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REVIEWS: CURRENT TOPICS

Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people (Review)

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Abstract

Whey proteins and amino acid supplements have a strong position in the sports nutrition market based on the purported quality of proteins and amino acids they provide. Recent studies employing stable isotope methodology demonstrate the ability of whey proteins or amino acid mixtures of similar composition to promote whole body and muscle protein synthesis. Other developing avenues of research explore health benefits of whey that extend beyond protein and basic nutrition. Many bioactive components derived from whey are under study for their ability to offer specific health benefits. These functions are being investigated predominantly in tissue culture systems and animal models. The capacity of these compounds to modulate adiposity, and to enhance immune function and anti-oxidant activity presents new applications potentially suited to the needs of those individuals with active lifestyles. This paper will review the recent literature that describes functional properties of essential amino acids, whey proteins, whey-derived minerals and other compounds and the mechanisms by which they may confer benefits to active people in the context that exercise is a form of metabolic stress. The response to this stress can be positive, as with the accretion of more muscle and improved functionality or greater strength. However, overall benefits may be compromised if immune function or general health is challenged in response to the stress. From a mechanistic standpoint, whey proteins, their composite amino acids, and/or associated compounds may be able to provide substrate and bioactive components to extend the overall benefits of physical activity. © 2003 Elsevier Inc. All rights reserved.

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1. Introduction

People who engage in active lifestyles are likely to seek benefits from diet and nutritional supplements that can enhance physical well being. Many are familiar with whey proteins and amino acid supplements and their suggested role in increasing muscle mass in conjunction with appropriate training. While early studies relied on indirect measures to show an anabolic effect of protein or amino acid supplementation, more recent studies demonstrate directly that whey proteins and their constituent amino acids efficiently promote protein synthesis [1-3]. Calcium and nonfat dry milk have been shown to regulate nutrient partitioning, adiposity, and body composition, suggesting another manner by which whey and whey components may optimize body composition [4,5]. Additionally, whey and whey-derived bioactive compounds have been studied for their ability to enhance general health and well being [6,7]. This paper reviews the literature that describes these functions and presents potential mechanisms by which whey and amino acids may enhance health and physical status in a manner applicable to and of specific interest to physically active individuals.

2. Whey, amino acids, and mechanisms of muscle anabolism

Physical activity exerts a metabolic stress in the sense that many physiological changes occur including release of stress hormones and consequent shifts in fuel availability and use. Body stores are broken down in order to meet the

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exercise-related demand for energy, carbohydrate, fat, and, to some degree protein [8]. This catabolic state during exercise is typically balanced by an anabolic phase in the recovery period following exercise [9]. There are several means by which whey proteins or essential amino acids might shift the balance towards anabolic processes to promote recovery from exercise and theoretically enhance training and, ultimately, performance. Much of the scientific effort to demonstrate benefits of proteins or amino acids has focused on their ability to stimulate muscle protein synthesis.

Factors that influence the efficiency with which amino acids stimulate protein synthesis primarily include the dose and composition of the amino acid mixture or protein [10]. Several studies support the notion that only indispensable or essential amino acids are necessary to stimulate muscle protein synthesis and suggest that a protein which provides a high proportion of these amino acids will be efficient in promoting muscle growth [11-13]. Skeletal muscle protein synthesis was significantly elevated in human volunteers who consumed a mixture of 13.4 g of essential amino acids with carbohydrate [3]. However, the specificity of the response to essential amino acids may be obscured by the moderately high dose provided or the carbohydrate-induced insulin response. A follow up study found a significantly greater (approximately two-fold) response to 6 g of essential amino acids compared to 6 g mixed amino acids (3 g essential plus 3 g non-essential) [14]. This observation, and others from supportive animal and tissue culture studies [13,15,16], confirms that ingestion of only essential amino acids is sufficient for the acute stimulation of muscle protein synthesis.

Whey proteins have a high protein quality score and contain a relatively high proportion of branched-chain amino acids (BCAA, ~26%) [17,18]. The abundance of leucine in whey is of particular interest in this regard. Leucine plays a distinct role in protein metabolism and has been identified as a key signal in the translation initiation pathway of muscle protein synthesis [15]. Leucine is involved in the reversible phosphorylation of proteins that control mRNA binding to the 40S ribosomal subunit. This highly regulated step is dependent upon availability of eukaryotic initiation factors (eIF's). While the precise signals for the response in skeletal muscle are not fully characterized, amino acids stimulate initiation via an enhanced formation of the complex of several of these factors to enable binding of the mRNA to the ribosomal subunit thereby allowing protein synthesis to proceed (Figure 1). Additionally, leucine enhances phosphorylation of the 70-kDa ribosomal protein S6 kinase, thereby up-regulating protein synthesis in skeletal muscle by enhancing both activity and synthesis of proteins integral to mRNA translation [15]. Evidence that leucine may stimulate muscle protein synthesis by additional intracellular signaling pathways [13] invites speculation that the anabolic effectiveness of a protein may, in part, be positively related to the abundance of leucine.

There is no conclusive evidence *in vivo* to show that the relationship between amino acid availability and muscle protein synthesis is mediated by phosphorylation status of initiation factors in humans. However, Yoshizawa and associates [19] have shown in rats that formation of the active eIF4E-eIF4G complex increases in response to a meal containing 20% complete protein. An isocaloric, protein free meal failed to stimulate protein synthesis. Deprivation of essential amino acids in animals *in vivo* impacts protein synthesis directly by repressing synthesis of most proteins. Additionally, the capacity to synthesize proteins is inhibited via repressed translation of mRNA's encoding components of the translation machinery [13]. Thus, leucine and other essential amino acids play two important roles in the protein synthetic pathway, first as signaling molecules for initiation and, secondly, as substrate for the synthesis of new proteins.

Based on the data described above, intracellular amino acid availability is presumably a key promoter of muscle protein synthesis, although extracellular amino acid concentrations are likely to be equally or more important [20]. Plasma concentrations of amino acids increase following ingestion of protein or amino acid infusion [20-23] whereas tissue concentrations have been reported to increase [3] or remain unchanged [20]. Milk proteins specifically elicited a greater increase in branched chain amino acid (BCAA) concentrations in peripheral tissues compared to soy protein [2] suggesting that the amino acid profile or other constitutive differences in dietary proteins influence their utilization. Importantly, animal [11,16] and human [2,24,25] studies provide evidence that poor quality or imbalanced dietary proteins increase nitrogen losses and limit protein synthesis due to inefficient utilization of the indispensable amino acids. The anabolic effect of a complete mixture of amino acids infused in rats was matched by provision of BCAA only [16], again reflecting the primacy of BCAA in protein synthesis. An interesting consideration with regard to the capacity of whey proteins to stimulate muscle protein synthesis derives from comparing the proportion of amino acids supplied by whey relative to the amino acid composition of human skeletal muscle [26-28]. Amino acid composition of whey proteins is very similar to that of skeletal muscle, providing almost all of the amino acids in approximate proportion to their ratios in muscle. Logically, one might suppose that this compatibility would position whey as an effective anabolic supplement, although we recognize that the non-essential amino acids contribute little to the overall response. Whey proteins must be compared directly to other dietary proteins in a proper study utilizing methodology as described in [2,14] to address this question.

Another point, beyond the composition of amino acids present in whey, is the manner in which whey and other intact proteins are absorbed and utilized relative to each other as well as to free amino acid solutions. [29,30]. Recent studies have addressed the significance of patterns of pro-

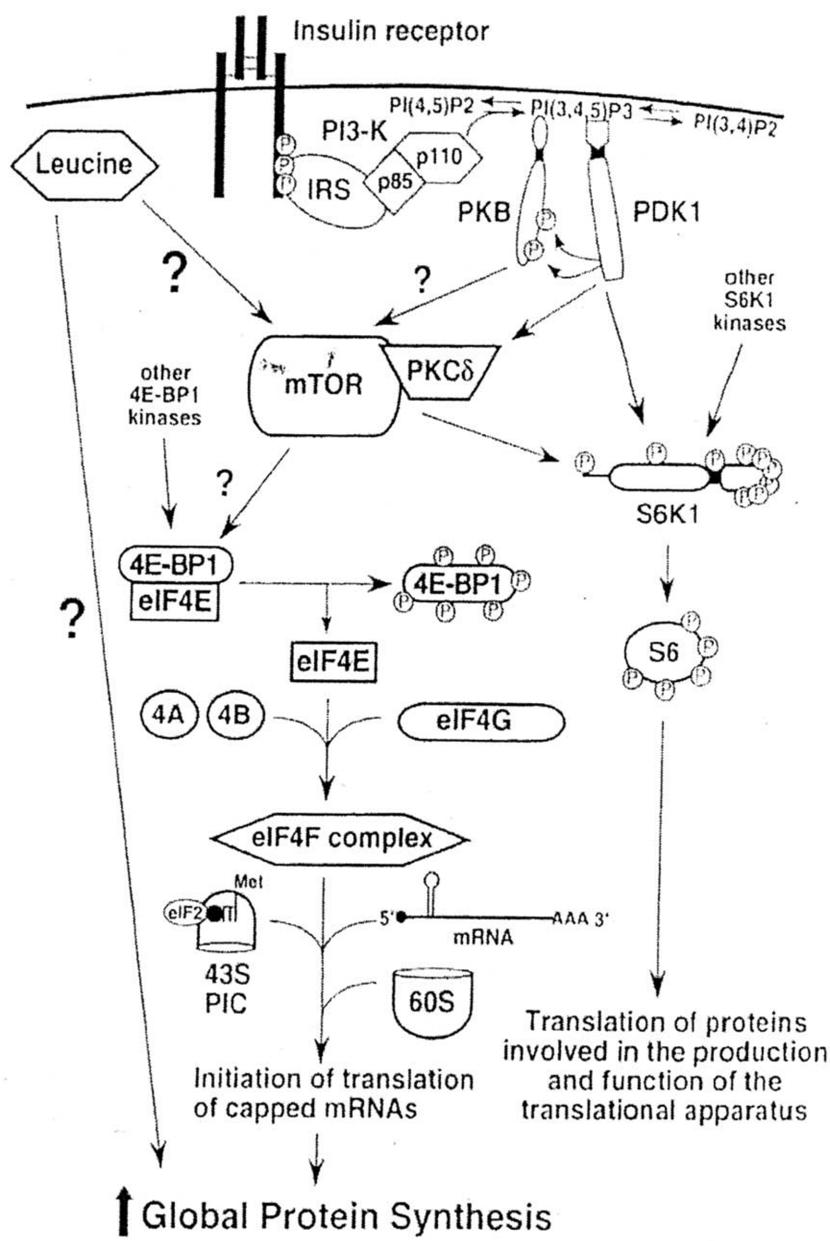

Fig. 1. Molecular mechanisms of initiation of protein synthesis (reprinted with permission from reference 15, Anthony et al. 2001)

tein absorption in terms of human nitrogen and protein metabolism [21,31,32]. Whey proteins have been compared to casein, and a distinct difference is that whey is rapidly absorbed compared to casein, a protein which exits the stomach relatively slowly [29]. The practical implications of this distinction with regard to effects on protein metabolism must be considered in the context of recently published data. Bohé et al [33] reported that continuous stimulation of muscle protein synthesis (via a constant infusion of amino acids) resulted in saturation of the response within two hours. Thus, an effective protein source would be one that could stimulate a response in the periods between feeding which would be additive to the net accumulation of muscle protein in the course of a day. Preliminary studies confirm that an acute amino-acid induced increase in muscle protein synthesis does contribute to protein synthesis in an additive fashion over the course of a day [34]. Studies of bolus ingestion of free amino acid solutions show a rapid, transient response which returns to basal values within an hour [14,27]. The metabolic response to a bolus of whey is rapid and of significant magnitude and therefore, should suffice to increase muscle protein synthesis without preempting the response to a subsequent meal. Preliminary data show this rapid absorption pattern to be beneficial to overall nitrogen balance and post-prandial protein utilization in elderly volunteers [31] but further studies are needed to explore advantages or disadvantages in other populations [22,32]. Muscle uptake of labeled nitrogen from simulta-

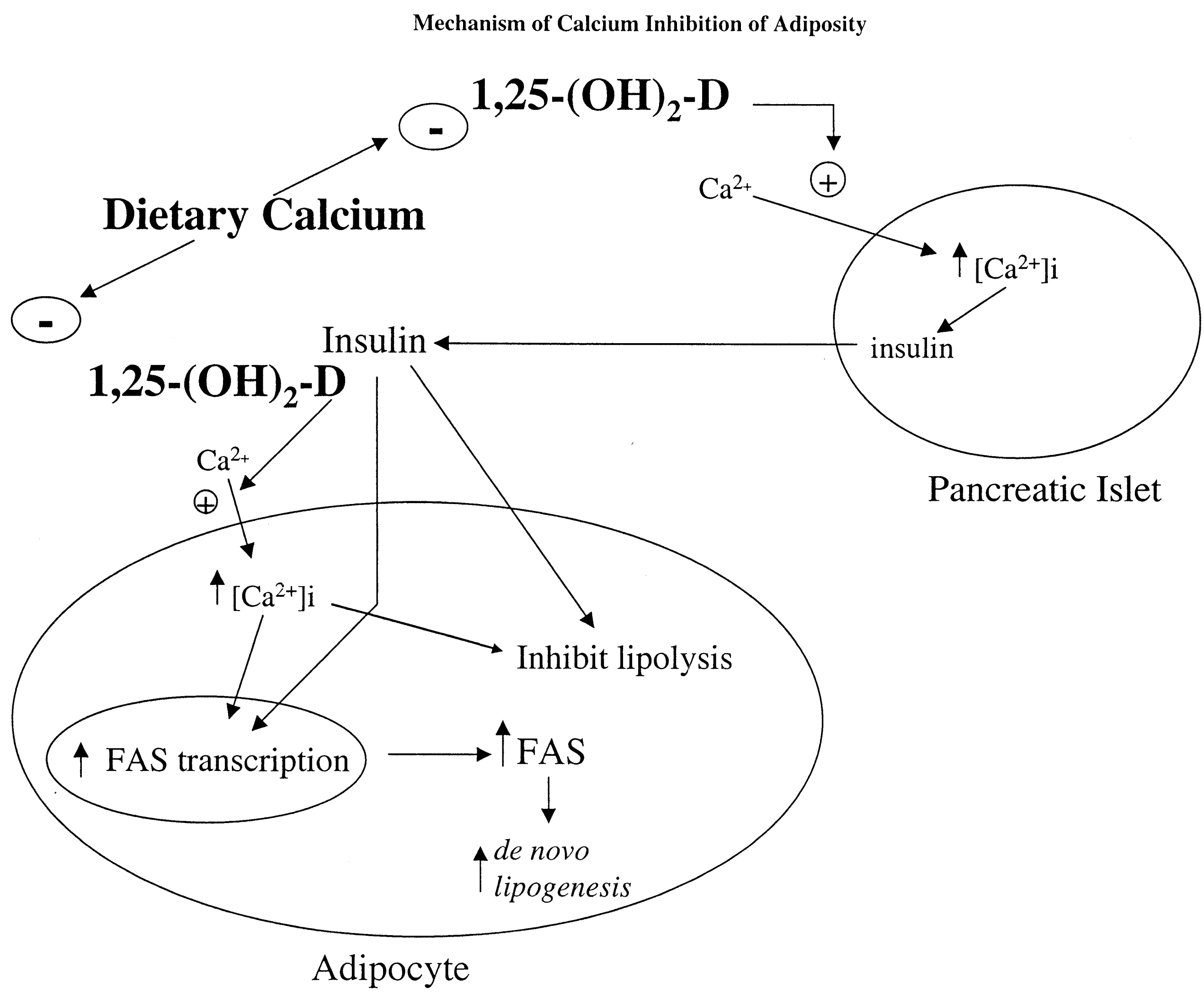

Fig. 2. Mechanisms of calcium inhibition of adiposity.

neously ingested whey and casein in healthy, young volunteers showed no source-related difference in delivery of amino acids to the tissue two hours after ingestion of the proteins [35]. This preliminary observation demonstrates the need to further explore the practical physiological implications of consuming proteins with varying rates of absorption. To summarize, advantages of whey proteins in terms of muscle anabolism may relate to their rapid rate of absorption and leucine abundance to initiate synthesis, as well as their amino acid composition to provide substrate for protein synthesis.

3. Whey components and mechanisms of adiposity

Whey may offer a nutritional advantage towards achieving a desirable body composition beyond that of promoting lean body mass accretion. Recent studies show that calcium [4,5,36-38] and the mineral mix provided by dairy products [4,5,36,39,40] decreases accumulation of body fat and accelerates weight and fat loss during energy restriction. This effect, along with some preliminary evidence showing a satiety effect of milk and certain whey derived peptides [41,42], suggests that whey supplements might facilitate achieving a favorable body weight and composition. The physiological mechanism whereby high calcium intakes decrease fat storage in adipocytes has been elucidated in human adipocytes and in a mouse model of obesity by Zemel et al [4,5,39]. These data demonstrate that the calcitrophic hormones (i.e. parathyroid hormone and 1,25-$(OH)_2$-D) which respond to low calcium diets promote adipose tissue lipid storage, and that suppression of these hormones via high calcium diets inhibits adiposity. Calcitrophic hormones stimulate calcium influx and thereby increase adipocyte intracellular calcium [4,5,39,43]. Intracellular calcium, in turn, has recently been identified as a key regulator of adipocyte lipid metabolism, with increased intracellular calcium increasing lipogenic gene expression and *de novo* lipogenesis and inhibiting lipolysis [4,5,39,45], thereby resulting in increased lipid storage (Figure 2). Conversely, suppressing calcitrophic hormones by increasing dietary calcium results in inhibition of lipogenesis, increased lipolysis and net lipid mobilization, thereby resulting in a shift in energy partitioning from adipose to lean [4,5,36,39,43]. Consequently, low calcium diets promote adipose tissue lipid storage, while high calcium diets promote a leaner phenotype at any given level of energy intake and accelerate weight and fat loss during caloric restriction. While a similar association between whey and body weight/composition has not yet been shown directly, most whey products provide a quantity and composition of minerals and amino acids comparable to fat-free milk [26].

The claim that certain whey peptides suppress appetite is being used to market some products but well designed studies to validate this effect in humans are lacking. A recent study failed to show any satiety enhancing effect of the milk protein casein macropeptide in humans [46]. While appetite control is not necessarily an issue for all active people, some individuals in "body-weight specific sports" may have interest in this application. More importantly, this function could be beneficial to people starting an exercise program with the intent of losing excess body weight. In summary, most individuals desire a high proportion of muscle to fat mass, and this is especially the case in physically active people seeking to maximize performance. Whey provides amino acids to promote muscle protein synthesis in combination with dairy minerals which can assist in limiting body fat accumulation and, therefore, may confer specific advantages towards optimizing body composition. Interestingly, those studies which have directly compared supplemental sources of calcium to dairy sources of calcium clearly demonstrate a marked advantage of dairy calcium in modulating body composition [4,5,36,39,40] suggesting that additional factors, such as whey proteins, may confer additional benefit beyond that of calcium *per se.* Increased muscle protein accretion in response to whey and decreased adiposity in response to dairy interventions have been shown independently in various populations but remain to be empirically and simultaneously assessed in physically active populations.

4. Bioactive amino acids and whey components

Certain amino acids and whey-derived bioactive compounds offer the potential to extend health benefits to active people beyond body composition. Many of the functions associated with these amino acids and whey components involve the immune system [6,18] and may therefore be of particular importance to athletes in intensive training, as several studies demonstrate an immunosuppressive effect of vigorous or excessive training [47,48]. Respiratory tract infections, gastrointestinal health, and free radical production are issues of concern given the evidence that implicates physical exertion as an influential factor in these conditions.

4.1. Immune function

In general, active people are less prone to illness than their sedentary counterparts. However, the dose-response relationship between exercise workload and infection risk fits a J-shaped curve, such that moderate activity enhances immunity while exhaustive exercise suppresses immune function [47]. A number of studies report an increased incidence of upper respiratory tract infections (URTI) in athletes following bouts of strenuous exercise. Nieman et al [48] reported a two-fold greater incidence of infectious episodes in runners who trained more heavily for a marathon. The authors also noted a five-fold greater incidence of URTI in those runners who competed in the marathon compared to similarly experienced runners who did not participate. These observations suggests that heavy training and challenging endurance events may increase risk of infection although they do not provide conclusive evidence of compromised immunity. Other studies offer insight into the nature of immunological changes that follow prolonged, exhaustive exercise. Gleeson et al reports that salivary immunoglobulin A (IgA) decreases after intense exercise and that lower concentrations of IgA after exercise appear to be correlated with the appearance of respiratory tract infection [49]. Immunoglobulins have been isolated from whey and currently, processing techniques to increase amounts and types of immunoglobulins present in whey are being explored [50]. Glutamine concentrations are also depressed after intensive exercise and in "over trained" athletes [47]. Glutamine is a fuel for rapidly dividing cells and has been considered to be "conditionally-essential" during times of metabolic stress or illness [51]. In recognition of its importance during physiological stress, several manufacturers are exploring benefits of supplementing whey, which is naturally enriched with glutamine [26], with additional free glutamine. In spite of plausible physiological mechanisms by which glutamine could optimize immune function, strong evidence for its efficacy in countering exercise or stress-induced immune suppression is lacking [51,52].

It has been proposed that exhaustive exercise or periods of heavy training may lead to an open window of impaired immunity. This period is marked by increased inflammatory activity and an acute phase response during which the athlete may be vulnerable [47]. In addition to being a source of IgA and glutamine, whey may provide other "immunonutrients" to protect against infection. Lactoferrin and its peptide product, lactoferricin, demonstrate strong anti-microbial activity in tissue culture and animal studies. These compounds, in combination with lysozome, another element that can be isolated from whey, provide a "cocktail" with apparently synergistic protective activity against viral and bacterial organisms [18]. Other whey based immune-modulating compounds include β-lactoglobulin, the major protein fraction of whey, β-lactalbumin, and their associated peptides [18,50]. Whey also provides a high proportion of cysteine-rich proteins which are required for synthesis of proteins involved in the acute phase response [18]. Immune-enhancing effects of these compounds have not yet been definitively demonstrated in humans, and healthy humans in particular.

Amino acid supplementation may modulate immunity in exercising individuals. Parry-Billings et al [53] found that branched-chain amino acids prevented an exercise-induced decline in glutamine, presumably by providing nitrogen to support *de novo* synthesis of the amino acid. A few studies support the notion that glutamine availability may influence risk of infection since glutamine-supplemented athletes have exhibited indirect indices of enhanced immune function [52]. However, no single study has demonstrated a link between amino acid availability and functional immune-related outcomes. Consequently, further studies are needed to determine the role of bioactive factors found in whey in modulating immune function.

5. Gastrointestinal health

Gastrointestinal disorders are another concern of athletes and of runners in particular. Many runners experience gastrointestinal distress such as cramps, bloating, and diarrhea during or after events such as marathon competition [54]. The etiology of the problem is not well understood. It has been suggested that damage to the intestinal wall occurs as a consequence of decreased blood flow to the gut while blood is directed towards the active, exercising muscle. This period of relative "nutrient deprivation" can increase intestinal permeability which may contribute to the symptoms [54]. Alternatively, the athlete may simply experience problems associated with poor digestion of foods consumed prior to competition. Whey, whey components, and several amino acids including glutamine are proposed to have some functionality in the gut. Factors such as glycomacropeptides have been identified which exhibit prebiotic and/or probiotic activity and may also stimulate cholecystokinin (CCK) release from intestinal cells and inhibition of toxin binding [18]. Lactose, a major constituent of whey (but not whey protein concentrates), can be enzymatically hydrolyzed to form galacto-oligosaccharides which are readily utilized by bifidobacteria, thus contributing to better functioning of the digestive tract [6]. The use of whey as a vehicle for other probiotics has attracted the interest of supplement manufacturers. Glutamine, ingested either as a supplement or a component of enriched parenteral and enteral diets, has been proposed to be an important fuel source for the small intestine although results of its therapeutic use indicate limited efficacy [51]. While there is a plausible mechanism whereby the athlete or individual with gastrointestinal dysfunction may benefit from whey or whey plus probiotics, this theory has not yet been tested.

6. Free radical production

Another concern associated with exercise regards consequences of excess free radical production. The production of reactive oxidative species (ROS) is an outcome of the obligatory increased oxidative metabolism associated with exercise. In addition to established concerns regarding ROS and chronic disease risk, some investigators believe that ROS accumulation delays muscle recovery and may impair performance [55]. Lactoferrin and lactoferricin, two minor protein in whey, function as antioxidants via their iron binding capacity. Lactoferrin is only 8-30% saturated in its native state, a condition that enables chelation of iron and subsequent inhibition of bacterial growth or oxidative reactions. Whey might also enhance antioxidant capacity by contributing cysteine rich proteins which are pivotal in the synthesis of glutathione, a major intracellular antioxidant [18]. Lands et al [55] reported improvement in muscular performance (assessed by leg isokinetic cycling) in subjects who consumed a whey-based supplement for 30 days compared to a casein control group. Lymphocyte GSH, measured as an index of intracellular GSH, was significantly increased in the whey supplemented group. Noting that oxidative stress contributes to muscular fatigue, the authors propose that increased biosynthesis of intracellular glutathione and its antioxidant activity was the mechanism behind performance improvement. Furthermore, HIV patients who consumed whey supplements effectively increased plasma glutathione concentration although the ultimate functional consequences of this change are not clear [56]. Studies to date are encouraging in terms of the ability of bioactive whey components to influence mediators in these pathways although the physiological implications of these changes must be identified.

7. Summary

Whey protein and amino acid supplements are positioned as high quality protein and amino acid sources and as a potential means to enhance lean body mass in conjunction with appropriate training. Recent findings suggest that benefits of whey may extend beyond muscle anabolism. The calcium and mineral mix found in certain whey products can potentially mediate body composition by shifting nutrient partitioning from adipose to lean tissue. Individual amino acids and bioactive compounds isolated from whey may also improve immune function and gastrointestinal health although many of these functions are just now being defined in model systems.

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