protein and carbohydrate beverage consumed 2 h before and immediately after resistance exercise resulted in increased testosterone immediately after exercise followed by a sharp decrease to values that were significantly below baseline (37). This same response was reproduced on three consecutive days, emphasizing the consistency and reproducibility of the response (37). Chandler et al. (10) showed that the reduction in testosterone after resistance exercise is evident when supplements containing either protein alone, carbohydrate alone, or a combination of protein and carbohydrate are consumed immediately after resistance exercise. Bloomer et al. (7) extended these findings by showing that postresistance exercise testosterone responses were reduced after consumption of a mixed meal, an isocaloric beverage of similar nutrient content, and an isocaloric carbohydrate beverage. One study did not show a difference in free testosterone responses to resistance exercise between carbohydrate and a water placebo consumed during exercise (56). Collectively, these studies indicate that pre- and postresistance exercise meals lead to a prolonged decrease in testosterone levels during the postresistance exercise period compared with fasting. This could be due in part to a decrease in the synthesis/secretion of testosterone and/or an increase in metabolic clearance. The decrease in postexercise testosterone is not associated with a decrease in LH, arguing against a decrease in the rate of testosterone secretion (10); however, this does not rule out a decrease in the...
testicular responsiveness to LH. Because postexercise meals increase muscle-specific protein synthesis during recovery (45,58,59), the lower testosterone levels could be due in part to increased uptake in active skeletal muscle. In support of this hypothesis, recent work in our laboratory has shown that the meal-induced decrease in testosterone after resistance exercise corresponds with an increase in skeletal muscle androgen receptor content measured 60 min after exercise.

**Cortisol.** Cortisol is an adrenal steroid hormone that is regulated by pituitary adrenocorticotropic (ACTH), which in turn is under the influence of hypothalamic corticotropin-releasing hormone (CRH). This hypothalamic-pituitary-adrenal (HPA) axis is sensitive to a variety of different stressors including feeding. Metabolically, cortisol increases hepatic lipolysis and proteolysis to fuel hepatic gluconeogenesis, which among many functions protects blood glucose and glycogen levels. Postprandial cortisol measurements must be interpreted in the context of the normal diurnal variation of the hormone. For example, a morning glucose tolerance test results in a decrease in postprandial cortisol for several hours (47,48). However, postprandial cortisol values are higher compared with the normal diurnal drop in the morning hours (48). In general, feeding has been shown to increase cortisol and the response is particularly evident at the noon meal (17,33), which may serve to synchronize the diurnal rhythm in HPA axis activity (39).

Although few studies have addressed the influence of meal composition on the HPA axis, it appears that protein has the greatest stimulatory effect on cortisol (20,27,53). Other studies have shown that meals containing 20–40% protein enhance postprandial cortisol levels compared with meals with high carbohydrate or fat content and that protein-free glucose or fat-rich meals do not stimulate cortisol release (27,53). Only a few studies have examined the effects of feeding on postresistance exercise cortisol responses. These studies indicate that intake of carbohydrate or carbohydrate combined with protein before and after resistance exercise does not alter the cortisol response compared with placebo (7,34,37,67). In contrast, one study showed that carbohydrate intake during an acute bout of resistance exercise significantly blunted the cortisol response (56). This study further showed that the reduction in postresistance exercise cortisol was significantly related to increases in muscle fiber hypertrophy. This study is unique because it is one of few that have linked acute changes in the pathway of adaptation (i.e., decrease in postexercise cortisol) to chronic adaptations to training (i.e., increased muscle size).

It is clear that feeding and meal composition significantly alter postprandial and postresistance exercise hormonal responses. Yet the acute and chronic effects of these hormonal changes on muscle remain largely unknown. Given the important regulatory functions of hormones on nearly all muscle processes, the endocrine system is likely a critical component in the pathway of adaptation that links diet to acute and chronic responses to resistance exercise.

**SUMMARY**

Diet can favorably influence key steps in the pathway of adaptation, which can optimize adaptations to resistance training. A primary mechanism via which diet can exert these effects is by providing key energy substrates (e.g., glucose, amino acids, lipid substrates, etc.) at precise times and by altering the hormonal environment to favor anabolism. Resistance exercise substantially elevates protein turnover and ingestion of essential amino acids before and after exercise stimulates transport of amino acids into skeletal muscle and protein synthesis. Although there have been some enlightening studies on the role of insulin in mediating responses to diet and exercise interventions, the influence of other hormones (e.g., GH, IGF-I, testosterone, cortisol) that have potent effects on carbohydrate, protein, and lipid balance remain unclear. Future studies should consider how the entire hormonal milieu responds to different diet and exercise configurations with an emphasis on target tissue effects. Because most studies are focused on a single outcome (e.g., protein balance, glycogen resynthesis, body composition, etc.), one challenge in the future will be to integrate the findings from these varying perspectives into a unified model. The pathway of adaptation presented in this article is one example of such a model. Another challenge in the future will be to match the varying nutritional demands associated with periodized resistance training programs with periodized diet strategies. Ultimately, “timing or cycling” of diet on an hourly, daily, weekly, or monthly basis to match the unique demands of each workout or phase of training may enhance adaptations to training.

**REFERENCES**


