

## **Protein Intake**

Protein intake that exceeds the recommended daily allowance is widely accepted for both endurance and power athletes. However, considering the variety of proteins that are available much less is known concerning the benefits of consuming one protein versus another. The purpose of this paper is to identify and analyze key factors in order to make responsible recommendations to both the general and athletic populations. Evaluation of a protein is fundamental in determining its appropriateness in the human diet. Proteins that are of inferior content and digestibility are important to recognize and restrict or limit in the diet. Similarly, such knowledge will provide an ability to identify proteins that provide the greatest benefit and should be consumed. The various techniques utilized to rate protein will be discussed. Traditionally, sources of dietary protein are seen as either being of animal or vegetable origin. Animal sources provide a complete source of protein (i.e. containing all essential amino acids), whereas vegetable sources generally lack one or more of the essential amino acids. Animal sources of dietary protein, despite providing a complete protein and numerous vitamins and minerals, have some health professionals concerned about the amount of saturated fat common in these foods compared to vegetable sources. The advent of processing techniques has shifted some of this attention and ignited the sports supplement marketplace with derivative products such as whey, casein and soy. Individually, these products vary in quality and applicability to certain populations. The benefits that these particular proteins possess are discussed. In addition, the impact that elevated protein consumption has on health and safety issues (i.e. bone health, renal function) are also reviewed.

### **INTRODUCTION**

The protein requirements for athletic populations have been the subject of much scientific debate. Only recently has the notion that both strength/power and endurance athletes require a greater protein consumption than the general population become generally accepted. In addition, high protein diets have also become quite popular in the general population as part of many weight reduction programs. Despite the prevalence of high protein diets in athletic and sedentary populations, information available concerning the type of protein (e.g. animal or vegetable) to consume is limited. The purpose of this paper is to examine and analyze key factors responsible for making appropriate choices on the type of protein to consume in both athletic and general populations.

#### **Role of Protein**

Proteins are nitrogen-containing substances that are formed by amino acids. They serve as the major structural component of muscle and other tissues in the body. In addition, they are used to produce hormones, enzymes and hemoglobin. Proteins can also be used as energy; however, they are not the primary choice as an energy source. For proteins to be used by the body they need to be metabolized into their simplest form, amino acids. There have been 20 amino acids identified that are needed for human growth and metabolism. Twelve of these amino acids (eleven in children) are termed nonessential, meaning that they can be synthesized by our body and do not need to be consumed in the

diet. The remaining amino acids cannot be synthesized in the body and are described as essential meaning that they need to be consumed in our diets. The absence of any of these amino acids will compromise the ability of tissue to grow, be repaired or be maintained.

### **Protein and Athletic Performance**

The primary role of dietary proteins is for use in the various anabolic processes of the body. As a result, many athletes and coaches are under the belief that high intensity training creates a greater protein requirement. This stems from the notion that if more protein or amino acids were available to the exercising muscle it would enhance protein synthesis. Research has tended to support this hypothesis. Within four weeks of protein supplementation (3.3 versus 1.3 g·kg<sup>-1</sup>·day<sup>-1</sup>) in subjects' resistance training, significantly greater gains were seen in protein synthesis and body mass in the group of subjects with the greater protein intake (Fern et al., 1991). Similarly, Lemon et al. (1992) also reported a greater protein synthesis in novice resistance trained individuals with protein intakes of 2.62 versus 0.99 g·kg<sup>-1</sup>·day<sup>-1</sup>. In studies examining strength-trained individuals, higher protein intakes have generally been shown to have a positive effect on muscle protein synthesis and size gains (Lemon, 1995; Walberg et al., 1988). Tarnopolsky and colleagues (1992) have shown that for strength trained individuals to maintain a positive nitrogen balance they need to consume a protein intake equivalent to 1.8 g·kg<sup>-1</sup>·day<sup>-1</sup>. This is consistent with other studies showing that protein intakes between 1.4 - 2.4 g·kg<sup>-1</sup>·day<sup>-1</sup> will maintain a positive nitrogen balance in resistance trained athletes (Lemon, 1995). As a result, recommendations for strength/power athletes' protein intake are generally suggested to be between 1.4 - 1.8 g·kg<sup>-1</sup>·day<sup>-1</sup>.

Similarly, to prevent significant losses in lean tissue endurance athletes also appear to require a greater protein consumption (Lemon, 1995). Although the goal for endurance athletes is not necessarily to maximize muscle size and strength, loss of lean tissue can have a significant detrimental effect on endurance performance. Therefore, these athletes need to maintain muscle mass to ensure adequate performance. Several studies have determined that protein intake for endurance athletes should be between 1.2 - 1.4 g·kg<sup>-1</sup>·day<sup>-1</sup> to ensure a positive nitrogen balance (Freidman and Lemon, 1989; Lemon, 1995; Meredith et al., 1989; Tarnopolsky et al., 1988). Evidence is clear that athletes do benefit from increased protein intake. The focus then becomes on what type of protein to take.

### **Protein Assessment**

The composition of various proteins may be so unique that their influence on physiological function in the human body could be quite different. The quality of a protein is vital when considering the nutritional benefits that it can provide. Determining the quality of a protein is determined by assessing its essential amino acid composition, digestibility and bioavailability of amino acids (FAO/WHO, 1990). There are several measurement scales and techniques that are used to evaluate the quality of protein.

### **Protein Rating Scales**

Numerous methods exist to determine protein quality. These methods have been identified as protein efficiency ratio, biological value, net protein utilization, and protein digestibility corrected amino acid score.

### **Protein Efficiency Ratio**

The protein efficiency ratio (PER) determines the effectiveness of a protein through the measurement of animal growth. This technique requires feeding rats a test protein and then measuring the weight gain in grams per gram of protein consumed. The computed value is then compared to a standard value of 2.7, which is the standard value of casein protein. Any value that exceeds 2.7 is considered to be an excellent protein source. However, this calculation provides a measure of growth in rats and does not provide a strong correlation to the growth needs of humans.

### **Biological Value**

Biological value measures protein quality by calculating the nitrogen used for tissue formation divided by the nitrogen absorbed from food. This product is multiplied by 100 and expressed as a percentage of nitrogen utilized. The biological value provides a measurement of how efficient the body utilizes protein consumed in the diet. A food with a high value correlates to a high supply of the essential amino acids. Animal sources typically possess a higher biological value than vegetable sources due to the vegetable source's lack of one or more of the essential amino acids. There are, however, some inherent problems with this rating system. The biological value does not take into consideration several key factors that influence the digestion of protein and interaction with other foods before absorption. The biological value also measures a protein's maximal potential quality and not its estimate at requirement levels.

### **Net Protein Utilization**

Net protein utilization is similar to the biological value except that it involves a direct measure of retention of absorbed nitrogen. Net protein utilization and biological value both measure the same parameter of nitrogen retention, however, the difference lies in that the biological value is calculated from nitrogen absorbed whereas net protein utilization is from nitrogen ingested.

### **Protein Digestibility Corrected Amino Acid Score**

In 1989, the Food & Agriculture Organization and World Health Organization (FAO/WHO) in a joint position stand stated that protein quality could be determined by expressing the content of the first limiting essential amino acid of the test protein as a percentage of the content of the same amino acid content in a reference pattern of essential amino acids (FAO/WHO, 1990). The reference values used were based upon the essential amino acids requirements of preschool-age children. The recommendation of the joint FAO/WHO statement was to take this reference value and correct it for true fecal digestibility of the test protein. The value obtained was referred to as the protein digestibility corrected amino acid score (PDCAAS). This method has been adopted as the preferred method for measurement of the protein value in human nutrition (Schaafsma, 2000). Table 1 provides a measure of the quantity of various proteins using these protein rating scales.

Although the PDCAAS is currently the most accepted and widely used method, limitations still exist relating to overestimation in the elderly (likely related to references

values based on young individuals), influence of ileal digestibility, and antinutritional factors (Sarwar, 1997).

Amino acids that move past the terminal ileum may be an important route for bacterial consumption of amino acids, and any amino acids that reach the colon would not likely be utilized for protein synthesis, even though they do not appear in the feces (Schaafsma, 2000). Thus, to get truly valid measure of fecal digestibility the location at which protein synthesis is determined is important in making a more accurate determination. Thus, ileal digestibility would provide a more accurate measure of digestibility. PDCAAS, however, does not factor ileal digestibility into its equation. This is considered to be one of the shortcomings of the PDCAAS (Schaafsma 2000).

Antinutritional factors such as trypsin inhibitors, lectins, and tannins present in certain protein sources such as soybean meal, peas and fava beans have been reported to increase losses of endogenous proteins at the terminal ileum (Salgado et al., 2002). These antinutritional factors may cause reduced protein hydrolysis and amino acid absorption. This may also be more effected by age, as the ability of the gut to adapt to dietary nutritional insults may be reduced as part of the aging process (Sarwar, 1997).

### **Protein Sources**

Protein is available in a variety of dietary sources. These include foods of animal and plant origins as well as the highly marketed sport supplement industry. In the following section proteins from both vegetable and animal sources, including whey, casein, and soy will be explored. Determining the effectiveness of a protein is accomplished by determining its quality and digestibility. Quality refers to the availability of amino acids that it supplies, and digestibility considers how the protein is best utilized. Typically, all dietary animal protein sources are considered to be complete proteins. That is, a protein that contains all of the essential amino acids. Proteins from vegetable sources are incomplete in that they are generally lacking one or two essential amino acids. Thus, someone who desires to get their protein from vegetable sources (i.e. vegetarian) will need to consume a variety of vegetables, fruits, grains, and legumes to ensure consumption of all essential amino acids. As such, individuals are able to achieve necessary protein requirements without consuming beef, poultry, or dairy. Protein digestibility ratings usually involve measuring how the body can efficiently utilize dietary sources of protein. Typically, vegetable protein sources do not score as high in ratings of biological value, net protein utilization, PDCAAS, and protein efficiency ratio as animal proteins.

### **Animal Protein**

Proteins from animal sources (i.e. eggs, milk, meat, fish and poultry) provide the highest quality rating of food sources. This is primarily due to the 'completeness' of proteins from these sources. Although protein from these sources are also associated with high intakes of saturated fats and cholesterol, there have been a number of studies that have demonstrated positive benefits of animal proteins in various population groups (Campbell et al., 1999; Godfrey et al., 1996; Pannemans et al., 1998).

Protein from animal sources during late pregnancy is believed to have an important role in infants born with normal body weights. Godfrey et al. (1996) examined the nutrition behavior of more than 500 pregnant women to determine the effect of nutritional intake on placental and fetal growth. They reported that a low intake of protein from dairy and meat sources during late pregnancy was associated with low birth weights.

In addition to the benefits from total protein consumption, elderly subjects have also benefited from consuming animal sources of protein. Diets consisting of meat resulted in greater gains in lean body mass compared to subjects on a lactoovovegetarian diet (Campbell et al., 1999). High animal protein diets have also been shown to cause a significantly greater net protein synthesis than a high vegetable protein diet (Pannemans et al., 1998). This was suggested to be a function of reduced protein breakdown occurring during the high animal protein diet.

There have been a number of health concerns raised concerning the risks associated with protein emanating primarily from animal sources. Primarily, these health risks have focused on cardiovascular disease (due to the high saturated fat and cholesterol consumption), bone health (from bone resorption due to sulfur-containing amino acids associated with animal protein) and other physiological system disease that will be addressed in the section on high protein diets.

## **Whey**

Whey is a general term that typically denotes the translucent liquid part of milk that remains following the process (coagulation and curd removal) of cheese manufacturing. From this liquid, whey proteins are separated and purified using various techniques yielding different concentrations of whey proteins. Whey is one of the two major protein groups of bovine milk, accounting for 20% of the milk while casein accounts for the remainder. All of the constituents of whey protein provide high levels of the essential and branched chain amino acids. The bioactivities of these proteins possess many beneficial properties as well. Additionally, whey is also rich in vitamins and minerals. Whey protein is most recognized for its applicability in sports nutrition. Additionally, whey products are also evident in baked goods, salad dressings, emulsifiers, infant formulas, and medical nutritional formulas.

## **Varieties of Whey Protein**

There are three main forms of whey protein that result from various processing techniques used to separate whey protein. They are whey powder, whey concentrate, and whey isolate. Table 2 provides the composition of Whey Proteins.

## **Whey Protein Powder**

Whey protein powder has many applications throughout the food industry. As an additive it is seen in food products for beef, dairy, bakery, confectionery, and snack products. Whey powder itself has several different varieties including sweet whey, acid whey (seen in salad dressings), demineralized (seen primarily as a food additive including infant formulas), and reduced forms. The demineralized and reduced forms are used in products other than sports supplements.

### **Whey Protein Concentrate**

The processing of whey concentrate removes the water, lactose, ash, and some minerals. In addition, compared to whey isolates whey concentrate typically contains more biologically active components and proteins that make them a very attractive supplement for the athlete.

### **Whey Protein Isolate (WPI)**

Isolates are the purest protein source available. Whey protein isolates contain protein concentrations of 90% or higher. During the processing of whey protein isolate there is a significant removal of fat and lactose. As a result, individuals who are lactose-intolerant can often safely take these products (Geiser, 2003). Although the concentration of protein in this form of whey protein is the highest, it often contains proteins that have become denatured due to the manufacturing process. The denaturation of proteins involves breaking down their structure and losing peptide bonds and reducing the effectiveness of the protein.

Whey is a complete protein whose biologically active components provide additional benefits to enhance human function. Whey protein contains an ample supply of the amino acid cysteine. Cysteine appears to enhance glutathione levels, which has been shown to have strong antioxidant properties that can assist the body in combating various diseases (Counous, 2000). In addition, whey protein contains a number of other proteins that positively effect immune function such as antimicrobial activity (Ha and Zemel, 2003). Whey protein also contains a high concentration of branched chain amino acids (BCAA) that are important for their role in the maintenance of tissue and prevention of catabolic actions during exercise. (MacLean et al., 1994).

### **Casein**

Casein is the major component of protein found in bovine milk accounting for nearly 70-80% of its total protein and is responsible for the white color of milk. It is the most commonly used milk protein in the industry today. Milk proteins are of significant physiological importance to the body for functions relating to the uptake of nutrients and vitamins and they are a source of biologically active peptides. Similar to whey, casein is a complete protein and also contains the minerals calcium and phosphorous. Casein has a PDCAAS rating of 1.23 (generally reported as a truncated value of 1.0) (Deutz et al. 1998).

Casein exists in milk in the form of a micelle, which is a large colloidal particle. An attractive property of the casein micelle is its ability to form a gel or clot in the stomach. The ability to form this clot makes it very efficient in nutrient supply. The clot is able to provide a sustained slow release of amino acids into the blood stream, sometimes lasting for several hours (Boirie et al. 1997). This provides better nitrogen retention and utilization by the body.

### **Bovine Colostrum**

Bovine colostrum is the "pre" milk liquid secreted by female mammals the first few days following birth. This nutrient-dense fluid is important for the newborn for its ability to provide immunities and assist in the growth of developing tissues in the initial stages of life. Evidence exists that bovine colostrum contains growth factors that stimulate cellular growth and DNA synthesis (Kishikawa et al., 1996), and as might be expected with such properties, it makes for interesting choice as a potential sports supplement.

Although bovine colostrum is not typically thought of as a food supplement, the use by strength/power athletes of this protein supplement as an ergogenic aid has become common. Oral supplementation of bovine colostrum has been demonstrated to significantly elevate insulin-like-growth factor 1 (IGF-1) (Mero et al., 1997) and enhance lean tissue accretion (Antonio et al., 2001; Brinkworth et al., 2004). However, the results on athletic performance improvement are less conclusive. Mero and colleagues (1997) reported no changes in vertical jump performance following 2-weeks of supplementation, and Brinkworth and colleagues (2004) saw no significant differences in strength following 8-weeks of training and supplementation in both trained and untrained subjects. In contrast, following 8-weeks of supplementation significant improvements in sprint performance were seen in elite hockey players (Hofman et al., 2002). Further research concerning bovine colostrum supplementation is still warranted.

### **Vegetable Protein**

Vegetable proteins, when combined to provide for all of the essential amino acids, provide an excellent source for protein considering that they will likely result in a reduction in the intake of saturated fat and cholesterol. Popular sources include legumes, nuts and soy. Aside from these products, vegetable protein can also be found in a fibrous form called textured vegetable protein (TVP). TVP is produced from soy flour in which proteins are isolated. TVP is mainly a meat alternative and functions as a meat analog in vegetarian hot dogs, hamburgers, chicken patties, etc. It is also a low-calorie and low-fat source of vegetable protein. Vegetable sources of protein also provide numerous other nutrients such as phytochemicals and fiber that are also highly regarded in the diet.

### **Soy**

Soy is the most widely used vegetable protein source. The soybean, from the legume family, was first chronicled in China in the year 2838 B.C. and was considered to be as valuable as wheat, barley, and rice as a nutritional staple. Soy's popularity spanned several other countries, but did not gain notoriety for its nutritional value in The United States until the 1920s. The American population consumes a relatively low intake of soy protein (5g-day<sup>-1</sup>) compared to Asian countries (Hasler, 2002). Although cultural differences may be partly responsible, the low protein quality rating from the PER scale may also have influenced protein consumption tendencies. However, when the more accurate PDCAAS scale is used, soy protein was reported to be equivalent to animal protein with a score of 1.0, the highest possible rating (Hasler, 2002). Soy's quality makes it a very attractive alternative for those seeking non-animal sources of protein in their diet and those who are lactose intolerant. Soy is a complete protein with a high concentration of BCAA's. There have been many reported benefits related to soy proteins relating to health and performance (including reducing plasma lipid profiles, increasing LDL-

cholesterol oxidation and reducing blood pressure), however further research still needs to be performed on these claims.

### **Soy Protein Types**

The soybean can be separated into three distinct categories; flour, concentrates, and isolates. Soy flour can be further divided into natural or full-fat (contains natural oils), defatted (oils removed), and lecithinated (lecithin added) forms (Hasler, 2002). Of the three different categories of soy protein products, soy flour is the least refined form. It is commonly found in baked goods. Another product of soy flour is called textured soy flour. This is primarily used for processing as a meat extender. See Table 3 for protein composition of soy flour, concentrates, and isolates.

Soy concentrate was developed in the late 1960s and early 1970s and is made from defatted soybeans. While retaining most of the bean's protein content, concentrates do not contain as much soluble carbohydrates as flour, making it more palatable. Soy concentrate has a high digestibility and is found in nutrition bars, cereals, and yogurts.

Isolates are the most refined soy protein product containing the greatest concentration of protein, but unlike flour and concentrates, contain no dietary fiber. Isolates originated around the 1950s in The United States. They are very digestible and easily introduced into foods such as sports drinks and health beverages as well as infant formulas.

### **Nutritional Benefits**

For centuries, soy has been part of a human diet. Epidemiologists were most likely the first to recognize soy's benefits to overall health when considering populations with a high intake of soy. These populations shared lower incidences in certain cancers, decreased cardiac conditions, and improvements in menopausal symptoms and osteoporosis in women (Hasler, 2002). Based upon a multitude of studies examining the health benefits of soy protein the American Heart Association issued a statement that recommended soy protein foods in a diet low in saturated fat and cholesterol to promote heart health (Erdman, 2000). The health benefits associated with soy protein are related to the physiologically active components that are part of soy, such as protease inhibitors, phytosterols, saponins, and isoflavones (Potter, 2000). These components have been noted to demonstrate lipid-lowering effects, increase LDL-cholesterol oxidation, and have beneficial effects on lowering blood pressure.

### **Isoflavones**

Of the many active components in soy products, isoflavones have been given considerably more attention than others. Isoflavones are thought to be beneficial for cardiovascular health, possibly by lowering LDL concentrations (Crouse et al., 1999) increasing LDL oxidation (Tikkanen et al., 1998) and improving vessel elasticity (Nestel et al., 1999). However, these studies have not met without conflicting results and further research is still warranted concerning the benefits of isoflavones.

### **Soy Benefits for Women**



An additional focus of studies investigating soy supplementation has been on women's health issues. It has been hypothesized that considering that isoflavones are considered phytoestrogens (exhibit estrogen-like effects and bind to estrogen receptors) they compete for estrogen receptor sites in breast tissue with endogenous estrogen, potentially reducing the risk for breast cancer risk (Wu et al. 1998). Still, the association between soy intake and breast cancer risk remains inconclusive. However, other studies have demonstrated positive effects of soy protein supplementation on maintaining bone mineral content (Ho et al., 2003) and reducing the severity of menopausal symptoms (Murkies et al., 1995).

### **High Protein Diets**

Increased protein intakes and supplementation have generally been focused on athletic populations. However, over the past few years high protein diets have become a method used by the general population to enhance weight reduction. The low-carbohydrate, high protein, high fat diet promoted by Atkins may be the most popular diet used today for weight loss in the United States (Johnston et al., 2004). The basis behind this diet is that protein is associated with feelings of satiety and voluntary reductions in caloric consumption (Araya et al., 2000; Eisenstein et al., 2002). A recent study has shown that the Atkins diet can produce greater weight reduction at 3 and 6 months than a low-fat, high carbohydrate diet based upon U.S. dietary guidelines (Foster et al., 2003). However, potential health concerns have arisen concerning the safety of high protein diets. In 2001, the American Heart Association published a statement on dietary protein and weight reduction and suggested that individuals following such a diet may be at potential risk for metabolic, cardiac, renal, bone and liver diseases (St. Jeor et al., 2001).

### **Protein Intake and Metabolic Disease Risk**

One of the major concerns for individuals on high protein, low carbohydrate diets is the potential for the development of metabolic ketosis. As carbohydrate stores are reduced the body relies more upon fat as its primary energy source. The greater amount of free fatty acids that are utilized by the liver for energy will result in a greater production and release of ketone bodies in the circulation. This will increase the risk for metabolic acidosis and can potentially lead to a coma and death. A recent multi-site clinical study (Foster et al., 2003) examined the effects of low-carbohydrate, high protein diets and reported significant elevation in ketone bodies during the first three months of the study. However, as the study duration continued the percentage of subjects with positive urinary ketone concentrations became reduced, and by six months urinary ketones were not present in any of the subjects.

### **Dietary Protein and Cardiovascular Disease Risk**

High protein diets have also been suggested to have negative effects on blood lipid profiles and blood pressure, causing an increase risk for cardiovascular disease. This is primarily due to the higher fat intakes associated with these diets. However, this has not been proven in any scientifically controlled studies. Hu et al., (1999) have reported an inverse relationship between dietary protein (animal and vegetable) and risk of cardiovascular disease in women, and Jenkins and colleagues (2001) reported a decrease in lipid profiles in individuals consuming a high protein diet. Furthermore, protein intake

has been shown to often have a negative relationship with blood pressure (Obarzanek et al., 1996). Thus, the concern for elevated risk for cardiovascular disease from high protein diets appears to be without merit. Likely, the reduced body weight associated with this type of diet is facilitating these changes.

In strength/power athletes who consume high protein diets, a major concern was the amount of food being consumed that was high in saturated fats. However, through better awareness and nutritional education many of these athletes are able to obtain their protein from sources that minimizes the amount of fat consumed. For instance, removing the skin from chicken breast, consuming fish and lean beef, and egg whites. In addition, many protein supplements are available that contain little to no fat. It should be acknowledged though that if elevated protein does come primarily from meats, dairy products and eggs, without regard to fat intake, there likely would be an increase in the consumption of saturated fat and cholesterol.

### **Dietary Protein and Renal Function**

The major concern associated with renal function was the role that the kidneys have in nitrogen excretion and the potential for a high protein diet to over-stress the kidneys. In healthy individuals there does not appear to be any adverse effects of a high protein diet. In a study on bodybuilders consuming a high protein (2.8 g·kg<sup>-1</sup>) diet no negative changes were seen in any kidney function tests (Poortsman and Dellalieux, 2000). However, in individuals with existing kidney disease it is recommended that they limit their protein intake to approximately half of the normal RDA level for daily protein intake (0.8 g·kg<sup>-1</sup>·day<sup>-1</sup>). Lowering protein intake is thought to reduce the progression of renal disease by decreasing hyperfiltration (Brenner et al., 1996).

### **Dietary Protein and Bone**

High protein diets are associated with an increase in calcium excretion. This is apparently due to a consumption of animal protein, which is higher in sulfur-based amino acids than vegetable proteins (Remer and Manz, 1994; Barzel and Massey, 1998). Sulfur-based amino acids are thought to be the primary cause of calciuria (calcium loss). The mechanism behind this is likely related to the increase in acid secretion due to the elevated protein consumption. If the kidneys are unable to buffer the high endogenous acid levels, other physiological systems will need to compensate, such as bone. Bone acts as a reservoir of alkali, and as a result calcium is liberated from bone to buffer high acidic levels and restore acid-base balance. The calcium released by bone is accomplished through osteoclast-mediated bone resorption (Arnett and Spowage, 1996). Bone resorption (loss or removal of bone) will cause a decline in bone mineral content and bone mass (Barzel, 1976), increasing the risk for bone fracture and osteoporosis.

The effect of the type of protein consumed on bone resorption has been examined in a number of studies. Sellmeyer and colleagues (2001) examined the effects of various animal-to- vegetable protein ratio intakes in elderly women (> 65 y). They showed that the women consuming the highest animal to vegetable protein ratio had nearly a 4-fold greater risk of hip fractures compared with women consuming a lower animal to vegetable protein ratio. Interestingly, they did not report any significant association

between the animal to vegetable protein ratio and bone mineral density. Similar results were shown by Feskanich et al (1996), but in a younger female population (age range = 35 - 59 mean 46). In contrast, other studies examining older female populations have shown that elevated animal protein will increase bone mineral density, while increases in vegetable protein will have a lowering effect on bone mineral density (Munger et al., 1999; Promislow et al., 2002). Munger and colleagues (1999) also reported a 69% lower risk of hip fracture as animal protein intake increased in a large (32,000) postmenopausal population. Other large epidemiological studies have also confirmed elevated bone density following high protein diets in both elderly men and women (Dawson-Hughes et al., 2002; Hannan et al., 2000). Hannon and colleagues (2000) demonstrated that animal protein intake in an older population, several times greater than the RDA requirement, results in a bone density accrument and significant decrease in fracture risk. Dawson-Hughes et al (2002), not only showed that animal protein will not increase urinary calcium excretion, but was also associated with higher levels of IGF-I and lower concentrations of the bone resorption marker N-telopeptide.

These conflicting results have contributed to the confusion regarding protein intake and bone. It is likely that other factors play an important role in further understanding the influence that dietary proteins have on bone loss or gain. For instance, the intake of calcium may have an essential function in maintaining bone. A higher calcium intake results in more absorbed calcium and may offset the losses induced by dietary protein and reduce the adverse effect of the endogenous acidosis on bone resorption (Dawson-Hughes, 2003). Furthermore, it is commonly assumed that animal proteins have a higher content of sulfur-containing amino acids per g of protein. However, examination of Table 4 shows that this may not entirely correct. If protein came from wheat sources it would have a mEq of 0.69 per g of protein, while protein from milk contains 0.55 mEq per g of protein. Thus, some plant proteins may have a greater potential to produce more mEq of sulfuric acid per g of protein than some animal proteins (Massey, 2003). Finally, bone resorption may be related to the presence or absence of a vitamin D receptor allele. In subjects that had this specific allele a significant elevation in bone resorption markers were present in the urine following 4-weeks of protein supplementation, while in subjects without this specific allele had no increase in N-telopeptide (Harrington et al., 2004). The effect of protein on bone health is still unclear, but it does appear to be prudent to monitor the amount of animal protein in the diet for susceptible individuals. This may be more pronounced in individuals that may have a genetic endowment for this. However, if animal protein consumption is modified by other nutrients (e.g. calcium) the effects on bone health may be lessened.

### **Protein Intake and Liver Disease Risk**

The American Heart Association has suggested that high protein diets may have detrimental effects on liver function (St. Jeor et al., 2001). This is primarily the result of a concern that the liver will be stressed through metabolizing the greater protein intakes. However, there is no scientific evidence to support this contention. Jorda and colleagues (1988) did show that high protein intakes in rats produce morphological changes in liver mitochondria. However, they also suggested that these changes were not pathological, but represented a positive hepatocyte adaptation to a metabolic stress.

Protein is important for the liver not only in promoting tissue repair, but to provide lipotropic agents such as methionine and choline for the conversion of fats to lipoprotein for removal from the liver (Navder and Leiber, 2003a). The importance of high protein diets has also been acknowledged for individuals with liver disease and who are alcoholics. High protein diets may offset the elevated protein catabolism seen with liver disease (Navder and Leiber, 2003b), while a high protein diet has been shown to improve hepatic function in individuals suffering from alcoholic liver disease (Mendellhall et al., 1993).

#### Comparisons between Different Protein Sources on Human Performance

Earlier discussions on protein supplementation and athletic performance have shown positive effects from proteins of various sources. However, only limited research is available on comparisons between various protein sources and changes in human performance. Recently, there have been a number of comparisons between bovine colostrum and whey protein. The primary reason for this comparison is the use by these investigators of whey protein as the placebo group in many of the studies examining bovine colostrum (Antonio et al., 2001; Brinkworth et al., 2004; Brinkworth and Buckley, 2002; Coombes et al., 2002; Hofman et al., 2002). The reason being that whey protein is similar in taste and texture as bovine colostrum protein.

Studies performed in non-elite athletes have been inconclusive concerning the benefits of bovine colostrum compared to whey protein. Several studies have demonstrated greater gains in lean body mass in individuals supplementing with bovine colostrum than whey, but no changes in endurance or strength performance (Antonio et al., 2001; Brinkworth et al., 2004). However, when performance was measured following prolonged exercise (time to complete 2.8 kJ·kg<sup>-1</sup> of work following a 2-hour ride) supplement dosages of 20 g·day<sup>-1</sup> and 60 g·day<sup>-1</sup> were shown to significantly improve time trial performance in competitive cyclists (Coombes et al., 2002). These results may be related to an improved buffering capacity following colostrum supplementation. Brinkworth and colleagues (2002) reported that although no performance changes were seen in rowing performance, the elite rowers that were studied did demonstrate an improved buffering capacity following 9-weeks of supplementation with 60 g·day<sup>-1</sup> of bovine colostrum when compared to supplementing with whey protein. The improved buffering capacity subsequent to colostrum supplementation may have also influenced the results reported by Hofman et al., (2002). In that study elite field hockey players supplemented with either 60 g·day<sup>-1</sup> of either colostrum or whey protein for 8-weeks. A significantly greater improvement was seen in repeated sprint performance in the group supplementing with colostrum compared to the group supplementing with whey protein. However, a recent study has suggested that the improved buffering system seen following colostrum supplementation is not related to an improved plasma buffering system, and that any improved buffering capacity occurs within the tissue (Brinkworth et al., 2004).

In a comparison between casein and whey protein supplementation, Boirie and colleagues (1997) showed that a 30-g feeding of casein versus whey had significantly different effects on postprandial protein gain. They showed that following whey protein ingestion

the plasma appearance of amino acids is fast, high and transient. In contrast, casein is absorbed more slowly producing a much less dramatic rise in plasma amino acid concentrations. Whey protein ingestion stimulated protein synthesis by 68%, while casein ingestion stimulated protein synthesis by 31%. When the investigators compared postprandial leucine balance after 7-hours post ingestion, casein consumption resulted in a significantly higher leucine balance, whereas no change from baseline was seen 7-hours following whey consumption. These results suggest that whey protein stimulates a rapid synthesis of protein, but a large part of this protein is oxidized (used as fuel), while casein may result in a greater protein accretion over a longer duration of time. A subsequent study showed that repeated ingestions of whey protein (an equal amount of protein but consumed over a prolonged period of time [4 hours] compared to a single ingestion) produced a greater net leucine oxidation than either a single meal of casein or whey (Dangin et al., 2001). Interestingly, both casein and whey are complete proteins but their amino acid composition is different. Glutamine and leucine have important roles in muscle protein metabolism, yet casein contains 11.6 and 8.9 g of these amino acids, respectively while whey contains 21.9 and 11.1 g of these amino acids, respectively. Thus, the digestion rate of the protein may be more important than the amino acid composition of the protein.

In a study examining the effects of casein and whey on body composition and strength measures, 12 weeks of supplementation on overweight police officers showed significantly greater strength and lean tissue accretion in the subjects ingesting casein compared to whey (Demling and DeSanti, 2000). Protein supplementation provided a relative protein consumption of 1.5 g·kg·day<sup>-1</sup>. Subjects supplemented twice per day approximately 8-10 hours apart.

Only one study known has compared colostrum, whey and casein supplementation (Fry et al., 2003). Following 12-weeks of supplementation the authors reported no significant differences in lean body mass, strength or power performances between the groups. However, the results of this study should be examined with care. The subjects were comprised of both males and females who were resistance training for recreational purposes. In addition, the subject number for each group ranged from 4-6 subjects per group. With a heterogeneous subject population and a low subject number, the statistical power of this study was quite low. However, the authors did analyze effect sizes to account for the low statistical power. This analysis though did not change any of the observations. Clearly, further research is needed in comparisons of various types of protein on performance improvements. However, it is likely that a combination of different proteins from various sources may provide optimal benefits for performance.

## **CONCLUSIONS**

It does appear that protein from animal sources is an important source of protein for humans from infancy until mature adulthood. However, the potential health concerns

associated with a diet of protein consumed primarily from animal sources should be acknowledged. With a proper combination of sources, vegetable proteins may provide similar benefits as protein from animal sources. Maintenance of lean body mass though may become a concern. However, interesting data does exist concerning health benefits associated with soy protein consumption.

In athletes supplementing their diets with additional protein, casein has been shown to provide the greatest benefit for increases in protein synthesis for a prolonged duration. However, whey protein has a greater initial benefit for protein synthesis. These differences are related to their rates of absorption. It is likely a combination of the two could be beneficial, or smaller but more frequent ingestion of whey protein could prove to be of more value. Considering the paucity of research examining various sources of protein in sport supplementation studies, further research appears warranted on examining the benefits of these various protein sources.

## **KEY POINTS**

Higher protein needs are seen in athletic populations.

Animal proteins is an important source of protein, however potential health concerns do exist from a diet of protein consumed from primarily animal sources.

With a proper combination of sources, vegetable proteins may provide similar benefits as protein from animal sources.

Casein protein supplementation may provide the greatest benefit for increases in protein synthesis for a prolonged duration.

Recovery from prolonged strenuous exercise requires that depleted fuel stores be replenished, that damaged tissue be repaired and that training adaptations be initiated. Critical to these processes are the type, amount and timing of nutrient intake. Muscle glycogen is an essential fuel for intense exercise, whether the exercise is of an aerobic or anaerobic nature. Glycogen synthesis is a relatively slow process, and therefore the restoration of muscle glycogen requires special considerations when there is limited time between training sessions or competition. To maximize the rate of muscle glycogen synthesis it is important to consume a carbohydrate supplement immediately post exercise, to continue to supplement at frequent intervals and to consume approximately 1.2 g carbohydrate·kg<sup>-1</sup> body wt·h<sup>-1</sup>. Maximizing glycogen synthesis with less frequent supplementation and less carbohydrate can be achieved with the addition of protein to the carbohydrate supplement. This will also promote protein synthesis and reduce protein degradation, thus having the added benefit of stimulating muscle tissue repair and adaptation. Moreover, recent research suggests that consuming a carbohydrate/protein supplement post exercise will have a more positive influence on subsequent exercise performance than a carbohydrate supplement.

## INTRODUCTION

Recovery from exercise is a complex process requiring the replenishment of the body's fuel stores, the repair of damaged muscle tissue and the initiation of training adaptations. This requires the body to switch from a predominantly catabolic state to a predominantly anabolic state. For this transition to occur efficiently and effectively requires not only that the proper nutrients be consumed, but also that they be consumed at the appropriate time.

The major source of fuel used by the skeletal muscles during prolonged aerobic exercise of a strenuous nature is muscle glycogen. The importance of muscle glycogen as a fuel source cannot be overstated. In general, it has been demonstrated that aerobic endurance is directly related to the initial muscle glycogen stores, that strenuous exercise cannot be maintained once these stores are depleted, and that perception of fatigue during prolonged intense exercise parallels the decline in muscle glycogen (Hermansen et al., 1965; Ahlborg, et al., 1967; Bergström and Hultman, 1967; Bergström et al., 1967). Because of the importance of muscle glycogen for sustaining prolonged intense exercise, there has been considerable research to establish the most efficient means for its replenishment once depleted. Early research focused on how to replenish the muscle glycogen stores on a daily basis in preparation for consecutive days of competition or exercise training. However, because many athletes may train or have to compete several times a day, more recent research has focused on how to replenishing the muscle glycogen stores within several hours after exercises. In this regard, questions that have been addressed include the most appropriate amount and frequency of carbohydrate supplementation, the most appropriate times to supplement, as well as the most appropriate supplements to use.

Aside from a reduction in the muscle glycogen stores, strenuous exercise will result in muscle tissue damage. This damage is due in part to the physical stress placed on the muscle, particularly during the eccentric phase of muscle contraction (Clarkson and Hubal, 2002; Evans, 2002), and hormonal changes that result in the breakdown of muscle protein, as well as fat and carbohydrate, to provide the fuel for powering muscle contraction (Walsh et al., 1998). However, muscle damage does not just occur during exercise, but can continue after exercise for many hours. This occurs as a result of a protracted exercise hormonal milieu, an increase in free radicals and acute inflammation. Not only will such tissue damage limit performance due to delayed onset muscle soreness, but it will also compromise the replenishment of muscle glycogen and limit muscle training adaptations (O'Reilly et al., 1987; Costill et al., 1990).

In this review the most efficient and appropriate means of rapidly replenishing the muscle glycogen stores post exercise will be discussed. Also discussed will be the means of limiting post exercise muscle damage and stimulating muscle protein synthesis. Finally, evidence will be presented that the procedures used to rapidly replenish the muscle glycogen stores and stimulate protein synthesis will favorably affect physical performance.

## **MUSCLE GLYCOGEN REPLENISHMENT POST EXERCISE**

The competitive nature of sports today requires many athletes to cross-train and train multiple times per day. Moreover, many athletes may be required to compete in several different contests over subsequent days or even on the same day. Recent research has suggested that for these situations athletes benefit from the rapid restoration of their muscle glycogen stores. Many factors will affect the rate of muscle glycogen storage after exercise. These include the timing of carbohydrate consumption, the amount and frequency of carbohydrate consumption, and the addition of protein to a carbohydrate supplement.

### **Timing of Carbohydrate Consumption After Exercise**

It has been found that muscle glycogen synthesis is more rapid if carbohydrate is consumed immediately following exercise as opposed to waiting several hours (Ivy et al., 1988a). When carbohydrate is consumed immediately after exercise the rate of glycogen synthesis averages between 6 to 8 mmol·kg<sup>-1</sup> wet wt·h<sup>-1</sup>; whereas, if the supplement is delayed several hours the rate of synthesis is reduced 50% (Mæhlum et al., 1977; Blom et al., 1987; Ivy et al. 1988a). The increased synthesis immediately post exercise is due in part to a faster rate of muscle glucose uptake as a result of an increase in muscle insulin sensitivity (Garetto et al., 1984; Richter et al., 1984; Cartee et al., 1989), and an increase in the concentration of glucose transporters associated with the plasma membrane of the muscle (Goodyear et al., 1990; Etgen et al., 1996). With time, however, the increase in insulin sensitivity and membrane glucose transporter concentration declines resulting in a slower rate of muscle glucose uptake and glycogen storage. For instance, Okamura et al. (1997) infused glucose at the same rate in dogs either immediately after exercise or 2-hours after exercise. Plasma glucose and insulin levels were significantly lower in the dogs infused immediately after exercise, but their rates of hindlimb glucose uptake were significantly greater. Levenhagen et al. (2001) found that leg glucose uptake was increased 3-fold above basal when supplemented immediately after exercise with carbohydrate, and increased only 44% above basal when supplemented 3-hours after exercise. This difference in rate of uptake occurred despite no differences in leg blood flow, or blood glucose and insulin concentrations between the two treatments.

It should also be pointed out that after exercise that depletes the body's carbohydrate stores, there is little if any increase in muscle glycogen storage until adequate carbohydrate is made available (Ivy et al., 1988a; Ivy et al., 1998b; Zawadzki et al., 1992). Therefore, early intake of carbohydrate after strenuous exercise is essential because it provides an immediate source of substrate to the muscle, while also taking advantage of the increased insulin sensitivity and membrane permeability of the muscle to glucose. Furthermore, supplementing immediately after exercise appears to delay the decline in insulin sensitivity, and with frequent supplementation, a relatively rapid rate of glycogen storage can be maintained for up to 8-hours post exercise (Blom et al., 1987; Ivy et al., 1988b).



### **Amount of Dietary Carbohydrate**

An important dietary factor affecting muscle glycogen replenishment is obviously the amount of carbohydrate consumed. When provided immediately post exercise, the rate of glycogen storage will decline as glucose availability decreases (Ivy et al., 1988a). However, Blom et al. (1987) demonstrated that this decline could be attenuated for up to 8-hours if supplements were continually provided at 2-hour intervals. They also found that supplementing with 0.7 g glucose·kg<sup>-1</sup> body wt appeared to maximize muscle glycogen storage, as there was no difference found between supplements containing 0.7 and 1.4 g glucose·kg<sup>-1</sup> body wt. Research from our laboratory, however, suggests that when providing carbohydrate supplementation at 2-hour intervals, 1.2 to 1.4 g of glucose·kg<sup>-1</sup> body wt (0.6 to 0.7 g carbohydrate·kg<sup>-1</sup> body wt·h<sup>-1</sup>) is required to maximize muscle glycogen storage (Ivy et al., 1988a; 1988b).

The rate of glycogen synthesis that is maintained by supplementing at 2-hour intervals, approximately 7 mmol·kg<sup>-1</sup> wet wt·h<sup>-1</sup>, does not appear to be the highest rate of muscle glycogen synthesis possible. Some studies have found that supplementing at increased frequency and the addition of protein to the carbohydrate supplement can positively influence the rate of synthesis (Doyle et al., 1993; Piehl-Aulin et al., 2000; van Hall et al., 2000).

### **Frequency of Carbohydrate Supplementation**

When carbohydrate supplementation occurs at frequent intervals such as every 15 to 30 minutes and in high amounts, the rate of muscle glycogen storage has been found to be approximately 30% higher than when supplementing every 2-hours (Doyle et al., 1993; Piehl-Aulin et al., 2000; van Hall et al., 2000). Doyle et al. (1993) reported glycogen storage rates of 10 mmol·kg<sup>-1</sup> wet wt·h<sup>-1</sup> during the first 4 hours of recovery from exercise when subjects received 0.4 g carbohydrate·kg<sup>-1</sup> body wt every 15 minutes (1.6 g carbohydrate·kg<sup>-1</sup> body wt·h<sup>-1</sup>). Similar rates were reported by van Hall et al. (2000) during a 4-hour recovery period when supplementation occurred at 15-minute intervals, and by Piehl-Aulin et al. (2000) during the first two hours of recovery when supplementing at 30-minute intervals. In these studies carbohydrate was provided at a rate of approximately 1.0 to 1.2 g·kg<sup>-1</sup> body wt·h<sup>-1</sup>. These studies suggest that supplementing at 15 to 30 minutes intervals may be preferable to supplementing every 2-hours for the rapid restoration of the muscle glycogen stores post exercise. They also suggest that when supplementing at frequent intervals, the optimal amount of carbohydrate is in the range of 1.2 g·kg<sup>-1</sup> body wt·h<sup>-1</sup>. Unfortunately, there have not been any studies conducted directly comparing the frequency of supplementation on the rate of glycogen storage.

### **Effect of Protein on Glycogen Storage**

Our laboratory was the first to study the combined effect of protein plus carbohydrate on muscle glycogen synthesis (Zawadzki et al., 1992). Comparisons were made for supplements consisting of 112 g of carbohydrate in a 21% w/v mixture and 112 g of carbohydrate with 40.7 g of protein provided immediately after and 2-hours after exercise. It was found that the addition of protein to the carbohydrate supplement

increased the rate of glycogen storage by approximately 38% over the first 4-hours of recovery. The greater rate of synthesis was believed due to a greater insulin response as a result of the addition of protein to the carbohydrate supplement (Pallotta and Kennedy, 1968; Spiller et al., 1987). Controversy arose, however, because the carbohydrate and carbohydrate/protein supplements we used were not isocaloric, and subsequent research from other laboratories failed to confirm our findings (Tarnopolsky et al., 1997; Carrithers et al., 2000; van Hall et al., 2000; Jentjens et al., 2001). The conflicting results, however, can probably be attributed to differences in experimental design such as the frequency of supplementation and the amount and types of carbohydrate and protein provided. In general, those studies that did not demonstrate a benefit of protein used more frequent feeding intervals (Tarnopolsky et al., 1997; Carrithers et al., 2000; van Hall et al., 2000; Jentjens et al., 2001), provided greater amounts of carbohydrate (van Hall et al., 2000; Jentjens et al., 2001), and in some studies less protein (Carrithers et al., 2000; Tarnopolsky et al., 1997). Support for this supposition comes from a recent study from our laboratory in which we tested the hypothesis that a carbohydrate-protein supplement would be more effective in the replenishment of muscle glycogen after exercise compared with a carbohydrate supplement of equal carbohydrate content or caloric equivalency when supplementing immediately and 2-hours post exercise (Ivy et al., 2002). After several hours of intense cycling to deplete the muscle glycogen stores, the subjects received, using a rank-ordered design, a carbohydrate protein (80 g CHO, 28 g Pro, 6 g fat), iso-carbohydrate (80 g CHO, 6 g fat), or isocaloric carbohydrate (108 g CHO, 6 g fat) supplement. After 4-hours of recovery, muscle glycogen was significantly greater for the carbohydrate/protein treatment ( $88.8 \pm 4.4 \text{ mmol}\cdot\text{l}^{-1}$ ) when compared with the iso-carbohydrate ( $70.0 \pm 4.0 \text{ mmol}\cdot\text{l}^{-1}$ ) and isocaloric ( $75.5 \pm 2.8 \text{ mmol}\cdot\text{l}^{-1}$ ) treatments. Glycogen storage did not differ significantly between the iso-carbohydrate and isocaloric treatments. Of interest was the very large difference in glycogen storage between treatments during the first 40 minutes of recovery. Glycogen storage was twice as fast after the carbohydrate/ protein treatment than after the isocaloric treatments, and four times faster than after the iso-carbohydrate treatment. This trend was also noted following the second feeding 2-hours into recovery.

The results indicate that the co-ingestion of protein with carbohydrate will increase the efficiency of muscle glycogen storage when supplementing at intervals greater than 1-hour apart, or when the amount of carbohydrate ingested is below the threshold for maximal glycogen synthesis. These results have important implications for athletes who wish to limit their carbohydrate intake in an effort to control body weight and for those athletes who participate in sports that have very short recovery periods during competition such as basketball, ice hockey and soccer.

## **LIMITING MUSCLE DAMAGE AND INITIATING MUSCLE PROTEIN ACCRETION**

During strenuous exercise there is generally damage to the active muscles and this damage can continue after exercise due to acceleration in protein degradation. For complete recovery, it is important to initiate protein synthesis while limiting protein degradation. Like muscle glycogen storage, muscle protein synthesis and degradation are affected by the types, amount and timing of nutrient supplementation.

#### Types of Supplementation Affecting Protein Synthesis and Degradation

Although the muscle can have residual catabolic activity following exercise, it is primed to shift into an anabolic state in the presence of the right nutrients. This is due, in part, to an increased sensitivity to insulin. Insulin is one of the most anabolic hormones in the body. Insulin increases muscle amino acid uptake and protein synthesis and reduces protein degradation. Following exercise, raising the plasma insulin level is key to limiting protracted muscle damage and stimulating protein accretion.

Roy et al. (1997) investigated the effect of carbohydrate supplementation on the fractional rate of protein synthesis following resistance exercise using one leg, with the opposite leg serving as a control. The subjects received 1g of carbohydrate·kg<sup>-1</sup> body wt immediately after and 1-hour after exercise or a placebo. Exercise alone did not result in a significant increase in protein synthesis. Carbohydrate supplementation, however, significantly elevated the plasma insulin level and increased protein synthesis by 36% in the exercised leg as compared to the non-exercised leg. Furthermore, urinary nitrogen and 3-methylhistidine were significantly reduced following carbohydrate supplementation suggesting a reduction in muscle tissue damage and protein degradation. Conversely, Levenhagen et al. (2002) found no increase in protein synthesis when a carbohydrate supplement was provided immediately post exercise. However, this finding may have been due to the lack of an appreciable insulin response resulting from the very small carbohydrate supplement (8g) provided.

Supplementation of a mixture of essential amino acids will also increase protein synthesis (Biolo et al., 1997; Tipton et al., 1999). Activation of protein synthesis by amino acids is most responsive immediately following exercise. Raising the plasma amino acid levels post exercise by infusion or oral supplementation has been reported to transition the muscle from a negative protein balance to a positive protein balance by stimulating protein synthesis (Rasmussen et al., 2000). When blood amino acid levels are reduced below normal, amino acids are released from the muscle and protein synthesis declines. Elevating the essential amino acid levels above normal, however, increases amino acid uptake and muscle protein synthesis (Wolfe, 2001).

While supplementing with either carbohydrate or amino acids post exercise may limit muscle damage and stimulate protein synthesis, there is increasing evidence that the combination can have an additive effect (Suzuki et al., 1999; Levenhagen et al., 2002; Miller et al., 2003). This is likely due to the synergist effect that a carbohydrate/amino acid or carbohydrate/protein supplement has on the plasma insulin response, and the fact that such supplements maintain an elevation in the plasma amino acid concentration. In this regard, Levenhagen et al. (2002) found that leg and whole body protein synthesis

increased 6-fold and 15%, respectively, when a carbohydrate/protein supplement was provided after 60 minutes of cycling at 60% VO<sub>2</sub>max. Net protein accretion was also positive. When a placebo or a carbohydrate supplement was provided, there was a release of muscle amino acids and protein degradation exceeded protein synthesis. In addition, Miller et al. (2003) assessed the independent and combined effects of carbohydrate and amino acid supplementation following leg resistance exercises. Supplements were provided 1- and 3-hours after exercise and protein synthesis across the leg was determined over a 3-hour recovery period. Both the plasma insulin response and protein synthesis rate were found to be greatest in response to the carbohydrate/amino acid supplement. The effect of the carbohydrate/amino acid supplement on net muscle protein synthesis was roughly equivalent to the sum of the independent effects of either the carbohydrate or amino acid supplement alone. These findings are supported by the research of Gautsch et al. (1998). These investigators found that a complete meal composed of protein and high glycemic carbohydrates provided post exercise would stimulate mRNA translation initiation for muscle protein synthesis, whereas a meal consisting of carbohydrate alone was insufficient.

### **Nutrient Timing on Protein Synthesis and Degradation**

As with the restoration of muscle glycogen after exercise, the timing of supplementation for the stimulation of protein accretion also appears critical. Okamura et al. (1997) appear to have been the first to investigate the effect on nutrient timing on muscle protein synthesis after exercise. They measured the rate of protein synthesis and degradation in dogs after treadmill exercise. All dogs were infused for 2-hours with a 10% amino acid and 10% glucose solution, with half of the dogs infused immediately after exercise and the other half infused 2-hours after exercise. During the pre-exercise period and during exercise there was a net protein breakdown. Only after initiating the infusion of the amino acids and glucose mixture did net protein balance become positive, with the increase in muscle amino acid uptake and protein synthesis greater when infused immediately after exercise compared to 2-hours after exercise.

Probably the study best illustrating the effect of nutrient timing on muscle tissue protein synthesis and accretion is that by Levenhagen et al. (2001). These researchers studied the effects of a carbohydrate/protein supplement on protein synthesis and degradation after a 60-minute moderate intensity exercise bout of cycling. Subjects were given the supplement immediately or 3-hours after exercise. Protein degradation was unaffected by supplement timing, but leg protein synthesis was increased approximately 3-fold above basal when supplementation occurred immediately post exercise. No increase in protein synthesis occurred when the supplement was delayed 3-hours, and only when the supplement was immediately provided after exercise was there a positive protein balance (the rate of protein synthesis exceeded the rate of protein degradation). It was also of interest to note that when supplementation occurred immediately compared to 3-hours after exercise, there was a greater fat oxidation. Levenhagen et al. (2001) concluded that ingesting a carbohydrate/ protein supplement early after exercise increases protein accretion as well as muscle glycogen storage.

## **PHYSICAL PERFORMANCE FOLLOWING RECOVERY**

Research suggests that providing a carbohydrate/ protein supplement at the appropriate times after exercise will have a significant impact on subsequent exercise performance. For example, we compared the effectiveness of a carbohydrate/protein supplement (15% carbohydrate - 4% protein) designed for recovery with that of a traditional sports drink (6% carbohydrate) (Williams et al, 2003). The supplements (355 ml of each) were provided immediately after and 2-hours after exercise. Degree of recovery was assessed by having the subjects exercise to exhaustion at 80% VO<sub>2</sub>max following a 4-hour recovery period. We found that muscle glycogen restoration was 128% greater and exercise performance 55% greater when consuming the carbohydrate/protein recovery drink as compared to the traditional sports drink. Obviously, from this study one cannot discern if the difference in performance between the two treatments was due to the type of supplement provided or the amount of carbohydrate consumed. However, the point that can be made is that a supplement designed for exercise recovery is much more effective than a traditional sports drink. Furthermore, two recent studies suggest that the addition of protein to a high carbohydrate recovery supplement is advantageous.

Niles et al. (2001) compared the effectiveness of isocaloric carbohydrate (carbohydrate, 152.7 g) and carbohydrate/protein (protein, 112 g; carbohydrate 40.4 g) supplements to promote recovery from strenuous aerobic exercise. Supplements were provided immediately and 1-hour after exercise, and recovery was assessed 3-hours after the last supplement by having the subjects run to exhaustion at an exercise intensity 10% about their anaerobic threshold. Run time to exhaustion was 21% longer when the subjects consumed the carbohydrate/protein supplement compared to the carbohydrate supplement. More remarkable are the findings of Saunders et al. (In Press). In their study, subjects received in random order 1.8 ml·kg<sup>-1</sup> body wt of a 7.3% carbohydrate or 7.3% plus 1.85% carbohydrate/protein supplement every 15 minutes while cycling at 75% VO<sub>2</sub>max to exhaustion, and 10 ml·kg<sup>-1</sup> body wt immediately after exercise. Twelve to fifteen hours after the last supplement, the subjects completed a second ride to exhaustion at 85% of VO<sub>2</sub>max. During the first cycling exercise the subjects rode 29% longer when consuming the carbohydrate/protein supplement compared with the carbohydrate supplement. Moreover, during the second ride performance was 40% longer when consuming the carbohydrate/protein supplement. Interestingly, plasma creatine phosphokinase (CPK) levels, an indication of muscle tissue damage, were 83% lower prior to the start of the second exercise in the subjects consuming the carbohydrate/protein supplement. It was concluded that the addition of protein to a carbohydrate supplement produces improvements in aerobic endurance and limits exercise muscle damage.

## **CONCLUSIONS**

The restoration of muscle glycogen after depletion by exercise is a central component of the recovery process. To maximize the rate of muscle glycogen storage during short-term recovery, it is important to consume a carbohydrate supplement as soon after exercise as possible. If consuming only carbohydrate, supplementation should occur frequently, such as every 30 minutes, and provide about 1.2 to 1.5 g of carbohydrate·kg<sup>-1</sup> body wt·h<sup>-1</sup>. However, the efficiency of muscle glycogen storage can be increased significantly with the addition of protein to a carbohydrate supplement. This will reduce both the amount of carbohydrate and frequency of supplementation required to maximize glycogen storage. If both carbohydrate and protein are consumed, it is recommended that 0.8 g carbohydrate·kg<sup>-1</sup> body wt plus 0.2 g protein·kg<sup>-1</sup> body wt be consumed immediately and 2-hours after exercise during a 4-hour recovery period. The addition of protein to a carbohydrate supplement also has the added advantage of limiting post exercise muscle damage and promoting muscle protein accretion. Along with a rapid increase in muscle glycogen, these processes can have a significant impact on subsequent exercise performance.

## **KEY POINTS**

For rapid recovery from prolonged exercise, it is important to replenish muscle glycogen stores and initiate muscle tissue repair and adaptation.

To maximize muscle glycogen replenishment, it is important to consume a carbohydrate supplement as soon after exercise as possible.

Consume the carbohydrate frequently, such as every 30 minutes, and provide about 1.2 to 1.5 g of carbohydrate·kg<sup>-1</sup> body wt·h<sup>-1</sup>.

Efficiency of muscle glycogen storage can be increased significantly with the addition of protein to a carbohydrate supplement (~4 to 1 carbohydrate to protein ratio).

The addition of protein to a carbohydrate supplement also has the added advantage of limiting post exercise muscle damage and promoting muscle protein accretion.