

Review

The Rationale for Consuming Protein Blends in Sports Nutrition

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Protein is considered by many to be the most important macronutrient for humans because of the numerous roles protein plays in the body. Protein needs have been compared across several population groups, including athletes and other exercising individuals. Many researchers have compared the effects of ingesting animal and vegetable protein sources and their implications on sports performance. Recently, blends of dairy protein and soy protein have appeared in commercial sports nutrition products such as nutrition bars and ready-to-drink and powdered beverages. This review will focus on the potential nutritional advantages of combining whey protein, casein, and isolated soy protein.

Key teaching points:

- Casein, whey protein, and isolated soy protein are all nutritionally complete proteins relative to U.S. Food and Drug Administration labeling guidelines, with a Protein Digestibility Corrected Amino Acid Score of 1.00.
- Casein, whey protein, and isolated soy protein appear to have different digestion rates, which may create the opportunity to meter delivery of digested amino acids to peripheral tissues during exercise recovery.
- Comparative studies between isolated soy protein and whey protein show similar increases in lean body mass.
- Isolated soy protein is rich in glutamine and arginine, whereas whey protein and casein contain more of the branched-chain amino acids.
- Isolated soy protein contains natural bioactive substances that enhance plasma antioxidant activity.

INTRODUCTION

Protein consumption has been one of the most researched areas in the nutrition field over the past few decades [1]. Over the past few years, a particular emphasis has been placed on the role of protein for weight management [2,3] and athletic performance [4,5]. Briefly, the global obesity epidemic has brought attention to the benefits of protein for satiety [2] and glycemic index [6]. Protein is the most satiating macronutrient [7]. When protein is ingested as a preload meal, energy intake at subsequent meals is significantly reduced [8]. Additionally, a food's glycemic index can be significantly reduced by replacing high glycemic index carbohydrates with protein [unpublished observations]. Benefits of consuming lower glycemic index foods and glycemic load diets include

improved blood glucose control and insulin sensitivity and reduced body weight [6].

An area of expanding research in recent years has been the comparative effects of different protein sources on physical activity: the advantages and disadvantages of animal versus vegetable protein sources [9] and their implications on athletic performance [10,11]. Recently, blends of the dairy proteins whey protein and casein and soy protein isolate (ISP) have appeared in commercial sports nutrition products, including nutrition bars and ready-to-drink and powdered beverages. This review will focus on the benefits of dairy proteins, specifically whey protein and casein, and ISP for athletes and active individuals engaged in resistance exercise. The potential nutritional advantages of combining these protein sources will also be addressed. Unless specifically noted, "whey protein"

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represents whey protein concentrates and whey protein isolate, and “casein” includes casein and various caseinates.

BACKGROUND

Dairy proteins and soy protein are the major protein sources used in sports nutrition products [12]. According to the *Nutrition Business Journal* [12], dairy proteins accounted for 77% of domestic powder protein sales in 2004, whereas soy protein accounted for 23%. Whey protein concentrate at 80% protein (WPC80) is the workhorse dairy protein in the sports nutrition industry. A 2007 report from 3A Business Consulting [13] indicates that 37% of WPC80 is used in sports nutrition; this is two- to fourfold more WPC80 than is used in any other market sector. Casein and caseinates are also used in nutritional drinks and bars, though to a lesser extent than whey protein, mostly because of formulation issues (e.g., less solubility) and a higher cost structure. Whey proteins originate from the soluble whey fraction removed primarily during cheese making, whereas casein comes from the solid fraction of skim milk after exposure to low pH. For vegetarian protein sources, ISP at 90% protein is the preferred choice because it delivers more protein per gram of product than other soy protein sources and is higher in terms of protein quality than other vegetable proteins [14].

Though many studies have examined the physiological effects of ISP, whey, and casein in isolation or against carbohydrate, systematic comparisons of the 3 proteins are lacking. Thus, few data exist to support the notion that casein, whey, or ISP is a superior protein source for athletes and other active individuals. Indeed, each protein source has unique attributes that may convey specific nutritional advantages compared to the others for gaining lean body mass and enhancing muscle recovery after exercise, including (1) an ability to stimulate skeletal muscle protein synthesis, which is related to different digestion rates, (2) divergent amino acid profiles, and (3) the presence of naturally occurring antioxidants.

Digestion Rate Influences Skeletal Muscle Protein Synthesis and Lean Body Mass Gain

Muscle Protein Synthesis. During a typical day, a nonexercising adult eating a balanced diet maintains skeletal muscle protein mass because the cumulative rate of skeletal muscle protein synthesis (SMPS) equals the cumulative rate of skeletal muscle protein breakdown (SMPB) [4]. Hypertrophy, or an increase in skeletal muscle mass, occurs when rates of SMPS exceed those of SMPB over time. During the transition from fasted to fed states, changes in SMPS rates greatly exceed changes in SMPB rates [15–17], leading to the thought that

protein synthesis serves as the likely regulatory site associated with feeding [18].

Resistance exercise, like feeding, stimulates SMPS to a greater extent than SMPB [15,19]. Biolo and others [15] found that 3 hours following resistance exercise, SMPS rates increased 108%, whereas SMPB rates increased 51%. Phillips et al. [19] measured rates of SMPS and SMPB 3 hours after resistance exercise and reported increases of 112% and 31%, respectively. Thus, similar to feeding, protein synthesis appears to be the primary control site for regulating muscle protein balance during the recovery period following resistance exercise.

Nutritional intervention leading to an increase in muscle amino acid availability early after resistance exercise is vital for promoting muscle growth. In addition to the stimulatory effect of exercise on SMPS, blood flow, and hence, nutrient delivery to exercised muscle is elevated [15,16]. When resistance exercise is followed by increased amino acids availability, the SMPS rate is increased more than that observed with either exercise or amino acids alone [16,20–22]. Hence, an “anabolic window” appears to exist within the first 3 hours after resistance exercise. This helps to explain why weight training combined with feeding protein during the early hours after exercise leads to significant increases in lean body mass compared to delaying the same feeding [23,24] and why the failure to increase muscle amino acid availability during recovery leads to muscle protein loss [19] and no lean body mass gain [23].

Digestion Rates. Whey protein, casein, and ISP appear to have different digestion rates. This may affect the ability of a postexercise protein feeding to promote lean body mass gain since the protein digestion rate is an independent factor regulating protein retention [25]. Whey protein has been referred to as a “fast” protein because it is rapidly digested and leads to a large, albeit temporary, rise in plasma amino acid levels [26]. Casein has been cited as a “slow” protein since the modest increase in plasma amino acid concentrations is more gradual and prolonged [26]. While within-study time course data are lacking for plasma amino acid responses after ingestion of ISP and whey protein, other data indicate that plasma amino acids peak later (150 minutes versus 75 minutes) and remain elevated longer after ISP ingestion than whey protein ingestion [27,28]. Thus, these data indicate that ISP is more of an “intermediate” protein in terms of digestion rate, based on plasma peak amino acid concentrations.

The rapid rise in plasma amino acid concentrations following ingestion of a “fast” protein stimulates amino acid oxidation [25,26]. Whey proteins are rapidly oxidized, causing protein balance to be lower when measured over several hours following ingestion compared to the more slowly digested casein [25,26]. Bos and others [27] showed that ISP is digested

Table 1. Comparison of Protein Sources for Lean Body Mass Gain

Author	Reference	Study Duration (wk)	Lean Body Mass Gain (kg)	Significantly Different ($p \leq 0.05$)
Brown et al., 2004	[10]	9	Whey protein (+1.3) ISP (+1.2)	No
Candow et al., 2006	[11]	6	Whey protein (+2.5) ISP (+1.7)	No
Kalman et al., 2007	[34]	12	Whey protein (+0.5) ISP (+0.5)	No
Hartman et al., 2004	[35]	12	Skim milk (+3.2) ISP (+2.9)	No
Cribb et al., 2006	[36]	10	Whey protein (+5.0) Casein (+0.8)	Yes

at a rate somewhat faster than milk protein (~80% casein; ~20% whey protein), but without the rapid plasma amino acid increase typical after whey protein ingestion. Nonetheless, amino acid oxidation, as indicative of greater transfer of dietary nitrogen into urea, was greater after ISP ingestion than milk protein ingestion because of the high casein content.

“Fast” and “slow” proteins also differentially affect SMPS and SMPB. Both ISP and whey protein similarly increase SMPS rates when fed immediately after exercise [29], indicating that ISP acts similarly to a “fast” protein in its ability to stimulate SMPS. Casein ingestion does not increase protein synthesis, but ameliorates protein breakdown [26].

Wilkinson and colleagues [30] hypothesized that combining “fast” and “slow” proteins would lead to a greater anabolic environment compared to a single protein source. This group showed that subjects consuming skim milk powder (~80% casein; ~20% whey protein) immediately after exercise had a greater rate of SMPS over 3 hours of recovery compared to subjects who consumed ISP. No treatment differences occurred after exercise for intramuscular essential amino acid concentrations, including leucine, the key amino acid regulating SMPS [20,31,32]. The researchers suggested that differences in essential amino acids content of the protein sources were not responsible for the different SMPS rates. This is not surprising considering that ISP, whey protein, and casein are all nutritionally complete proteins, each having a Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1.00 [33]. They did, however, conclude that differences in digestion rates of the whey protein and casein fractions in the skim milk powder and the ISP affected the pattern of amino acid appearance, ultimately leading to differences in SMPS rates.

Although muscle biopsies were taken 1, 2, and 3 hours after exercise, only SMPS rates at 3 hours were reported [30]. As others have shown that whey protein and ISP ingested immediately after exercise equally stimulate SMPS rates 1 hour after exercise [29], it is highly likely that the predominant protein in skim milk powder, the “slow” protein, casein, was the differentiating factor between the skim milk powder and ISP. Results from the Wilkinson study [30] clearly indicate that ingesting a combination of “fast” and “slow” proteins that initiate SMPS and prolong amino acid delivery to tissues is superior to ingesting a single protein source. Whether a combination of an “intermediate” protein like ISP with

whey protein and casein might improve upon a blend of “fast” and “slow” proteins requires additional research.

Lean Body Mass. Although resistance exercise studies directly comparing ISP, whey protein, or casein ingestion for lean body mass gain are lacking, data are available comparing one protein source to another. Several studies failed to show any significant difference between subjects consuming ISP or whey protein while undertaking a weight training program (Table 1) [10,11,34]; a similar result occurred in a comparison between milk protein and ISP [35]. In contrast, whey protein consumption appears to increase lean body mass during weight training compared to casein [36].

A MEDLINE search revealed no published reports of resistance training studies comparing ISP and casein ingestion for lean body mass gain. However, skim milk powder (the combination of whey protein and casein) ingestion after exercise, in addition to increasing SMPS [30], appears to add more lean body mass than ISP consumed as a single protein source [37]. Additionally, 40 g whey protein blended with 8 g casein consumed daily during a 10-week resistance training program enhanced lean body mass gain compared to 40 g of whey protein with 3 g of branched-chain amino acids (BCAAs) and 5 g of glutamine [38]. Because amino acids are more rapidly absorbed than whey protein after ingestion [25], these data indicate that the strategy of blending “fast” and “slow” proteins also adds more muscle mass than solely consuming “fast” proteins (i.e., the whey protein/amino acid blend).

In summary, ISP ingestion promotes muscle growth equal to that shown with whey protein, whereas whey protein outperforms casein. The combination of “fast” whey protein and “slow” casein is more anabolic than ISP alone or a combination of whey protein and amino acids. The latter findings indicate that protein combinations with a range of digestion rates produce superior gains in muscle mass compared with a single protein source or blends of protein with similar digestion rates.

Individual Amino Acids

Lay press and Internet sites covering sports nutrition often focus on 5 amino acids: the BCAAs leucine, isoleucine, and valine, as well as glutamine and arginine. Each of these amino

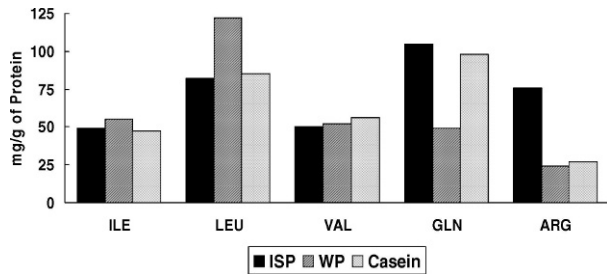


Fig. 1. Concentration of isoleucine (ILE), leucine (LEU), valine (VAL), glutamine (GLN), and arginine (ARG) in ISP, whey protein isolate (WP), and calcium caseinate (Casein). Values are expressed as grams per 100 g of protein and represent manufacturer's data and unpublished data (GLN only).

acids plays a critical role in the body during sports performance.

Branched-Chain Amino Acids. The BCAAs (leucine, isoleucine, and valine) are oxidized by muscle during exercise to provide energy once short-term glycogen stores are expended [39,40]. Wagenmakers [40] notes that only leucine and the leucine structure of isoleucine are actually oxidized by muscle; however, valine metabolism also provides important tricarboxylic acid cycle intermediates that help meet the increased energy demand for exercise.

Utilization of BCAAs as an energy source is related more with endurance exercise, but leucine, and its ability to stimulate SMPS, is of real interest to strength athletes. Although the topic is beyond the scope of this paper, others have detailed the role of leucine as a regulator of SMPS [20,41]. To briefly summarize, leucine activates the mammalian target of rapamycin (mTOR) signaling pathway that turns on the translational machinery essential for SMPS in both rodent and human muscle [41–43]. Resistance exercise similarly stimulates SMPS through the mTOR pathway [44,45], which may in part explain the synergistic stimulation of SMPS that is observed when resistance exercise is coupled with increased amino acid availability [16].

Whey protein has higher leucine and total BCAA concentrations [unpublished manufacturer's data] than casein and ISP (Fig. 1). These data support the notion that digestion rate is a bigger determinant of the anabolic response to ingestion of these proteins after exercise since milk protein and ISP ingestion yield similar intramuscular leucine concentrations [30] and whey protein and ISP elicit similar SMPS rates [29].

Glutamine and Arginine. Glutamine and arginine are considered to be conditionally essential amino acids as they are rate-limiting for protein synthesis under certain conditions [46]. All forms of stress, including exercise, significantly deplete plasma and muscle glutamine levels [46,47]. Arginine

becomes essential under a variety of catabolic conditions and when growth is accelerated [46]. ISP and casein contain more glutamine [unpublished data] than whey protein does, whereas ISP supplies 3 times more arginine than either dairy protein (Fig. 1) [unpublished manufacturer's data].

Muscle glutamine concentrations are directly correlated with SMPS rates [48], suggesting that glutamine plays a role in regulating muscle protein balance. Glutamine is the most important amino acid for kidney ammonia and bicarbonate production, and thus is important for maintaining acid-base balance [49,50]. Oral administration of glutamine increases plasma bicarbonate concentrations and enhances renal acid secretion [50,51]. Although elevated plasma bicarbonate levels (from bicarbonate feeding) can improve exercise performance by buffering against blood acidosis from lactic acid production during supramaximal exercise [52], supplemental glutamine has not had this effect [53]. Whether a similar amount of glutamine delivered in intact protein versus as an amino acid would attenuate blood acidosis requires further study.

Glutamine ingestion is accompanied by an increase in growth hormone release [51], which may be of particular interest to strength athletes, given the anabolic effects of growth hormones. Ninety minutes after ingesting only 2 g of glutamine, plasma growth hormone concentrations increased 4.3-fold. It is suggested that glutamine indirectly affects growth hormone secretion through initial conversion to citrulline in the small intestine [46]. Recent work indicates that 83% of circulating plasma citrulline comes from glutamine [54]. In the kidney, citrulline can be converted to arginine. In fact, 64% of arginine derived from *de novo* synthesis comes from citrulline generated via glutamine [54].

Arginine is a known stimulus for growth hormone release [55]. Oral ingestion of 5–9 g of arginine increases resting growth hormone levels by at least 100% [56]. Using an animal model, Tan and others [57] demonstrated that supplemental arginine increased body weight gain by 6.5% and skeletal muscle content by 5.5%, while decreasing fat content by 11%. Significant lean body mass and strength gains have been observed in men participating in a 5-week weight training program who ingested 1 g of supplemental arginine and 1 g of ornithine daily [58].

The anabolic response associated with arginine ingestion may be an indirect effect exerted through stimulation of nitric oxide (NO) synthesis and a resulting increase in muscle blood flow [59]. An increase in muscle blood flow would supply a higher proportion of amino acids to the muscle, provided an exogenous supply was present. Importantly, an augmented muscle blood flow without an attendant increase in plasma amino acid concentrations decreases, rather than increases, SMPS rates [59]. Alone, arginine supplementation does not appear to affect SMPS rates, but when taken with other amino acids or as part of a meal, arginine effectively stimulates SMPS [59]. Further

research is need to understand whether arginine supplementation as an amino acid or within a protein matrix would augment muscle blood flow during the early hours after resistance exercise when muscle blood flow is already elevated.

Both glutamine and arginine play a role in immunity. Glutamine is utilized at a high rate by cells of the immune system, including neutrophils, lymphocytes, and macrophages, and is a precursor of glutathione, an antioxidant that protects cells from free radical damage [46]. Plasma glutamine concentrations are depressed in overtrained or chronically fatigued athletes compared to healthy, trained athletes and nonathletes [60]. Overtrained athletes exhibiting depressed plasma glutamine concentrations would be predicted to have impaired immune function, but data supporting this theory are lacking [47].

Arginine plays a role in T-cell-mediated immune responses and rapidly normalizes T-cell function in postoperative patients [46,61]. Arginine supplementation has been suggested as a strategy to boost the immune system to enhance recovery after exercise and improve training by reducing the risk of overtraining, but as with glutamine, convincing data are absent.

Glutamine and arginine are clearly important amino acids for individuals looking to add muscle mass. Their ability to stimulate growth hormone secretion and protein synthesis, whether direct or via an NO-mediated increase in muscle blood flow, is well documented. Other benefits, including buffering against lactic acid build-up and enhanced immune function, lack such support but present interesting areas for further research.

Naturally Occurring Antioxidants

ISP contains naturally occurring compounds, including isoflavones and saponins that possess antioxidant activity [62–65], as well as copper, which is a component of various antioxidant enzymes [66]. Providing antioxidant protection might help to minimize exercise-induced free radical damage to muscle, thus aiding the recovery process. Short-term free radical production during exercise may affect fatigue and muscle injury, but long-term negative effects on health are more concerning [67].

The relationship between free radical generation, lipid peroxidation, and exercise-induced muscle damage has been known for over 20 years [68,69], and antioxidants, alone or in combination, have been employed by physically active subjects in an effort to minimize exercise-induced tissue damage for at least that long. Several studies show that when men and women participating in weight training programs consume supplemental ISP, antioxidant protection is significantly greater compared to when they consume whey protein [10,67,70]. In one 9-week trial [10], subjects displayed an

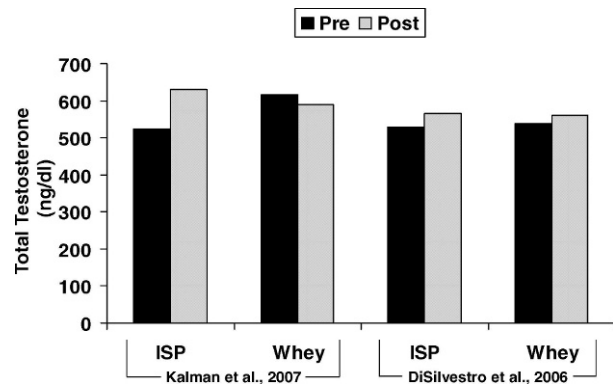


Fig. 2. Comparison of circulating total testosterone concentrations reported as ng/dl in active males consuming either whey protein or isolated soy protein. Subjects in the Kalman study [34] participated in a 12-week resistance training program while supplementing 50 g/d of either whey protein or ISP. Total testosterone was measured in serum. Subjects in the DiSilvestro study [74] were highly active college males aged 18–30 years who consumed 40 g/70 kg BW/d of either whey protein or isolated soy protein for 4 weeks. Total testosterone was measured in plasma. No significant differences were found in either study for within-group or between-group changes from baseline.

increase in markers that measure antioxidant status and a decrease in markers of oxidative stress while consuming ISP relative to whey protein. Endurance athletes show similar effects. Rossi et al. [71] supplemented the diet of healthy, college-aged males with 40 g of soy or whey protein for 3 weeks following strenuous exercise and reported a decrease in myeloperoxidase, a marker indicative of oxidative stress.

When considered together, these findings suggest that physically active individuals who consume ISP may be afforded a protective advantage against the muscle damage and muscle soreness associated with physical activity. More recent data from Fanti et al. [72] indicating that isoflavones possess strong anti-inflammatory potential lend further credence to these assertions. To date, however, no studies have looked directly at the impact of ISP on exercise-induced free radical generation, muscle damage, and inflammation, suggesting a fertile area for additional research.

Hormonal Responses

Although some athletes and the general public have shied away from soy products because of purported effects on hormonal balance, research overwhelmingly shows no negative impact of ISP consumption on hormone levels in men or women [73]. Results from 2 studies that specifically looked at circulating testosterone concentrations in highly active males are presented in Fig. 2 [34,74]. After 12 weeks of intense weight training, Kalman and others [34] found no significant differences between or within groups for serum concentrations of free or total testosterone and serum hormone binding globulin in subjects who consumed either 50 g of supplemental

	Complete Protein	Digestion Rate	Adds Lean Body Mass	High in Leucine	High in Glutamine	High in Arginine	Anti-oxidant Activity
Soy Protein Isolate	✓	Intermediate	✓		✓	✓	✓
Whey Protein	✓	Fast	✓	✓			
Casein	✓	Slow			✓		
Combination	✓	Prolonged	✓	✓	✓	✓	✓

Fig. 3. Prospective benefits derived from blending ISP, whey protein, and casein based on their unique attributes. Each is a high-quality, complete protein. Based on digestion rates and peak plasma total amino acid concentrations, whey protein is considered a “fast” protein, ISP an “intermediate” protein, and casein a “slow” protein. Data directly comparing one protein source to another indicates that whey protein and ISP ingestion produce similar lean body mass increases, but that whey protein is superior to casein. Whey protein contains a greater concentration of leucine than casein or ISP, but casein and ISP contain more glutamine. ISP contains 3 times as much arginine as either whey protein or casein and is the only one of the 3 proteins to possess antioxidant activity.

ISP (delivering ~83 mg total isoflavones) or whey protein. Similar results were reported by DiSilvestro et al. [74] in highly active college-aged men (aged 18–30 years). Plasma total testosterone concentrations were unchanged after 4 weeks, while subjects consumed either 40 g/70 kg body weight (BW) whey protein or ISP that contained 164 mg total isoflavones/70 kg BW. These data add to the plethora of studies showing that ISP does not depress testosterone levels in men and corroborates the conclusion reached by a National Institutes of Health Expert Panel, which states: “In diet intervention trials, no significant association was observed between soy product exposure and circulating testosterone or 17-β estradiol levels.” [75].

CONCLUSION—RATIONALE FOR PROTEIN BLENDS

In review (Fig. 3), it can be stated that:

- ISP, casein, and whey protein are equal in protein quality, each with a PDCAAS of 1.00.
- Whey protein is rapidly digested, whereas casein is slowly digested.
- ISP ingestion delays peak blood total amino acid concentration compared to whey protein, but is more rapidly digested than casein.
- Rapidly digested proteins quickly stimulate SMPS, but increase amino acid oxidation.
- Protein digestion rates influence protein retention.

- Casein and whey protein contain higher BCAA concentrations compared to ISP.
- ISP contains higher glutamine and arginine concentrations compared to casein and whey protein.
- ISP contains bioactive compounds with antioxidant activity.
- ISP ingestion does not affect circulating or free testosterone concentrations in men.

Even though rapidly digested proteins may stimulate SMPS, the associated high amino acid oxidation rates negatively affect protein retention over time [25,26]. This finding is one driver for sports nutrition manufacturers to use blends of proteins with different digestion rates as opposed to a single protein source. Whey protein, with its high leucine concentration, quickly delivers leucine to muscles and stimulates SMPS. However, the rapid increase in blood leucine concentration triggers leucine oxidation, slowing delivery of leucine and other amino acids to muscle. ISP similarly stimulates protein synthesis but appears to be digested slightly slower than whey protein. As casein is digested and absorbed, amino acid oxidation rates are attenuated and delivery of amino acids to muscle is prolonged.

The timed release of amino acids into the circulation is associated with greater SMPS rates and lean body mass gains [30,37,38]. These authors attributed the benefits of the protein blend to differences in digestion rates that prolonged the increase in blood amino acid levels, resulting in greater muscle amino acid uptake.

Blending ISP, casein, and whey protein also creates a more balanced amino acid profile, specifically for BCAAs, glutamine, and arginine. This may confer an advantage because a more balanced amino acid profile might provide for a wider range of benefits (e.g., acid-base balance, growth hormone release, enhanced muscle blood flow, immunity) than a single protein source rich in only 1 or 2 of these key amino acids.

The Optimal Blend

Because ISP, casein, and whey protein each has a PDCAAS of 1.00 [33], any combination of the 3 will be a nutritionally “complete protein.” The optimal formulation for a blend of ISP, casein, and whey protein should provide enough whey protein to stimulate SMPS without causing high levels of amino acid oxidation. ISP may help to bridge the appearance of amino acids between the time of digestion of the whey protein and casein. The exact ratio of protein sources to accomplish this scenario has not been reported. Moreover, the possibility exists that endurance athletes, because of their specific needs, would benefit from a different protein blend than the blend used by strength athletes. With so many potential benefits for an incredibly dedicated consumer base, protein blends should be a fruitful area for future research.

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