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The Effect of Silk Amino Acid Supplementation on the Level of Blood Energy Substrates and Hormones during Prolonged Exercise

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ABSTRACT

The silk amino acid supplementation is unknown to affect the release of several hormones related to energy production and metabolism during prolonged exercise. This study examined the effects of silk amino acid supplementation on the level of blood amino acid, energy substrates and hormones level during prolonged treadmill exercise in college taekwondo player. A prolonged treadmill test was carried out 60 min at 65% of maximal heart rate on 8 athletics. Blood samples were obtained form antecubital vein of subjects at rest bed 30 minute before test, after exercise and rest 1 hour. The subjects were supplemented silk amino acid (6,390 mg/day) for 4 week. The silk amino acid supplementation did not produce significant changes on the levels of blood lactate, ammonia, amino acid, glucose, triglyceride, total cholesterol, HDL, LDL, seratonin and leptin at rest bed 30 minute before test, after exercise and rest 30 minute. The silk amino acid 4 week supplementation did not affect the levels of blood amino acid, energy substrates and hormones during prolonged treadmill exercise.

INTRODUCTION

Amino acids probably supply negligible amounts of energy in short term exercise, but can supply 5～10% of the total energy in prolonged exercise (Poortmans, 1984). This minor role as a substrate, the wide spectrum of specific proteins, their diverse half-lives, and the numerous metabolic pathways are the lack of research attention. Nevertheless, it is becoming more obvious that amino acid metabolism is an important aspect of the metabolic responses of active skeletal muscle. There is a great deal or integration between amino acid and both carbohydrate and fat metabolism, both in terms of "shared" pathways (or more specifically the end product of one pathway being incorporated into another one) and in terms of metabolic by-products of one pathway modulating the regulatory enzymes of another pathway. It becomes apparent that it is only for convenience that we divide metabolism into sections labelled fat, carbohydrate, and protein. In recent years there have been excellent general reviews (Poortmans, 1984) as well as those focusing specifically on protein catabolism (Dohm, 1986), amino acid metabolism (Cheuvront et al., 2004; Hood and Terjung, 1990; Paddon-Jones et al., 2004; Ternopolsky M, 2004; Tipton & Wolfe, 2004; Wagenmakers et al., 1990; Watson et al., 2004), and the purine nucleotide cycle (Lowenstein, 1990; Tullson and Terjung, 1990).

The ammonia and amino acids metabolism were changed by exercise intensity (Borberg & Sahlin, 1988; Graham & saltin, 1989; Sollevi & Hassen, 1988), fiber type (Borberg et al., 1988; Borberg & Sahlin, 1988) metabolism level (Borberg et al., 1988; Norman et al., 1988) and training (Lo & Dudley, 1987). Ammonia $(NH_3)^2$ is released from skeletal muscle during both intense and prolonged submaximal exercise (Broberg and Sahlin, 1989; Graham et al., 1990; Graham et al., 1991; Wagenmakers et al., 1991). The rate of NH_3 release from skeletal muscle continually increases during prolonged, submaximal exercise (Broberg and Sahlin, 1989) and is reflected by a contin-

ually increasing venous plasma NH_3 concentration (MacLean et al., 1991). The oxidation of amino acids has been suggested to be an additional source of energy for skeletal muscle during fasting (Goldberg and Odessey, 1972), low energy diets (Munro, 1951), and prolonged exercise (Brooks, 1987). Furthermore, studies have shown that amino acid uptake and oxidation by skeletal muscle increases in response to exercise (Ahlborg et al., 1974; Brooks 1987; Dohm, 1986; Hagg et al., 1982; Wolfe et al., 1982).

Amino acids liberated from active muscle are important substrates for hepatic gluconeogenesis. Bjorkman and Wahren (1988) report that during the latter phase of prolonged exercise, gluconeogenesis can be responsible for 45% of the hepatic glucose production. Similarly, Lemon and Mullin (1980) found that protein catabolism could account for more than 10% of the energy requirement dyring prolonged exercise in a low muscle glycogen state. It is possible that the catabolism of certain amino acids has an anaplerotic function for tricarboxylic cycle (TCA) intermediates and that this could be important for sustaining high energy production from the oxidative processes. In addition, carious theories link ammonia and/or amino acids to central and peripheral fatigue during exercise.

Several studies have reported that physical exercise increases the levels of monoamines, particulary 5-HT, in the brain of experimental animal (Chaouloff et al., 1989). In previous studies it was suggested that an increase in the rate of synthesis, and hence the level of 5 HT, in specific parts of the brain could contribute to fatigue, mainly central/mental fatigue but also fatigue (Blomstrand et al., 1989).

Leptin is a hormone produced by the fat cells, and is thought to play a key role in the control of body weight. Recent studies suggest a complex interrelationship between leptin and insulin or insulin resistance (Tritos & Mantzors, 1997). Leptin is hypothetically involved in a feedback loop linking fat and a satiety/energy expenditure center in the hypothalamus. This feedback loop appears to be disturbed in obesity. Recent investigations suggest that the leptin involve a complex interplay of insulin, corticosteroids, free fatty acids, and food intake factors (Cohen et al., 1996; Cusin et al., 1995; DeVos et al., 1995; Saladin et al., 1995), But little is known about the role of exercise, exercise represents the most variable fraction of energy expenditure in humans. Few studies have examined in a comprehensive fashion the effect of most metabolic syndrome components and leptin concentration on the effects of exercise and endurance training on leptin levels (Gutin et al., 1999; Haluzik et al., 1999; Hickey et al., 1996a; Hickey et al., 1996b; Korht et al., 1996; Zhang et al., 1996).

Silk protein consist of two major protein, fibroin and sericin (Makoto, 2004). Silk protein role as a immune, muscle, antioxidant, information of neurotransmitters, blood circulation, reduction of blood pressure, blood glucose, total cholesterol, and insulin secretion. Silk peptide and dietary fiber supplementation-added routine diet improved fat distribution, total cholesterol, triglyceride (Lee et al., 2003).

There is very little data available on silk amino acid supplementation. This study is one of the first to examine amino acid and NH_3 responses sixty minutes exercise in humans and the possible impact altered dietary silk amino acid intakes may have on these metabolites during prolonged, submaximal exercise. The present study was undertaken to examine the silk amino acid supplementation on the level of blood energy substrates and hormones responses to altered dietary intakes prolonged, submaximal exercise.

METHODS

Subjects

The subjects were 8 healthy college taekwondo players. The average ages of subjects were 20.25 ± 0.70 yrs, weight 70.28 ± 10.08 kg and the height 175.87 ± 6.12 cm. The BMI of the subjects was between 19.40 and 25.30. All of the subjects were informed of the nature, purpose, and risks involved in the study before their participation

Preliminary test

Before the subjects' prolonged treadmill test, the pre-exercise $V°O_2max$ tests were done (Vmax229, Korea). Maximal oxygen uptake ($V°O_2max$), whether estimated from the work rate achieved or measured directly by gas analysis, provides important information about cardiovascular fitness and prognosis. In the absence of untoward signs or symptoms, patients should be encouraged to give their best effort so that maximal exercise tolerance can be determined. Bruce protocol were used on $V°O_2max$ test which gives warm-up speed of 2.0 km/h, on the degree of 3%, with a three minute exercise. Every three minutes speed increased (2.7, 4.0, 5.5, 6.8, 8.0 km/h). Grade were 10% on stage one and increased 2% every three minute. Eight subjects were tested until all out. During $V°O_2max$ test, no symptoms and signs of cardiopulmonary were observed. The subjects' max heart rate and $V°O_2max$ ranged from 199.69 ± 6.69 beats/min and 59.11 ± 2.53 mL/kg/min.

Experimental procedure

The experiment were conducted by well-trained exercise physiologist and nurse. Laboratory was properly equipped and the testing personnel were appropriately trained. Emergency team were standby for aid in providing effective and timely advanced CPR when necessary. The exercise regimen consisted of treadmill for 60 min. at 65% of maximal heart rate on 8 competitive athletics. Warm-up for the prolonged exercise test was done at speed 3.0 km/h on degree of 5% within three minute. After 3 min speed and degree increased at 5.5 km/h and 14.0%. All eight competitive athletics exercised on treadmill (Intertrack 6025, Korea) 60 minute. The heart rate was monitored continuously via telemetary (Polar Electro OY, Finland). Ratings of perceived exertion The subjects were carefully instructed to rate their overall degree of perceived exertion on the 15 degree (6-20) category scale devised by Borg (1970). After the test done, each individual supplemented silk amino acid (6.390 mg/day) for 4 weeks. After 4 weeks, same experiment were operated for 8 athletics.

Components of silk amino acid

Table 1 shows the elements of silk amino acid.

Blood analysis

Blood sample were obtained from subjects at rest bed 30 minute before test, just after the exercise and rest 30 minute. Blood was drawn 24 mL from the medial cubital vein into sterile, SST-treated tubes (Sekisui, Japan). Just

Table 1. Components of silk amino acid

	Essential amino acid			Nonessential amino acid	
	Content (%)	Rate (%)		Content (%)	Rate (%)
Isoleucine	0.24	0.26	Alanine	27.74	30.62
Leucine	0.18	0.20	Aspartic acid	2.79	3.08
Lysine	0.09	0.10	Arginine	0.10	0.11
Methionine	0.02	0.02	Cysteine	0.06	0.07
Phenylalanine	0.12	0.13	Glutamic acid	3.06	3.37
Threonine	1.32	1.46	Glycine	35.65	39.34
Tryptophane	0.18	0.20	Glutamine	-	-
Valine	1.91	2.11	Hydroxyproline	-	-
Histidine	0.58	0.64	Proline	0.55	0.61
	-	-	Serine	8.48	9.36
	-	-	Tyrosine	7.54	8.32

The glutamine and hydroxyproline were not contained in silk amino acid.

after the tubes were centrifuged (at 3,000×g for 15 min at 20°C) to obtain plasma, the sample were stored at -80°C until further analysis. Blood components were measured at Green Cross Reference Laboratory in Seoul Korea. Amino acid were measured by HPLC method (Biochrome 20, UK). Glucose concentrations were measured by enzymatic method (ADVIA 1650, Japan). Ammonia and lactic acid were measured enzymatic method (Cobas Integra 800, Swizerland) using different reagent. Triglyceride (TG) were measured with Lipase, GK, GPD, Colorimetry method (ADVIA 1650, Japan). Total cholesterol and HDL-cholesterol were measured enzymatic, colorimetry (ADVIA 1650, U.S.A) using different reagent. LDL-cholesterol were measured by enzymatic, colorimetry (Hitachi 7150, Japan) using LDL-cholesterol reagent. Insulin concentrations were measured by radioimmunoassay (Cobra 5010, Quantum, U.S.A). Serotonin concentration were measured by High-Performance Liquid Chromatography (HPLC system by BIO-RAD, U.S.A).

Data analysis

All statical analyses were managed by SPSS (Version 12.0) in personal computer. Data were analyzed using means, standard variation and paired T-test. All data are reported as means±SD. The significant level for all tests was set at $p<.05$.

RESULTS

The results of the tests are as follows.

Changes of heart rate and RPE-Scale in submaximal exercise (Table 2). As show in Table 2, in RPE and heart rate, the ratings of mental fatigue increased during exercise in both trials. But between the supplementation and non-supplementation were insignificant difference.

The concentration of NH_3 in plasma before and after supplementation increased during 65% submaximal exercise ($p<0.01$). In a 30 minute rest after a submaximal exercise, ammonia concentration was lower than that of before the exercise ($p<0.01$). The NH_3 concentration changes in two groups before and after supplementation was not significant. A significant increase in plasma alanine was observed between before the submaximal exercise and after exercise ($p<0.01$), but no significant changes appeared between before and after supplemented group. BCAA (leucine, isoleucine and valine) were decreased more than other amino acids between resting and after exercise state ($p<0.05$). However, no significant changes found between before and after supplemented group. Lysine and ammonia were lower ($p<0.05$) in the after supplemented group, but there were no other differences between two groups (before and after supplement) in plasma amino acid concentrations at rest. The plasma amino acids were generally extremely constant during the 60 min of submaximal exercise. Both groups (before and after supplement) had small declines in hydroxyproline. In contrast to these modest changes, the larger declines ($p<0.05$) were found

Table 2. Changes of HR (beats/min) and RPE-Scale in submaximal exercise

Variables	Group	Rest	After exercise	Rest 30 min
Heart rate	Before supp.	69.37±5.75[1)]	169.62±11.68	68.24±5.34
	After supp. (4 week)	68.62±6.96	165.00±8.41[a**]	68.12±6.83[b**]
RPE	Before supp.	6.00±0	17.1±0.89	6.00±0
	After supp. (4 week)	6.00±0	17.0±1.10[a**]	6.00±0[b**]

[1)]Value represents mean±SD.

[a]Significant difference between rest vs. after exercise.

[b]Significant difference between after exercise vs. rest 30 min.

**p<0.01.

Table 3. Changes of plasma amino acid (μmol/L) and ammonia (μg/dL) concentration in submaximal exercise

Variables	Group	Rest	After exercise	Rest 30 min
Alanine	Before supp.	313.79±30.13[1)]	420.34±31.24	316.53±28.49
	After supp. (4 week)	316.93±34.57	415.87±32.15[a**]	316.67±32.57[b*]
Arginine	Before supp.	68.43±25.12	70.21±15.67	68.91±18.23
	After supp. (4 week)	69.12±28.32	69.59±13.12	68.25±16.34
Aspartic acid	Before supp.	15.34±8.72	12.56±4.45	14.68±5.26
	After supp. (4 week)	15.35±7.98	13.78±5.10	14.02±6.17
Cystine	Before supp.	71.33±8.35	63.46±5.21	69.83±6.21
	After supp. (4 week)	72.48±7.12	65.31±5.89[a*]	71.39±7.36[b*]
Glutamic acid	Before supp.	41.64±9.88	37.87±7.32	37.11±6.53
	After supp. (4 week)	38.82±7.74	35.48±5.67	34.24±7.14
Glutamine	Before supp.	622.14±18.79	621.38±7.32	621.19±13.47
	After supp. (4 week)	620.86±21.89	620.36±9.52	619.78±10.03
Glycine	Before supp.	215.14±15.79	204.35±12.69	209.47±11.85
	After supp. (4 week)	214.86±17.89	205.85±13.80[a*]	210.37±12.87
Histidine	Before supp.	88.99±5.77	88.97±7.34	87.29±7.42
	After supp. (4 week)	89.67±8.97	88.52±6.10	88.10±5.21
Hydroxyproline	Before supp.	12.73±4.32	8.49±3.78	10.63±3.50
	After supp. (4 week)	13.67±5.34	7.98±4.25	10.02±4.01
Isoleucine	Before supp.	57.23±2.20	50.49±4.26[a*]	52.53±3.58
	After supp. (4 week)	58.76±4.01	51.81±3.05[a*]	56.31±4.12
Leucine	Before supp.	136.43±15.73	129.37±10.07[a*]	135.83±13.50
	After supp. (4 week)	133.79±18.34	124.28±13.75[a*]	130.56±12.48
Lysine	Before supp.	178.45±11.67	175.80±10.21	176.92±9.02
	After supp. (4 week)	171.57±9.29[c*]	174.68±10.71	174.03±9.14
Methinonie	Before supp.	18.35±2.34	17.96±1.31	17.72±1.13
	After supp. (4 week)	16.21±1.08	16.02±0.86	16.03±1.10
Phenylalanine	Before supp.	48.44±2.30	47.81±1.16	47.01±1.24
	After supp. (4 week)	51.89±1.89	50.90±0.87	50.19±1.06
Proline	Before supp.	162.17±9.54	154.86±15.25	160.25±8.64
	After supp. (4 week)	160.67±10.78	151.19±14.70[a*]	157.03±9.34
Serine	Before supp.	118.56±6.19	116.43±9.46	117.52±4.31
	After supp. (4 week)	121.16±5.10	118.78±9.03[a*]	120.12±4.92
Threonine	Before supp.	114.58±5.32	111.34±10.48	112.92±7.54
	After supp. (4 week)	115.89±4.30	113.28±9.25	112.32±4.21
Tryptopane	Before supp.	62.18±6.35	62.97±5.65	61.79±3.02
	After supp. (4 week)	60.27±4.36	61.40±5.55	60.10±4.68
Tyrosine	Before supp.	49.37±5.31	50.89±4.14	49.72±4.84
	After supp. (4 week)	46.20±4.45	48.02±5.11	47.25±5.05
Valine	Before supp.	259.19±7.30	252.30±6.41[a*]	254.04±5.20
	After supp. (4 week)	258.17±6.49	250.80±4.19[a*]	250.12±5.00
Ammonia (μg/dL)	Before supp.	105.13±44.69	145.25±38.84	75.0±20.57
	After supp. (4 week)	91.37±29.70[c*]	143.37±43.48[a**]	75.12±19.64[b**]

[1)]Value represents mean±SD.
[a]Significant difference between rest vs. after exercise.
[b]Significant difference between after exercise vs. rest 30 min.
[c]Significant difference between before supplement vs. after supplement.
*p<0.05, **p<0.01.

in two amino acids: glycine and proline.

The lactic acid concentration (Table 4) increased during submaximal exercise and dropped down at rest 30 minute. But no significant changes between before and after supplement group ($p<.05$). The glucose concentration change before and after supplement in submaximal exercise was slightly lower than resting point (Table 4). However, no significant change occurred between two groups (before and after supplement). The triglyceride concentration increased ($p<0.05$) during 65% submaximal exercise and then dropped to the level of resting time on rest 30 minute. The total cholesterol concentration were increased during the exercise ($p<0.05$). There were small decrease in resting point after supplementation, but no significant change between two groups (before and after supplement) at the end of exercise and rest 30 minute. On the other hand the concentration at the time of 30 minute rest was lower than after submaximal exercise ($p<0.05$). The HDL and LDL concentration were increased both before-, after supplement group during submaximal exercise ($p<0.05$) and slightly decreased in 30 min. rest.

Plasma insulin, serotonin and leptin concentration (Table 5) were not different between two groups (before supplement and after supplement) at any time. Insulin decreased progressively to ~55% of the rest value, whereas serotonin increased approximately 50%. The plasma leptin concentration and its correlation to the variances before-, after exercise and 30 minute rest were not significant.

DISCUSSION

The concentration of ammonia in plasma before and after the supplementation increased during 65% submaximal exercise. In 30 minute rest after submaximal exercise, ammonia concentration was lower than that of before exercise. The subjects of this experiment was college taekwondo player. The mean of taekwondo player carrier was 12 yrs. Lo and Dudley (1987-30) reported that endurance trained subjects had lower plasma ammonia concentrations than untrained subjects at the same absolute power output in a short-term incremental protocol. This was confirmed by Snow et al. (1991). During intense exercise the dominant source of NH_3 in human skeletal

Table 4. Changes of plasma lactic acid, glucose, triglyceride, T. cholesterol, HDL and LDL concentration in submaximal exercise (mg/dL)

Variables	Group	Rest	After exercise	Rest 30 min
Lactic acid	Before supp.	8.77±2.45[1)]	19.16±7.97	10.13±4.50
	After supp. (4 week)	8.68±2.19	18.63±8.44[a*]	9.63±3.96[b*]
Glucose	Before supp.	106.12±15.55	93.00±9.84	94.25±10.03
	After supp. (4 week)	105.62±16.61	93.37±7.32[a*]	93.12±7.77
Triglyceride	Before supp.	93.12±37.71	100.62±31.65	85.25±22.72[b*]
	After supp. (4 week)	93.87±35.87	103.62±21.90[a*]	94.37±14.34[b*]
T. cholesterol	Before supp.	148.25±16.63	157.25±16.99	149.75±16.91
	After supp. (4 week)	140.62±16.63[c*]	156.62±18.02[a*]	148.50±17.71[b*]
HDL	Before supp.	42.50±7.87	46.00±8.68	43.87±7.86
	After supp. (4 week)	42.62±8.36	46.37±8.08[a*]	43.00±5.12
LDL	Before supp.	83.25±18.54	91.00±18.36[a*]	89.00±16.64
	After supp. (4 week)	81.75±15.67	88.62±16.26[a*]	86.87±16.56

[1)]Values represent means±SD.

[a]Significant difference between rest vs. after exercise.

[b]Significant difference between after exercise vs. rest 30 min.

[c]Significant difference between before supplement vs. after supplement.

*$p<0.05$.

Table 5. Changes of plasma serotonin, insulin and leptin concentration in submaximal exercise

Variables	Group	Rest	After exercise	Rest 30 min
Serotonin (pg/mL)	Before supp.	5.77±1.75[1)]	11.28±8.91	5.55±1.44
	After supp. (4 week)	5.73±1.62	11.22±8.09[a*]	5.58±1.40[b*]
Insulin (ulU/mL)	Before supp.	12.62±11.62	5.98±3.47	3.62±2.24
	After supp. (4 week)	13.27±11.05	5.56±2.22[a*]	3.97±2.07
Leptin (ng/mL)	Before supp.	2.00±1.11	2.11±1.08	2.07±1.00
	After supp. (4 week)	2.10±1.28	2.18±1.15	2.15±0.89

[1)]Values represent means±SD.
[a]Significant difference between rest vs. after exercise.
[b]Significant difference between after exercise vs. rest 30 min.
*$p<0.05$.

muscle in the deamination of AMP to IMP and ammonia by AMP deaminase as one of the reactions of the purine nucleotide cycle (PNC) (Graham et al., 1990). It has been suggested that the same mechanism is also responsible for the NH_3 produced by human skeletal muscle during prolonged, submaximal exercise (Brogerg and Sahlin, 1989). The present result are in agreement with this finding.

In this experiment after the exercise, many amino acids, including tyrosine, phenylalanine, the branched-chain amino acids, glutamate and glutamine levels were diminished. A significant increase in plasma alanine was observed between before and at the end of submaximal exercise, and there was insignificant changes in the level between before and after the supplemented group.

By far the most important of amino acids are glutamine, glutamate, and alanine. Alanine is the only amino acid to have dramatic shifts in its intramuscular concentration during exercise. Because the silk amino acid contain 27.74% of Alanine. Another possible source of NH_3 formation in skeletal muscle is from the deamination of the branched chain amino acids (BCAA), isoleucine, leucine, and valine. This involves the coupling of a BCAA transferase with glutamate dehydrogenase (GDH) forming a transdeamination reaction and subsequently producing NH_3 Approximately 60 to 80% of the total amino acid nitrogen is transported via the release of alanine and glutamine (Felig, 1981). The output of these from muscle far exceeds their percentage composition as found in muscle protein (Ruderman, 1975). Thus, these 2 amino acids have attracted considerable attention with regard to the sources of the carbon ad nitrogen required for their synthesis, as well as for their roles as amino nitrogen transporters from peripheral tissues to the liver. There is a net efflux of essential amino acids from muscle during prolonged exercise (Graham et al., 1995; MacLean et al., 1994) while the intramuscular pool was constant, and recently we found that this could be dampened if circulating levels of BCAA were increased (MacLean et al., 1994). To evaluate which proteins are catabolized, the release of specific amino acids can be used.

Kasperek et al. (1992) found that active rat muscle did not release 3-methyl fistidine during contractions, but did release the essential amino acid tyrosine, suggesting that the soluble proteins are selectively degraded. Investigators have reported activation of lysosomal enzymes (Kasperek et al., 1992).

It appears to be generally accepted that, despite the wide variety of exercise protocols and methodologies employed, rates of protein syntheses are depressed in muscle, liver and throughout the body during acute exercise conditions (Booth & Watson, 1985; Dohm et al., 1980; Rennie et al., 1981; Wolfe et al., 1982). Hood DA & Terjung RL- An increase in Protein degradation would also add to the tissue pool size, but reports on the directional changes of protein degradation in response to exercise are more conflicting. The response measured appears to depend on the methodology employed, as well as the intensity and duration of the exercise conditions (Booth & Watson, 1985; Dohm et al., 1985). However, an increase in liver protein loss has been documented

(Kasperek et al., 1980), as well as a consistent release of the branched-chain amino acids from the liver (Ahlborg et al., 1974) during exercise. These processes would also tend to increase the availability of amino acids for catabolism in skeletal muscle.- Hood DA & Terjung RL In support fo this view, 65% of max heart rate of 60 min exercise illustrated that the production of ammonia, glutamine, and alanine is not restricted to times of intense metabolic demands. The arterial concentration of these metabolites frequently have little or even no change during such exercise. Hood DA & Terjung RL And it is also consistent with our study. Similar results for glutamate and glutamine have been found in skeletal muscle of humans by Rennie et al. (1981). The reduction of muscle glutamate levels during exercise reflects this molecule's central position in amino acid metabolism.

Glutamate is produced along with oxoacids from oxoglutarate in various transamination reactions and it has a central position in amino acid metabolism. This glutamate can be converted to oxoglutarate and NH_3 by glutamate dehydrogenase, in which case NH_3 is produced within the muscle. However, the glutamate can also combine via glutamine synthetase with an additional free NH_3 to form glutamine. The effects of exercise and training on glutamine metabolism are not well established. However, depending on the intensity, exercise produces a condition of metabolic (lactate) acidosis, as well as enhanced ammoniagenesis via the purine nucleotide cycle (Meyer & Terjung, 1979). Greater substrate (NH_3) availability and acidosis are important factors (Ruderman & Berger, 1974) which could promote glutamine release from muscle during exercise. Babij et al. (1983) showed a linear increase in plasma glutamine concentration as a function of workload in man. This suggests that glutamate acts as a sink for NH_3 in the formation of gltamine during enhanced NH_3 production in exercise. It should be noted that the observed decrease in muscle glutamate concentration during exercise (Meyer & Terjung, 1979) reflects a competition for the glutamate pool in synthesizing either alanine, glutamine or aspartate. The extent to which glutamate follows each of these pathways during exercise, or following endurance training is not established. While aspartate remains inside the cell and may, under some conditions, be transaminated back to glutamate, the result of alanine and glutamine formation and efflux from muscle is probably a depletion of the intramuscular α amino nitrogen pool (Babij et al., 1983), as well as a loss of potentially oxidisable Krebs cycle carbon from the cell.

Furthermore the alanine aminotransferase reaction can result in the glutamate being converted into alanine. Thus, alanine formation can represent an equimolar NH_3 equivalent and glutamine can represent two NH_3 equivalents. This situation becomes even more complex because it is not essential that the glutamate for the glutamine synthetase and alanine aminotransferase reactions be derived from the transamination of oxoglutarate. Glutamate exists in the intracellular amino acid pool and can also be derivde from endogenous protein. Furthermore, it can be taken up from the plasma in considerable quantities. In these situations any resulting alanine formation does not represent any NH_3 production and any glutamine production represents only one rather than two NH_3 molecules. Thus unless one measures NH_3, alanine, and glutamine formation and also considers glutamate availability from intramuscular protein and the free amino acid pool as well as from the plasma, it is not possible to quantify the true NH_3 production rate in skeletal muscle.

Alanine appears to be oxidised at the highest rate quantitatively, approximately 30 to 70% higher than leucine oxidation (Goldberg & Odessey, 1972; White & Brooks, 1981). In support to this view, our study was constant with Goldberg & White. Information on the effect of endurance training on alanine formation is lacking. Ji et al. (1987) have demonstrated incresed plasma levels of *alanine* during exercise in trained *vs* untrained animals. This may indicate an accelerated removal of alanine from the plasma in the trained state, since preliminary data (Hood & Terjung, in preparation) have indicated that the output of alanine from muscle during moderately intense contraction conditions is increased in comparison to untrained controls. This may emphasize the greater impor-

tance of gluconeogenesis in trained animals.

Whether or not regular exercise would obligate an increase in the minimum daily leucine intake requirement. In humans, the leucine requirement for adults is 14 mg/kg bodyweight/day (Young & Bier, 1987). This corresponds to a rate of intake of 107 mol/kg/day, or 4.5 mol/kg/h. The dietary recommendations of leucine in humans are inadequate, particularly in individuals who regularly participate in endurance-type physical activities.

The glucose concentration decreased, whereas the latic acid, trigltceride, total cholesterol, HDL-C and LDL-C concentrations increased during exercise in both trials. The difference noted between the two conditions was for the before silk amino acid supplementation trial while there was no significant change during exercise in the the silk amino acid supplementation. Altering the availability of muscle glycogen or blood glucose as a substrate also influences the rate of protein catabolism during exercise (Lemon and Mullin, 1980; Wagenmakers et al., 1991). It has been proposed that NH_3 production, via the PNF, is greater when initial muscle glycogen content is low (Broberg and Sahlin, 1989). However, the declining concentration of muscle glycogen may progressively enhance BCAA catabolism and NH_3 production, because skeletal muscle NH_3 production continually increases during submaximal exercise (Graham et al., 1991) and the highest plasma NH_3 concentrations occur at exhaustion when muscle glycogen is depleted.

Our results indicated that the plasma insulin, serotonin and leptin concentration were not different between two groups (before supplement and after supplement) at any time.

The mechanism behind the increased level of 5-HT during sustained exercise is thought to be an increase in the plasma ratio of free (not albumin-bound) tryptophan/other large neutral amino acids (LNAAs), which occurs during sustained exercise and is very marked after exercise (Blomstrand et al., 1988, Davis et al., 1992).

This concentration ratio is considered to be important since the LNAAs (including tryptophan and the branched chain amino acids [BCAAs]) are transpitred into the brain by the same carrier mechanism and competition between these amino acids for entry into the brain can occur (Pardridge, 1977). Studies of the effect of physical exercise on the brain level of tryptophan support the view that it is the free tryptophan concentration, rather than the total concentration, which governs the uptake of tryptophan by the brain (Chaouloff et al., 1986; Blomstrand et al., 1989).

In keeping with the well-documented influence of physical exercise upon lipolysis, acute physical exercise increases blood free tryptophan and decreases albumin-bound tryptophan both in (trained) animals (Blomstrand et al., 1989; Chaouloff, 1985) and humans (Davis et al., 1992). Because in most cases circulating total (free plus bound) tryptophan level is not affected by exercise, it is likely that the increase in free tryptophan during exercise is solely due to lipolysis and that hepatic tryptophan pyrrolase activity undergoes weak changes. Acute physical exercise affects the blood levels of those amino acids competing with tryptophan for entry into the brain is a question that has received some attention. Unfortunately, animal and human studies have led to the proposal that exercise decreases (Blomstrand et al., 1991) increases (Blomstrand et al., 1989), or does not affect (Davis et al., 1992) branched-chain amino acid levels. This result thus shows that acute exercise differs from other models (e.g., carbohydrate ingestion, immobilization stress) where increases in brain tryptophan are dependent upon 1) increases in blood total (rather than free) tryptophan, 2) decreases in the competition for entry into the brain between tryptophan and the other neutral amino acids, or 3) unspecific increases in blood-brain barrier permeability (12).

We found that the plasma leptin concentration insignificantly inecresed after exercise. our results were suported by Kraemer et al. (1999), our findings are difficult to explained by Hickey et al. (1996a) and Haluzik et al.

(1999). This is consistent with studies showing that long or intense single bouts of exercise significantly reduce leptin concentration(Landt et al., 1997; Kohrt et al., 1996). Thus, the changes in leptin may have reflected changes in energy balance in addition to changes in adiposity; negative energy balance resulting from physical training may reduce leptin concentrations. Lower levels of leptin were also found in highly trained trained women versus controls (Ryan and Elahi, 1996). The relationship found between training hours and leptin may suggest that exercise effectively results in reduced leptin secretion or an elevated elimination of leptin. Perhaps the clear difference in exercise protocol (intensity and duration per training per training session in the present study were lower and shorter) and participating subjects (normal subjects vs. cerebral palsy in the study) can explain the differences found in relation to training and leptin.

CONCLUSION

In summary, we have shown that the RPE and heart rate, the ratings of mental fatigue increased during exercise in both trials. But between the supplementation and non-supplementation were insignificant difference.

The concentration of NH_3, lactic acid, triglyceride, total cholesterol, HDL-C, LDL-C in blood before and after supplementation significantly increased during 65% submaximal exercise. But there were no other differences between two groups (before and after supplement) after exercise and at rest. The plasma amino acids were generally insignificantly decreased during the 60 min of submaximal exercise. except alanine. Also, no significant changes found between before and after supplemented group.

In conclusion, this study shows that the 4 wks silk amino acid supplementation did not significant change the level of blood amino acid, energy substrates and hormones level during prolonged treadmill exercise.

Direction for future research, the components, and amounts of each component, that would optimally achieve the desired goal should then be predicted on the basis of results from metabolic studies in which the responses of muscle protein synthesis and breakdown are quantified. Factors yet to be determined are the optimal composition of a supplement (eg, type of protein, composition of amino acid mixture, nature of nonprotein energy), the optimal timing of ingestion in relation to exercise, and the amount of protein or amino acids per serving. When a theoretically optimal supplement is designed, then a long-term (eg, 6-mo) outcome study should be performed in which pertinent outcome variables (eg, muscle strength) are measured. Only when an optimal supplement is evaluated under controlled conditions (ie, comparable levels of exercise intensity, training duration, and other nutritional intake) can the question of protein requirements during exercise be definitively answered.

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