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# Leucine Supplementation and Intensive Training

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## Abstract

Leucine, isoleucine and valine, the branched-chain amino acids (BCAA), make up about one-third of muscle protein. Of these, leucine has been the most thoroughly investigated because its oxidation rate is higher than that of isoleucine or valine. Leucine also stimulates protein synthesis in muscle and is closely associated with the release of gluconeogenic precursors, such as alanine, from muscle. Significant decreases in plasma or serum levels of leucine occur following aerobic (11 to 33%), anaerobic lactic (5 to 8%) and strength exercise (30%) sessions. In skeletal muscle, there is a decrease in leucine level and a reduction in glycogen stores during exhaustive aerobic exercise. Basal fasting serum leucine levels decrease by 20% during 5 weeks of speed and strength training in power-trained athletes on a daily protein intake of 1.26 g/kg bodyweight.

The leucine content of protein is assumed to vary between 5 and 10%. There are suggestions that the current recommended dietary intake of leucine be increased from 14 mg/kg bodyweight/day to a minimum of 45 mg/kg bodyweight/day for sedentary individuals, and more for those participating in intensive training in order to optimise rates of whole body protein synthesis.

Consumption of BCAA (30 to 35% leucine) before or during endurance exercise may prevent or decrease the net rate of protein degradation, may improve both mental and physical performance and may have a sparing effect on muscle glycogen degradation and depletion of muscle glycogen stores. However, leucine supplementation (200 mg/kg bodyweight) 50 minutes before anaerobic running exercise had no effect on performance. During 5 weeks of strength and speed training, leucine supplementation of 50 mg/kg bodyweight/day, supplementary to a daily protein intake of 1.26 g/kg bodyweight/day, appeared to prevent the decrease in the serum leucine levels in power-trained athletes.

According to 1 study, dietary supplementation of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB) 3 g/day to humans undertaking intensive resistance training exercise resulted in an increased deposition of fat-free mass and an accompanying increase in strength. Muscle proteolysis was also decreased with HMB, accompanied by lower plasma levels of enzymes indicating muscle damage and an average 50% decrease in plasma essential amino acid levels. Furthermore, BCAA supplementation (76% leucine) in combination with moderate energy restriction has been shown to induce significant and preferential losses of visceral adipose tissue and to allow maintenance of a high level of performance.

Caution must be paid when interpreting the limited number of studies in this area since, in many studies, leucine has been supplemented as part of a mixture of BCAA. Consequently, further research into the effects of leucine supplementation alone is needed.

The roles and contributions of amino acids in the body are various, ranging from a primary role in the synthesis of protein to the ultimate contribution of catabolism to energy and nitrogenous waste products. The role of amino acids in protein synthesis is well understood and has been extensively studied.[1,2] While much is known about the mechanism behind the incorporation of amino acids into protein, questions remain about the physiological regulation of this process, differences among tissues, and controls exerted by factors such as diet, exercise and training. Catabolism of amino acids has also been studied extensively, with the degradative pathways for most amino acids being well defined.[3] However, its physiological regulation and the relationships between this process in individual tissues within the body remain to be investigated.

Leucine, isoleucine and valine – the branched-chain amino acids (BCAA) – are essential components of the human diet, since enzymes for *de novo* synthesis of BCAA are not present in human cells.[4] Of the 20 amino acids in the body, only the BCAA, alanine, glutamate and aspartate are oxidised at a significant rate in skeletal muscle.[5] Quantitatively, alanine appears to be oxidised at the highest rate (30 to 70% higher than leucine).[5,6] As BCAA comprise about one-third of muscle protein, they can easily supply the substrate for building alanine and glutamine.[7] The study by Ahlborg et al.[8] indicated that BCAA were taken up by active skeletal muscle during submaximal aerobic exercise, whereas all other amino acids were taken up by the liver. Thus, the metabolism of BCAA is initiated in skeletal muscle rather than the liver. Of the 3 BCAA, leucine has been the most thoroughly investigated as its oxidation rate is higher than that of isoleucine or valine. Leucine can also stimulate protein synthesis in muscle[9,10] and it has been closely associated with the release of gluconeogenic precursors, such as alanine, from muscle.[11]

Recently, amino acids have become a popular nutritional supplement marketed to athletes. Some of the most popular are the BCAA as well as the various forms of leucine.[12-14] The aim of this article is to examine the role of leucine supplementation in intensive athletic training and to evaluate reports that have investigated its effects when consumed during exercise sessions or training periods. Because there is insufficient data available regarding leucine supplementation alone, caution must be taken when interpreting these data.

## 1. Structure of Leucine

As with all amino acids, the essential amino acid leucine contains a positively charged amino group ($-NH_3+$) and a negatively charged carboxyl group ($-COO^-$) attached to the same carbon atom, the α-carbon, when within the physiological pH range (pH 5.0 to 8.0). The α-carbon also has bonds with hydrogen and a side-chain group, the latter determining the identity of the particular amino acid. For leucine, the side chain is a branched nonpolar hydrocarbon group (fig. 1).

## 2. Human Amino Acids

Most amino acids in the body exist within protein structures. Only 0.5 to 1.0% of the total amino acid content of the body is present as free amino acids, in plasma or the intracellular and extracellular spaces.[2] However, the small amounts of amino acids present in the 'free pools' are responsible for the metabolic or substrate influences of all amino acids. Amino acid levels change in response to food

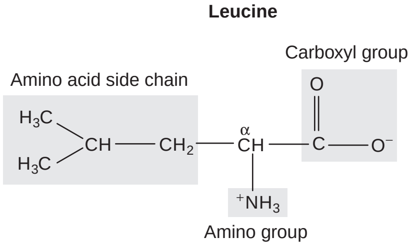

**Fig. 1.** Structure of leucine predominating at pH 7.4.

**Table I.** Fasting blood free amino acid levels in untrained individuals, power-trained athletes and endurance athletes[16-18]

| Amino acid | Untrained[a] (μmol/L) | Power-trained[b] (μmol/L) | Endurance[c] (μmol/L) |
|---|---|---|---|
| Leucine | 133 ± 39 | 183 ± 44 | 145 ± 6 |
| Taurine | 94 ± 48 | 80 ± 52 | 59 ± 4 |
| Aspartate | 7 ± 4 | 31 ± 11 | |
| Threonine | 145 ± 39 | 165 ± 34 | 112 ± 7 |
| Serine | 113 ± 30 | 133 ± 31 | 114 ± 5 |
| Asparigine | 62 ± 19 | 80 ± 28 | |
| Glutamate | 34 ± 13 | 58 ± 19 | |
| Glutamine | 656 ± 146 | 863 ± 241 | 718 ± 36 |
| Glycine | 232 ± 44 | 298 ± 66 | 222 ± 8 |
| Alanine | 360 ± 69 | 508 ± 188 | 299 ± 21 |
| Citrulline | | 55 ± 10 | |
| Valine | 264 ± 79 | 315 ± 53 | 236 ± 8 |
| Methionine | 24 ± 8 | 30 ± 7 | 27 ± 2 |
| Isoleucine | 64 ± 23 | 87 ± 13 | 72 ± 3 |
| Tyrosine | 84 ± 19 | 76 ± 24 | 58 ± 4 |
| Phenylalanine | 58 ± 14 | 73 ± 19 | 62 ± 3 |
| Ornithine | 66 ± 17 | 94 ± 26 | |
| Lysine | 192 ± 44 | 205 ± 48 | 164 ± 10 |
| Histidine | 94 ± 14 | 118 ± 30 | 73 ± 7 |
| Arginine | 94 ± 20 | 123 ± 40 | 79 ± 3 |

a Mean plasma value ± standard deviation (n = 10; 5 males and 5 females).

b Mean serum value ± standard deviation (n = 10 males).

c Mean plasma value ± standard error (n = 7 males).

intake and exercise, for example, but their levels remain within relatively narrow limits. Estimates of the size of the free amino acid pool in humans show that there is a free nonessential amino acid pool of 2.4 mmol/kg bodyweight which has an amino acid flux of 12 mmol/kg bodyweight/day and a half-life of 200 minutes.[2] However, large differences exist among the individual amino acids. For example, lysine, which has a relatively large and stable free pool, has a half-life within the free pool of approximately 10 hours, whereas leucine, which is much more metabolically active, has a half-life of 45 minutes. The amount of amino acid moving through the free pool each day is many times the actual free pool size. In humans, the total free pool is replaced approximately 6 times per day.

### 2.1 Free Amino Acids in Blood

The nonessential amino acids glutamine, alanine and glycine have the highest plasma levels in both untrained individuals and athletes. Of the essential amino acids, valine, leucine, lysine and threonine have the highest levels. Einspahr and Tharp[15] found that endurance runners had significantly higher plasma levels of leucine (41%), isoleucine (27%) and tyrosine (23%) than untrained individuals. To confirm these preliminary observations, more research is needed also using diary records of nutrition and training. Table I presents the fasting blood free amino acid levels in untrained individuals, power-trained athletes and endurance athletes.[16-18]

### 2.2 Free Amino Acids in Skeletal Muscle

The fasting amount of free amino acid in the skeletal muscle of untrained and endurance-trained males are described in table II. Resting levels of taurine, glutamine, glutamate and alanine are high in both nonathletes and endurance-trained athletes where as levels of leucine are quite low in both groups.

**Table II.** Mean (± standard error) fasting skeletal muscle free amino acid content in vastus lateralis intracellular fluid of untrained males (n = 4) and male endurance athletes (n = 7)[18,19]

| Amino acid | Untrained (µmol/L) | Endurance (µmol/kg wet weight) |
|---|---|---|
| Leucine | 0.17 ± 0.03 | 168 ± 6 |
| Taurine | 16.43 ± 1.93 | 12300 ± 1480 |
| Aspartate | 0.66 ± 0.11 | 457 ± 73 |
| Threonine | 0.90 ± 0.04 | 327 ± 16 |
| Serine | 0.99 ± 0.05 | 416 ± 16 |
| Asparigine | 0.58 ± 0.05 | |
| Glutamate | 4.25 ± 0.42 | 2210 ± 190 |
| Glutamine | 18.90 ± 1.15 | 9540 ± 870 |
| Proline | 1.13 ± 0.10 | |
| Glycine | 1.85 ± 0.15 | 861 ± 41 |
| Alanine | 2.36 ± 0.20 | 1180 ± 100 |
| Citrulline | 0.05 ± 0.02 | |
| Valine | 0.30 ± 0.03 | 247 ± 16 |
| Methionine | 0.07 ± 0.01 | 33 ± 4 |
| Isoleucine | 0.10 ± 0.01 | 92 ± 7 |
| Tyrosine | 0.09 ± 0.01 | 61 ± 4 |
| Phenylalanine | 0.07 ± 0.01 | 61 ± 3 |
| Ornithine | 0.31 ± 0.05 | |
| Lysine | 1.04 ± 0.02 | 294 ± 27 |
| Histidine | 0.47 ± 0.03 | 225 ± 38 |
| Arginine | 0.60 ± 0.03 | 159 ± 26 |

In a study of rat skeletal muscle, the total concentration 23 free amino acids was found to be twice as high in slow-twitch as in fast-twitch muscle.[20] There are no corresponding human data in the literature.

## 3. Effect of Exercise and Training on Leucine and Other Free Amino Acids

### 3.1 Effect of Exercise Bouts

#### *3.1.1 Aerobic Exercise*

It has been shown that during a 20-minute bout of exercise at 70% of maximal oxygen uptake ($\dot{V}O_{2max}$), untrained male individuals had increased plasma levels of alanine, glutamine, arginine, tyrosine and phenylalanine, whereas levels of most of the other amino acids were decreased [leucine levels decreased significantly (22%)].[19] In a later study by Blomstrand et al.,[18] endurance-trained male cyclists with reduced muscle glycogen stores showed increases in plasma levels of glutamate, taurine, tyrosine and phenylalanine during an 80-minute exhaustive exercise session. Significant decreases in plasma tryptophan and leucine levels were also observed, the latter by 11%.

Strüder et al.[21] reported that plasma levels of 11 out of 14 analysed amino acids (serine, histidine, glycine, threonine, alanine, valine, methionine, tryptophan, isoleucine, phenylalanine and leucine) decreased significantly in 8 male players during a continuous 4-hour tennis tournament; the measured decrease in leucine levels was 33% and no changes were observed for glutamate, arginine and tyrosine. In runners, plasma leucine, isoleucine, valine and alanine levels were also significantly decreased after completing a marathon.[22] Over the 24 hours after the marathon, plasma levels of these 4 amino acids recovered to pre-marathon levels in 1 runner but not in the other three, even after 48 hours. These results suggest that there is an increased uptake of leucine by the liver and/or muscle during bouts of aerobic exercise.

With respect to the amount of amino acid in skeletal muscle, a study of untrained male individuals showed that the amount of alanine and glutamine increased and that of glutamate decreased significantly during a 20-minute exercise session at 70% $\dot{V}O_{2max}$.[19] A small nonsignificant increase was observed in skeletal muscle aspartate and small nonsignificant decreases occurred in the amount of skeletal muscle glycine, asparagine, tyrosine, phenylalanine, ornithine and lysine. Skeletal muscle leucine content increased nonsignificantly by 19%.

In endurance-trained male cyclists with reduced muscle glycogen stores, there were significant increases in alanine and tyrosine and significant decreases in the amount of glutamate, valine, isoleucine and leucine (22%) in the vastus lateralis muscle following an exhaustive bout of aerobic endurance exercise.[18]

Therefore, it seems that during bouts of aerobic exercise there is no change in skeletal muscle leucine content, except in those individuals with reduced muscle glycogen stores. In these individuals, skeletal muscle leucine may be used as a fuel for muscle contraction. Skeletal muscle BCAA content has been found to remain more or less unchanged during short term aerobic exercise in muscle with normal glycogen stores.[23]

This lack of change in muscle with normal glycogen stores is notable since it has been clearly demonstrated using radioisotope studies that the oxidation of leucine is significantly increased in humans during aerobic exercise.[24] *In vitro*, a tendency for the BCAA content to be lower in stimulated muscle than control muscle has never been demonstrated.[25] This suggests the existence of a protective mechanism in muscle which maintains endogenous BCAA levels even during periods of high energy demand coinciding with an insufficient supply of external BCAA. It also suggests that the increased metabolism of BCAA in skeletal muscle during high energy demand can be achieved only when there is a supply of exogenous BCAA or reduced muscle glycogen stores.

#### *3.1.2 Anaerobic Exercise*

There is little information in the literature regarding free amino acid levels in plasma during or following anaerobic lactic exercise session. In the study by Mero et al.,[26] male sprinters and jumpers performed a track exercise consisting of 3 sets of 3 × 60-metre short runs with recovery periods of 2 minutes between runs and 6 minutes between sets. The speed of the sets was 91, 93 and 95% of the repetition maximum, respectively. A second exercise consisted of 20-second long runs on a treadmill with a recovery period of 100 seconds between runs. The initial treadmill speed (4.08 metres/second on a 4° incline) was increased by 0.38 metres/second for each consecutive run until exhaustion. Running speeds ranged from 56 to 100% of the speed of the previous run. Total running time was 92 ± 9 seconds for the short runs and 166 ± 19 seconds for the long runs. Peak blood lactate levels were higher following the long runs than following the short runs (16.4 ± 1.3 and 13.8 ± 1.9 mmol/L, respectively).

A comparison with pre-exercise values showed that after the short runs there were decreases in plasma levels of leucine (8%), tryptophan, ornithine and glycine, but increases in plasma levels of alanine (43%) and glutamate. Following the long runs, there were decreases in plasma leucine (5% nonsignificant), valine, tryptophan, threonine, ornithine, glycine, asparagine and tyrosine levels but increases in plasma alanine (34%), glutamate and taurine levels. These results indicate that there are decreases in the plasma level of most amino acids following bouts of anaerobic exercise, with that for leucine ranging from 5 to 8%. The large rise in plasma alanine level suggests that the alanine-glucose cycle is activated in anaerobic exercise.

#### *3.1.3 Strength Exercise Bouts*

Although protein and amino acid supplements are very popular among strength athletes, little data exists in the literature regarding the relationship between bouts of strength exercise and amino acid levels. One study by Mero et al.[27] reported that serum BCAA levels were significantly decreased in 9 male sprinters and jumpers who performed a bout of heavy strength exercises for 90 minutes. A similar result was observed in a later study by Mero et al.,[28] in which 16 male sprinters and jumpers also performed a bout of heavy strength exercises lasting 90 minutes. The decrease in serum BCAA level was 24% and both leucine and isoleucine levels decreased by 30%. Alanine was the only amino acid level (out of 20 measured) which, on average, increased (4%), but this rise was not significant. These data indicate that the serum levels of amino acids are lowered following a session of strength exercises, with particularly large decreases occurring for leucine and isoleucine.

### 3.2 Effect of Training Period

As discussed in section 2, there is evidence that endurance-trained individuals may have higher basal skeletal muscle and plasma amino acid levels than untrained individuals.[15,23] Both training and nutrition affect the basal levels of amino acids. Ten adult male sprinters and jumpers were studied over a 5-week training programme (sprinting, jumping exercises and weight training) training 6 days per week (approximately 12 hours total per week).[17,29] The comparison of levels measured before and after the programme showed that the basal fasting level of total serum amino acids (21 amino acids) decreased by 19%. Significant decreases were noticed in levels of 12 amino acids – leucine decreased by 20%, isoleucine by 21% and valine by 18%. These results indicate that with a daily protein intake of 1.26 g/kg bodyweight, serum levels of amino acids are considerably lowered during a period of intensive training. This suggests that with a daily protein intake of 1.26 g/kg bodyweight, whole body protein synthesis during intensive training may not be optimal. This conclusion is consistent with the recommended daily intake of protein for strength athletes of 1.5 to 2.0 g/kg bodyweight.[30]

## 4. Leucine Supplementation

### 4.1 Leucine Requirement and Turnover

Leucine is primarily used by the body for protein synthesis but it also acts as a muscle energy substrate, providing 3 to 4% of muscle energy at rest and 1% during exercise.[31] Young and Bier[32] have suggested that the recommended dietary intake (RDI) for leucine for the general population should be increased from 14 to 30 mg/kg bodyweight/day to optimise rates of whole body protein synthesis. Data presented in the review by Hood and Terjung[31] indicate that a minimum 3- to 4-fold increase in the RDI for leucine should be adopted (a minimum of 45 mg/kg bodyweight/day) even for sedentary individuals. Consequently, the RDI for leucine should be increased in persons who are regularly physically active. Assuming that the leucine content of protein varies from 5 to 10%,[33,34] the proposed higher RDI for leucine could be met by an average protein intake of 0.45 to 0.90 g/kg bodyweight/day. Thus, viewed solely from the point of view of leucine, the recommended protein intake of 0.8 g/kg bodyweight/day for adult sedentary men[35] is inadequate to meet the leucine requirement, depending on the type of protein habitually ingested. For active individuals, it generally appears that a protein intake of 1.4 to 1.7 g/kg bodyweight/day should be adequate.[36] Strength athletes should consume about 1.5 to 2.0 g/kg bodyweight/day of protein.[30] Consequently, this means that the RDI for leucine should be greatly increased from the recommended values for sedentary people.

Hagg et al.[37] used an intravenous, constant-rate infusion of a trace amount of leucine in sedentary individuals and found that oxidation of leucine as an energy source increases during exercise whereas utilisation of this amino acid as a substrate for protein synthesis decreases. Using a similar infusion technique, Lamont et al.[38] observed that endurance training results in increased leucine and/or protein turnover, which may contribute to the increased resting energy expenditure. Phillips et al.[39] also employed a similar study method and concluded that higher leucine oxidation in male endurance-trained athletes at rest and during exercise could result in their having an even higher protein requirement than female endurance-trained athletes. A 3.5-day fast has also been found to increase leucine oxidation during low intensity exercise.[40]

Leucine infusion (270 μmol/min), performed in male volunteers at rest over 150 minutes (during which time whole blood leucine levels rose by about 6-fold), was accompanied by significant decreases in whole blood levels of tyrosine (35%), phenylalanine (35%), methionine (50%), valine (40%) and isoleucine (55%).[41] Arterial glucose levels fell slightly (5%) and insulin levels increased by 20% during the infusion. Infusion of a mixture of BCAA (70% leucine, 20% valine and 10% isoleucine; 300 μmol/min over 150 minutes) resulted in marked decreases in levels of tyrosine (50%), phenylalanine (50%) and methionine (35%). The decreased amino acid levels remained low for 2 hours after the end of the infusion. These investigators concluded that infusion of a mixture of BCAA gives results similar to those obtained with leucine infusion alone.[41] They speculated that the reason for this may be due to either a role of leucine in stimulating net skeletal muscle protein synthesis or the same amino acid transport system (the *l*-system) for the amino acids which were most influenced by the leucine infusion.[42,43] Leucine has the strongest role in the *l*-system, increasing its own intracellular levels and thus stimulating cellular uptake of the other amino acids transported by the *l*-system.

Alvestrand et al.[44] studied the influence of leucine infusion on intracellular free amino acid pools in individuals at rest. A continuous intravenous infusion of *l*-leucine (300 μmol/min) was administered to 12 healthy females over 150 minutes. During infusion, plasma leucine levels rose 6-fold. Approximately 40% of the infused amount was taken up by skeletal muscle. It was concluded that approximately 40% of the leucine taken up by skeletal muscle accumulated in the intracellular free pool, some 20% may have been incorporated into protein and 40% was probably oxidised.

### 4.2 Leucine Supplementation in Aerobic Exercise and Training

In a study by Blomstrand and Newsholme,[45] a mixture of BCAA was consumed by well-trained endurance athletes during 2 types of sustained, intensive running exercise; a 30km cross-country race and a full marathon. The total amount of BCAA given to each athlete was 7.5g (leucine 35%, valine 50% and isoleucine 15%) during the cross-country race and 12g (leucine 35%, valine 40% and isoleucine 25%) in the marathon. When BCAA were taken during exercise, the plasma levels and vastus lateralis concentrations of these amino acids increased, while in the placebo groups the levels of BCAA decreased in plasma and remained unchanged in the muscle. In the placebo group, both types of exercise caused a 20 to 40% increase in the amount of the aromatic amino acids tyrosine and phenylalanine in the muscle, and the plasma levels of these amino acids were increased after the marathon. Since tyrosine and phenylalanine are neither taken up nor metabolised by skeletal muscle, the increased amount of them in muscle might indicate net protein degradation during exercise. These results suggest that intake of BCAA during exercise may slightly prevent or decrease the net rate of protein degradation caused by intense endurance exercise.

In another study,[46] a mixture of BCAA (7.5g in a 5% carbohydrate solution; leucine 35%, valine 50% and isoleucine 15%) was consumed by participants during a 30km cross-country race. With the BCAA supplement, post-race mental performance was improved compared with that for the placebo group. When BCAA (16g in plain water; leucine 30%, valine 50% and isoleucine 20%; carbohydrate drinks were also allowed) were consumed by participants during a marathon, running performance was improved for the slower runners (3.05 to 3.30 hours). The results show that both mental and physical performance can be improved by intake of BCAA during endurance exercise.

To investigate whether a pre-exercise intravenous infusion of BCAA modifies exercise performance during incremental endurance exercise when muscle glycogen is reduced, a mixed solution of BCAA [300 mg/kg bodyweight (leucine 15.7 g/L, valine 12.0 g/L and isoleucine 12.9 g/L in 500ml of saline), infusion rate 260 mg/kg/h] was administered 70 minutes before exercise to exhaustion.[47] The investigators summarised that in conditions of reduced muscle glycogen content, after a short period of fasting (20 to 21 hours), BCAA infusion had no significant effect on the total work that could be performed during a graded incremental exercise.

In another study, 7 male endurance-trained cyclists with reduced muscle glycogen stores performed sustained exhaustive endurance exercise.[18] Before and during exercise, the cyclists were supplied with an aqueous solution of BCAA (7 g/L; leucine 35%, valine 40% and isoleucine 25%). The amount of BCAA consumed was 90 mg/kg bodyweight. Ingestion of BCAA caused the levels of these amino acids during exercise to increase by 135% in plasma and 57% in muscle tissue, whereas in the placebo trial there was no change or a slight decrease in plasma levels and a decrease of the amount of BCAA in muscle of 18%. Plasma levels of alanine increased by 48% during exercise when BCAA were ingested, and the increase in muscle alanine content during exercise was greater (70% *vs* 31% in the placebo trial), suggesting an increased rate of alanine production with BCAA ingestion. There was a significant decrease in muscle glycogen content during exercise in the placebo trial, whereas only a small decrease was observed in the BCAA trial [28 and 9 mmol/kg wet weight ($p < 0.05$) in the placebo and BCAA trials, respectively]. This might indicate that an increased supply of BCAA has a sparing effect on muscle glycogen degradation during exercise by individuals with reduced muscle glycogen stores. The probable mechanism for this is the activation of the alanine-glucose cycle.

### 4.3 Leucine Supplementation in Anaerobic Exercise and Training

The influence of leucine supplementation on serum amino acid levels and anaerobic running performance has been examined in trained male sprinters.[48] The sprinters performed 2 maximal anaerobic running tests on a treadmill (n × 20 seconds with a recovery of 100 seconds between the runs) until exhaustion at an interval of 7 days. The sprinters were randomised and consumed drinks containing leucine 200 mg/kg bodyweight or placebo 50 minutes before the tests. Leucine administration significantly increased the total serum levels of BCAA at both 10 minutes before the test and 10 minutes after the test. The serum levels of leucine were higher in the leucine than the placebo group in both before and after samples. The levels of isoleucine and valine in the pretest samples were similar in both groups but were decreased in post-test samples in the leucine group. Running speed and peak blood lactate levels were the same in the leucine and placebo groups (7.66 ± 0.28 m/sec, 20.7 ± 2.2 mmol/L and 7.63 ± 0.29 m/sec, 20.7 ± 2.4 mmol/L, respectively). These results indicate that leucine supplementation diminishes the serum isoleucine and valine levels during anaerobic running exercise but has no effect on performance. The reason for the decreased serum levels of isoleucine and valine may be the use of the same skeletal muscle amino acid transport system by the 3 BCAA;[42,43] hence, leucine stimulates cellular uptake of the amino acids with the same transport system.

Effects of a low dose amino acid supplement on adaptations to cycling training in untrained individuals were investigated in the study by Vukovich et al.[49] Participants in the experimental group received 2.9 g/day of an amino acid supplement (leucine 1132mg, isoleucine 832mg, valine 832mg, glutamine 67mg and carnitine 33mg). After 7 days of receiving the supplement there were no differences in cycling performance compared with the control group. The participants then embarked on 6 weeks of combined aerobic and anaerobic training on a cycle ergometer. Amino acid supplementation had no effect on either blood or muscle lactate accumulation during exercise but supplementation resulted in a faster adaptation in buffer capacity. Buffer capacity was calculated as the change in muscle lactate divided by the change in muscle pH. Performance during intensive exercise was not improved with amino acid supplementation. BCAA supplementation may have produced a protein-sparing effect in the experimental group, which would account for the greater buffer capacity as it has been shown that muscle proteins partially account for the increased buffer capacity associated with training.[50]

### 4.4 Leucine Supplementation in Strength Exercise and Training

To investigate the effects of leucine supplementation on amino acid profile during strength and speed training, 20 adult male track and field power athletes participated in a randomised, double-blind, crossover study during 10 weeks of training.[17] The athletes received leucine 50.0 ± 3.3 mg/kg bodyweight/day or placebo tablets. Measurements of amino acid levels were made before, in the middle of and after the 10-week training period. Blood samples were taken in the morning after 10 hours of fasting.

Serum leucine levels decreased by 20% in the placebo group during the first 5 weeks but not during the second 5 weeks (i.e. it stayed at the 5-week level). There were no changes in the serum leucine levels in the supplementation group. The total serum amino acid pool decreased in all athletes by 21%

during the 10-week training period. The decrease mostly occurred during the first 5 weeks. These results indicate that with a daily protein intake of 1.26 g/kg bodyweight, serum levels of amino acids are lowered considerably during intensive strength training. Supplementation with leucine 50 mg/kg bodyweight/day appears to prevent the decrease in the serum leucine levels seen during intensive training. This supports the estimate made by Golgan[12] that the daily leucine requirement for athletes in intensive training (at least 3 hours per day) is 60 mg/kg bodyweight. The respective estimations for valine and isoleucine were 50 and 20 mg/kg bodyweight/day.[12]

α-Ketoisocaproic acid (KIC) is the product of the transamination of leucine, and the first step in the complete degradation of leucine. Skeletal muscle has been demonstrated to be an important site of leucine degradation in humans.[51] Once KIC has been formed in skeletal muscle, it can be reaminated to form leucine,[52] completely oxidised in skeletal muscle[53] or released and decarboxylated within other tissues.[51]

Fielding et al.[54] observed that muscle KIC levels clearly increased but plasma KIC levels did not change during an intensive endurance exercise to exhaustion. Following this bout of exercise, plasma KIC levels rose significantly, with peak values occurring 15 minutes after exercise, and returned to pre-exercise values after 60 minutes of recovery. It was concluded that during intense exercise, leucine transamination in muscle may continue at a faster rate than the decarboxylation of KIC. In addition, plasma levels of KIC did not reflect the intracellular accumulation of KIC during exercise, suggesting a delay in the diffusion of KIC from muscle.

Nissen et al.[14] studied the effect of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB) on muscle metabolism during resistance exercise training. HMB is produced from KIC by the enzyme KIC dioxygenase and, at least in the pig, HMB is produced exclusively from leucine.[55] In the first study,[14] volunteers were randomised to 3 levels of HMB supplementation (0, 1.5 or 3.0 g HMB/day) and 2 protein intakes (normal, 117 g/day; or high, 175 g/day). The volunteers lifted weights for 1.5 hours on 3 days per week for 3 weeks. In the second study,[14] volunteers received either 0 or 3.0 g HMB/day and lifted weights for 2 to 3 hours on 6 days per week for 7 weeks.

In the first study,[14] HMB significantly decreased the exercise-induced rise in muscle proteolysis, as measured by urinary 3-methylhistidine levels, during the first 2 weeks of exercise. Plasma creatine phosphokinase levels were also decreased with HMB supplementation (week 3). No significant protein intake/HMB interactions were noted relative to any of the reported measures, suggesting that the effects of HMB on metabolism are additional to and independent of protein intakes. The weight lifted was higher in the HMB supplementation group compared with the nonsupplemented individuals for every week of the study. In the second study,[14] fat-free mass was significantly increased in HMB-supplemented individuals compared with the nonsupplemented group at 2 and 4 to 5 weeks of the study. The authors summarised that dietary supplementation with 3 g HMB/day to individuals participating in intensive resistance exercise training resulted in a significantly increased deposition of fat-free mass and an accompanying significant increase in strength.[14] Muscle proteolysis was also decreased with HMB supplementation, and this was accompanied by lower plasma levels of enzymes indicating muscle damage and an average 50% decrease in the plasma levels of essential amino acids. The mechanism by which HMB affects muscle proteolysis and function is currently unknown.

Mourier et al.[56] studied competitive wrestlers who restricted their energy intake (28 kcal/kg bodyweight/day) for 19 days, using 4 various diets, to determine the effects of caloric restriction on body composition and performance versus a control diet. A significantly greater loss of bodyweight (–4 kg) and decrease in percent body fat (–17%) was observed for members of the hypocaloric, high-BCAA group. This group followed a hypocaloric diet (24.4 kcal/kg bodyweight/day) comprising approximately protein 20%, carbohydrate 60% and fat 20%, with a protein dietary supplement enriched with BCAA 0.9 g/kg bodyweight/day (51.9 g BCAA/100 g protein: leucine 76%, isoleucine 19% and valine 5%). The control group diet was 40 kcal/kg bodyweight/day (carbohydrates 55%, proteins 12% and fat 33%). Individuals in the BCAA group exhibited a significant reduction (–34%) in abdominal visceral adipose tissue versus the control group. There were no changes in aerobic and anaerobic capacities or in muscular strength in the BCAA group. The authors concluded that, under the experimental conditions, the combination of moderate energy restriction and BCAA supplementation induced significant and preferential losses of visceral adipose tissue and allowed maintenance of a high level of performance.[56]

## 5. Conclusions

Significant decreases in plasma or serum levels of leucine occur following various exercise sessions. In skeletal muscle, there is no change in the leucine content during an exhaustive aerobic exercise session, except in individuals with reduced muscle glycogen stores in which case there is a decrease in leucine content. With a daily protein intake of 1.26 g/kg bodyweight, the basal fasting level of serum leucine decreased over 5 weeks of speed and strength training. Assuming that the leucine content of protein varies from 5 to 10%, there are some suggestions that, to optimise rates of whole body protein synthesis, the RDI for leucine should be increased from the current recommendation of 14 mg/kg bodyweight/day to a minimum of 45 mg/kg bodyweight/day for sedentary individuals and to a yet higher amount for those participating in intensive training. Supplementation of leucine (30 to 35% leucine in a mixture of BCAA) may prevent or decrease skeletal muscle protein degradation, improve both mental and physical performance and have a sparing effect on muscle glycogen degradation during endurance exercise with reduced muscle glycogen stores. BCAA supplementation (76% leucine) also increases the loss of visceral adipose tissue in conditions of moderate energy restriction and can allow maintenance of a high level of performance. According to one study, a dietary supplementation of the leucine metabolite HMB 3g/day during intensive resistance exercise training results in an increased deposition of fat-free mass, an increase in strength, a decrease in muscle proteolysis, lowered plasma levels of enzymes indicating muscle damage and a decrease of plasma levels of essential amino acids.

It is worth remembering, however, that these conclusions are based on limited research and that leucine has been administered as part of a mixture of BCAA in many of the studies described. Therefore, further research into the effects of leucine alone is needed. It is also important to record details of training and nutrition, and to obtain direct measures of changes in muscle mass and strength, together with levels of leucine metabolites.

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