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Hannu Pitkänen

# Amino Acid Metabolism in Athletes and Non-Athletes

## With Special Reference to Amino Acid Concentrations and Protein Balance in Exercise, Training and Aging

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Academic Dissertation

Neuromuscular Research Center,
Department of Biology of Physical Activity,
University of Jyväskylä

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# ABSTRACT

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Amino acids are the basic components of proteins, which are essential to maintain and develop muscle, bone, cartilage, skin and blood. As a response to a physical stress amino acids are mobilised from the body's free amino acid pool, which is located in the plasma and in cellular spaces. The pool represents only 2% of the total amino acids in the body of a 70-kg individual and approximately half of it exists in skeletal muscle. Despite the small size of the pool it has an important daily task in the protein metabolism. The present study was designed firstly to compare serum amino acid responses to different exercise sessions and to a training period with and without leucine (LEU) supplementation. Secondly, muscle protein balance after a strength training session was examined. The third purpose was to create a profile of the serum amino acid concentrations for aging men and women. The results indicated that the concentration of the sum of all serum amino acids decreased following a training period and following a strength exercise session but not after lactic anaerobic running exercise sessions with protein intake of 1.1-1.3 g / kg body weight / day. The amino acid concentration seemed to decrease following a speed and strength training period of five weeks even though increases were seen in testosterone, cortisol and testosterone / cortisol –ratio showing an anabolic state. LEU supplementation seemed to prevent both a training-induced and an exercise-induced decrease in the serum LEU concentration and to decrease the serum concentration of isoleucine and valine. However, the supplementation did not enhance performance. In fasting conditions, a strength exercise session induced different responses in femoral arterious, femoral venous and muscle concentrations of free amino acids. Both protein synthesis and breakdown increased in fasting conditions following a strength exercise session, but there were no changes in the protein net balance, which was catabolic. Furthermore, the results showed that the concentrations of amino acids decreased significantly with aging and the concentrations were greater in men than in women. This study provides evidence that there are changes in amino acid metabolism following exercise sessions and training periods and that LEU supplementation has an effect on these changes. In fasting conditions, protein breakdown is greater than protein synthesis during recovery following resistance exercise. Serum amino acid concentrations decrease with age and men have greater amino acid concentrations than women.

Key words: Protein metabolism, amino acids, athletes, sedentary people, exercise, age, gender

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Kankaanpää, November 2002
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## ORIGINAL PAPERS

This thesis is based on the following papers, which will be referred to by their Roman numerals. In addition some data not presented in these papers are also included.

I Pitkänen HT, Mero AA, Oja SS, Komi PV, Pöntinen PJ, Saransaari P and Takala T (2002) Serum amino acid responses to three different exercise sessions in male power athletes. Journal of Sports Medicine and Physical Fitness. In Press.

II Pitkänen HT, Mero AA, Oja SS, Komi PV, Rusko H, Nummela A, Saransaari P, and Takala T (2002) Effects of training on the exercise-induced changes in serum amino acids and hormones. Journal of Strength and Conditioning Research, 16: 390-398.

III Pitkänen HT, Oja SS, Rusko H, Nummela A, Komi PV, Saransaari PV, Takala T and Mero AA (2002) Leucine supplementation does not enhance acute strength or running performance but affects serum amino acid concentration. Amino Acids. In Press.

IV Mero AA, Pitkänen HT, Oja SS, Komi PV, Pöntinen PJ and Takala T (1997) Leucine supplementation and serum amino acids, testosterone, cortisol, and growth hormone in male power athletes during training. Journal of Sports Medicine and Physical Fitness, 37: 137-145.

V Pitkänen HT, Nykänen T, Knuutinen J, Lahti K, Keinänen O, Alen M, Komi PV and Mero AA (2002) Free amino acid pool and muscle protein balance after resistance exercise. Submitted.

VI Pitkänen HT, Oja SS, Kemppainen K, Seppä JM, and Mero AA (2002) Serum amino acid concentrations in aging men and women. Amino Acids. In Press.

# CONTENTS

## ABBREVIATIONS AND DEFINITIONS

| | |
|---|---|
| ALA | alanine |
| ARG | arginine |
| AcetylCoA | acetyl-coentzyme A |
| ASN | asparagine |
| ASP | aspartate or aspartic acid |
| ATP | adenosine triphosphate |
| AV | antecubital vein |
| a-v | arterio-venous |
| BCAAs | branched chain amino acids |
| CG | control group |
| CI | confidence interval |
| CIT | citrulline |
| CMJ | counter movement jump |
| -COOH | carboxyl group |
| COR | cortisol |
| CYS | cystine |
| DNA | deoxyribonucleic acid |
| GC / MS | gas chromatography / mass spectrometry |
| GH | growth hormone |
| GLN | glutamine |
| GLU | glutamate or glutamic acid |
| GLY | glycine |
| EAAs | essential amino acids |
| EG | exercise group |
| FA | femoral artery |
| FV | femoral vein |
| HIS | histidine |
| HMB | beta-hydroxy beta-methylbutyrate |
| HPLC | high performance liquid chromatography |
| ICG | indocyanine green |
| IGF-1 | insulin-like growth factor |
| ILE | isoleucine |
| KIC | alpha-keto-isocaproate |
| KOH | potassium hydroxide |
| L | leucine group |
| LEU | leucine |
| LRS | long-run session |
| LYS | lysine |
| MARE | maximal running exercise |
| MET | methionine |
| m-RNA | messenger-RNA |
| NEAAs | non-essential amino acids |

| | |
|---|---|
| $-NH_2$ | amino group |
| OPA | *orto*-pthaldehyde |
| ORN | ornithine |
| P | placebo group |
| PHE | phenylalanine |
| PRO | proline |
| RDA | recommended dietary allowances |
| RES | resistance exercise session |
| RIA | radioimmunoassay |
| RM | repetition maximum |
| RNA | ribonucleic acid |
| RPHPLC | reversed phase high performance liquid Chromatography |
| SD | standard deviation |
| SE | standard error |
| SEM | standard error of means |
| SER | serine |
| SES | strength exercise session |
| SRS | short-run session |
| TAAs | total amino acids |
| TAU | taurine |
| TCA-cycle | tricarboxylic acid -cycle |
| TE | testosterone |
| TE / COR -ratio | testosterone / cortisol -ratio |
| THR | threonine |
| t-RNA | transfer-RNA |
| TRP | tryptophan |
| TTR | tracer to tracee ratio |
| TYR | tyrosine |
| VAL | valine |
| $VO_{2max}$ | maximal oxygen uptake |

# 1 INTRODUCTION

Protein is an essential nutrient, which, excluding water, is the largest component in the human body, representing about 15% of body weight and primarily found in skeletal muscle (Lemon 1991a). Approximately 60% of the whole body protein mass is made up of skeletal muscle protein (Ballard and Tomas 1983). Proteins play an important role in transport and storage, catalysis, control of growth and differentiation, immune protection and in structural functions (Stryer 1988). The components of proteins are amino acids, which are essential for the synthesis of structural proteins, enzymes, neurotransmitters and some hormones. Amino acids occur in the body in the free form and in the form of the body proteins.

The free amino acid pool, about 50% out of which is located in skeletal muscle (Wagenmakers 1998), promotes amino acids for protein synthesis and oxidation and is replenished by protein breakdown or amino acids from diet. The pool (Fig. 1) plays an important role in overall protein turnover (protein synthesis and breakdown) during stressful situations (e.g. training, abnormal acclimation, disease, recovering) in providing amino acids where they are needed. Protein turnover is mediated by hormonal factors, caloric intake and amino acid availability. Branched chain amino acids (BCAAs) are unique among amino acids since they are oxidized in muscle directly to acetyl-coentzyme A (Acetyl CoA) (Wagenmakers 1998). The oxidation of LEU, which is one of the three BCAAs, is increased during exercise (Rennie and Tipton 2000). The importance of BCAAs in determining protein synthesis and decreasing exercise induced protein degradation via the oxidative by-product KIC (alpha-keto-isocaproate) (Tischler et al. 1982) has led to their supplementation in athletes. It is well known that sufficient protein intake is essential for optimal performance, but there are discrepancies in assessing the adequate intake to maintain the free amino acid pool under different kinds of stress situations and in different exercise sessions as well as during training seasons. Most studies concerning exercise loading (e.g. Einspahr and Tharp 1989, Strüder et al. 1995, Blomstrand et al. 1996) have concentrated on endurance exercise and performance and the available information is mainly

focused on male athletes. Recently new methods have been developed to assess the free amino acid transport and quantifying protein kinetics. Stable labelled isotopic tracers can provide information about protein synthesis, protein breakdown and de novo synthesis by calculations obtained from arterio-venous (a-v) catheterisation and muscle biopsies (Biolo et al. 1995a, 1995b).

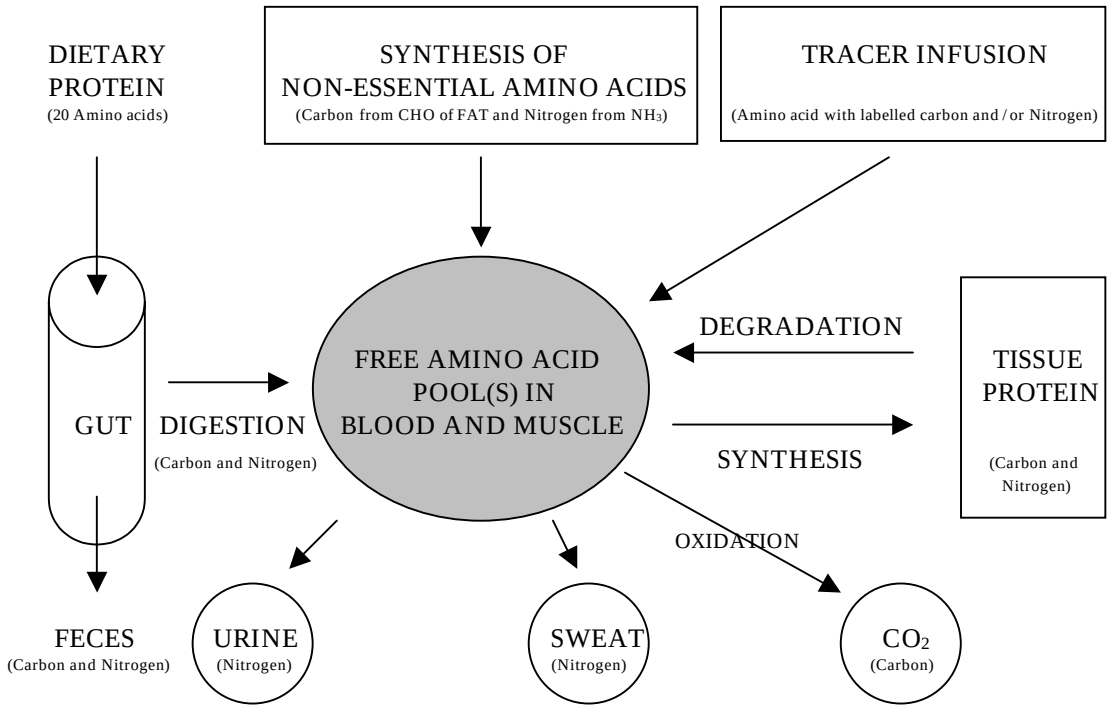


FIGURE 1 Protein kinetics (redrawn with modifications from Lemon 1998).

However, traditional techniques are still usable because of methodological ease and inexpensiveness. In this study both techniques have been used and the changes in the free amino acid pools as a response to different exercise sessions, training periods and recovery have been investigated. Attention was directed to the response in athletes but also in sedentary people of both genders with aging.

The general purpose of this study was to examine the changes in amino acid concentrations as a response to anaerobic work loading and further to examine whether this kind of exercise can be improved by LEU supplementation. Muscle protein synthesis and muscle protein degradation following resistance exercise were also examined. In addition, the changes in the amino acid profile in the elderly, both in men and women were looked for.

# 2 REVIEW OF THE LITERATURE

## 2.1 General aspects in amino acid and protein metabolism

In addition to protein synthesis, the amino acids provide precursors for several biological compounds for example cathecholamines, neurotransmitters and phospholipids (Kasperek 1989). The genetic code for protein synthesis is copied from deoxyribonucleic acid (DNA) to ribonucleic acid (RNA) in the process called transcription. The protein molecule is formed in ribosome in association with messenger-RNA (mRNA) and transfer-RNA (tRNA) in translation process (e.g. Guyton, 1986). The basic structure of an amino acid contains an amino group ($-NH_2$) and a carboxyl (-COOH) group (Stryer 1988). Some amino acids can be synthesized in the body (non-essential amino acids or dispensable = NEAAs) and some (essential amino acids or non-dispensable = EAAs) (Henry et al. 1974) must be eaten in a regular basis in the diet (Paul 1989) (TABLE 1).

TABLE 1 Essential and non-essential amino acids.

| Essential Amino Acids | Non-Essential Amino Acids |
|---|---|
| Arginine, ARG | Alanine, ALA |
| Histidine, HIS | Asparagine, ASN |
| Isoleucine, ILE* | Aspartate or aspartic acid, ASP |
| Leucine, LEU* | Citrulline, CIT** |
| Lysine, LYS | Cysteine, CYS |
| Methionine, MET | Glutamine, GLN |
| Phenylalanine, PHE | Glutamate or glutamic acid, GLU |
| Threonine, THR | Glycine, GLY |
| Tryptophan, TRP | Ornithine, ORN** |
| Valine, VAL* | Proline, PRO |
| | Serine, SER |
| | Taurine, TAU** |
| | Tyrosine, TYR |

* Branched chain amino acids; ** Conditionally (under certain conditions) essential amino acids

If EAAs are not included in diet, protein synthesis is impaired resulting in decrease in body protein content due to protein degradation. This reveals the actual requirement for select amino acids and not for protein per se (Lemon 1991b). Current recommended protein intake of 0.8 g/kg/day is based on nitrogen balance (nitrogen intake – nitrogen excretion) and is primarily focused on sedentary individuals (Lemon 1991a). There is evidence from different research techniques (based on nitrogen balance, labelled amino acid isotopes, urea production and 3-methylhistidine excretion) that protein metabolism is altered by exercise and that the recommended intake may be insufficient for physically active individuals (Lemon et al. 1984, Lemon 2000), but the allowances should be based on a percentage of daily energy intake rather than body weight (Paul 1989). The type, duration and intensity of exercise as well as previous training affect the requirement of dietary protein (Kasperek 1989). The proteins of the diet are digested to dipeptides or amino acids that can be absorbed into the blood (Guyton 1986). The end product of amino acid degradation, urea, is formed in liver via urea cycle and is removed from blood by the kidney and sweat glands (Brooks and Fahey 1984). The 3-methylhistidine excretion in urine indicates the degradation of contractile protein (Young and Munro 1978). All tissue proteins are constantly being "turn-overed" via synthesis and degradation. In nitrogen balance these processes are equal and there is no net gain or loss of protein. During catabolic conditions protein is mobilised by suppressed protein synthesis and/or intensified protein degradation (Dohm 1986).

Approximately 2% of the total amino acids exist in the free amino acid pool, which represents the body's "store" for the amino acids within the plasma and intra- and extra cellular spaces (Wagenmakers 1998). It is assumed that the free amino acid pool is in a state of equilibrium, since an increase in the metabolism of any compartment of the pool (muscle, liver, blood) will have a direct effect on the amino acids in the other compartments (Paul 1989). Despite its small size (about 200g in a 70-kg individual) the pool accounts for a continuous exchange of amino acids (protein turnover) due to events such as exercise or a change in dietary intake (Wagenmakers 1998). Some of the amino acids in the pool can be utilized for the synthesis (anabolism) of body proteins, some for energy demands through oxidation and some provide substrates for gluconeogenesis (Rennie and Tipton 2000). Skeletal muscle is the major site of the free amino acid deposition (Munro 1970). The rate of free amino acid consumption in muscle is the main determinant of the amount of the total free amino acid pool available (Viru 1987). Muscle protein balance can be determined by muscle protein synthesis and muscle protein degradation (Lemon 1991b). Altering one of these factors results in chance in balance and further in muscle structure and metabolism (Paul 1989).

## 2.2 Hormonal regulation in amino acid and protein metabolism

Several hormones, synthesized and secreted by endocrine system, have effects on muscle protein metabolism. The anabolic hormones that are involved in muscle growth and remodelling are growth hormone (GH), insulin, thyroid hormones, testosterone (TE) (Florini 1987) and insulin-like growth factor-1 (Di Pasquale 1997), whereas glucocorticoids and thyroid hormones are catabolic (Kuoppasalmi and Adlercreutz 1985). GH increases the rate of synthesis resulting in increased tissue proteins. Insulin accelerates the amino acid transport, which possibly stimulates protein synthesis. TE increases the deposition of protein in the tissues. Glucocorticoids are supposed to increase the rate of extra hepatic proteins (Guyton 1986). Thyroid hormones can have either anabolic (physiological levels) or catabolic (higher levels) effects (Di Pasquale 1997) and they affect skeletal muscle via secondary mechanisms (Florini 1987). Exercise has profound effects on protein synthesis, degradation and hormones, which further modulate the response to exercise (Goldberg et al. 1980). In general, exercise increases acutely the level of GH and cortisol (COR) and decreases or increases the level of TE. During recovery there is a decrease in COR and an increase in GH and TE. As a successful response to training, the levels of TE and GH slightly increase while the level of COR decreases (Di Pasquale 1997).

## 2.3 Glucose-alanine cycle

LEU, ILE, VAL, ASN, ASP and GLU are amino acids that are metabolised in resting muscle. They provide the amino groups required for the synthesis of GLN and ALA, which are released in post absorptive state and during protein intake (Wagenmakers 1998). Muscle has the mechanism that allows transport of nitrogen and carbon to the liver. After gluconeogenesis (in the liver), some of the carbon returns into the muscle as glucose and the exchange of ALA and glucose between liver and muscle (Munro and Crim 1988) is called the glucose-alanine cycle (FIG. 2). LEU can be oxidised in muscle cell and is mainly converted directly to acetylCoA, whereas the other carbon skeletons of amino acids are used for synthesis of tricarboxylic acid -cycle (TCA-cycle) intermediates and GLN (Wagenmakers 1998). After a meal the output of amino acids diminishes and the mechanism is reversed so that muscle actually gains protein (Munro and Crim 1988). There is lack of data, whether glucose-alanine cycle can be intensified.

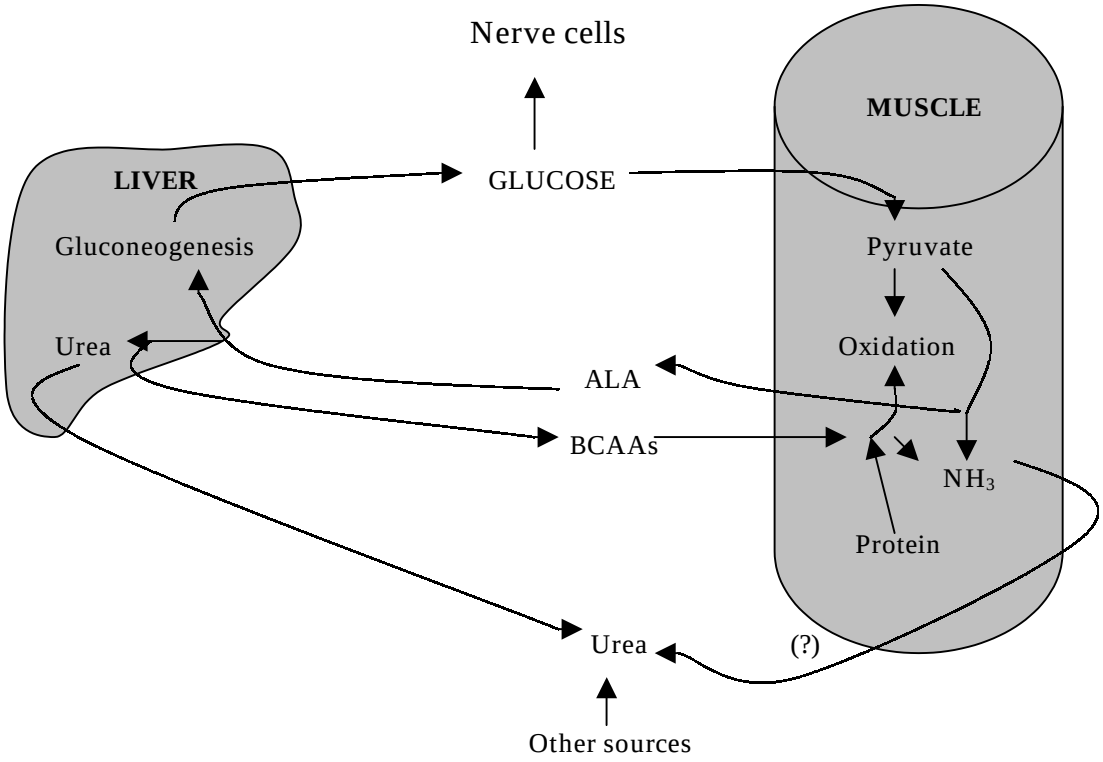


FIGURE 2 Glucose-alanine cycle (redrawn with modifications from Viru 1987).

## 2.4 Exercise sessions and amino acid metabolism

Since the musculature is the largest tissue of the body (e.g. Munro 1969), protein metabolism is affected to a great extent during exercise. Adaptation to muscular activity requires the metabolism and functional activities to adjust for the energy demands, while the mobilization of amino acids for the adaptive synthesis decreases (Viru 1987). During exercise the whole body protein synthesis has been estimated to decrease to 14-18% judged by labelled nitrogen production (Rennie et al. 1981). In an exhaustive 3-hour run protein synthesis was inhibited by 70% in rats (Dohm et al. 1982). In general, it seems that exercise suppresses protein synthesis and stimulates protein degradation in skeletal muscle. At rest the breakdown of protein contributes 2-5% of the body's total energy requirement and during exercise loading energy derived from protein may supply as much as 10 to 15% of the total energy requirement (Tipton and Wolfe 1998).

Dynamic exercise stimulates amino acid oxidation, mainly that of the BCAAs, since they contribute as energy substrates and as nitrogen donors to the synthesis of ALA, GLN and ASP (Hood and Terjung 1990). The increased output of ALA from working muscle is proportional to exercise intensity (Felig and Wahren 1971). However, the increased oxidation is due to the exercise

intensity-dependent activation of the limiting enzyme, branched-chain oxoacid dehydrogenase (Kasperek and Snider 1987). If exercise is intense enough, there is a net loss of muscle protein resulting from decreased protein synthesis and/or increased protein breakdown. Some of the amino acids are utilized as fuel oxidation, whereas some are associated with gluconeogenesis and acid based regulation (Rennie and Tipton 2000).

### 2.4.1 Endurance exercise session

The available studies concerning training effects on the concentrations of serum amino acids are still sparse and the subjects have mostly been endurance-trained athletes**.** For example, Einspahr and Tharp (1989) investigated 12 endurance runners (110 km/week) and 13 controls (< 5 km/week) and found significantly higher plasma levels of leucine (41%), isoleucine (27%) and tyrosine (23%) among the trained subjects at rest. However, the authors did not report the daily nutrition of the subjects. In addition, several studies (e.g. Wolfe et al. 1982, Carraro et al. 1994) have shown that the whole body protein breakdown, indicated by the increase in LEU oxidation, is increased during aerobic endurance exercise. This results from a need for amino acids to be oxidised in working muscles. However, urea production is not elevated during exercise, even though it reflects protein breakdown (Wolfe et al. 1982). Carraro et al. (1994) showed that the increased nitrogen flux is incorporated in acute phase plasma protein synthesis, which are available for later protein synthesis during recovery. The exercise induced increase in ALA (and thus in nitrogen) seen during and following endurance exercise may be partially incorporated in acute phase plasma protein synthesis rather than in urea (Carraro et al. 1990a). Whole body protein synthesis has been reported to be either decreased (Wolfe et al. 1982) or unchanged (Carraro et al. 1990a) immediately following endurance exercise. Insulin is the major hormone that regulates protein synthesis (Jeffersson 1980), since its decreased level, due to exercise, may inhibit protein synthesis (Guyton 1986). The concentration of total free amino acids have been reported to be lower, except TYR, which increased during heavy exercise of long duration (Haralambie and Berg 1976). Blomstrand et al. (1996) have reported significant decreases in plasma TRP and LEU concentrations following an 80-minute exercise session to exhaustion. In a study of Strüder et al. (1995) decreases were observed in 11 out of 14 amino acids after a 4-hour tennis tournament. Moreover, endurance exercise results in changes in intramuscular concentrations of amino acids: GLU decreases rapidly within the first minutes of exercise and then plateaus (Sahlin et al. 1990). In contrast ALA release increases during the initial period of exercise and then returns to resting level. Sustained level of GLN from muscle is also associated with prolonged exercise (MacLean et al. 1994). The decrease of GLN, which plays an important role in immune system, may be a factor in the high incidence of infections in athletes due to apparent immunosuppression (Castell and Newsholme 1998).

### 2.4.2 Short-term exercise session

The changes in arterial amino acid concentrations following short-term exercise are similar to muscle in GLU, which decreases and in ALA, which increases, but with regard to GLN the response exercise is different. The plasma concentration increases, whereas the intramuscular concentration remains almost constant (Sahlin et al. 1990). Babij et al. (1982) have investigated plasma amino acid levels during exercise of higher intensities and observed plasma ALA to increase exponentially with increasing workload, whereas GLN increased linearly. BCAA concentrations in plasma during short-term exercise have been found to be decreased (Bergström et al. 1985), unchanged (Eriksson et al. 1985) or increased (Sahlin et al. 1990). BCAA concentration of muscle has been found to remain unchanged with short-term exercise, at 50-70% of maximal oxygen uptake ($VO_2$max) (Bergström et al. 1985), even though LEU oxidation is increased during exercise (Wolfe at al. 1982). Literature lacks data about the changes in amino acid concentrations during heavy short-term anaerobic loading. Further it is difficult to quantify the degree of work done when compared to aerobic loading (expressed as percentage of $VO_2$max). However, the peak blood lactate level can be used to express the degree of anaerobic work loading.

### 2.4.3 Resistance exercise session

There are discrepant results among studies investigating the effects of a resistance exercise session on muscle protein metabolism, but it seems that muscle protein synthesis can be stimulated by exercise if there is enough challenge for the muscles i.e. the intensity of the exercise is enough to stimulate the muscle (Tipton and Wolfe 2001). In a study of Biolo et al. (1995b) an increase of 100% in protein synthesis and of 50% in protein breakdown was observed after resistance exercise. However, there is a negative muscle protein balance if there is an absence of food intake (Biolo et al. 1995b). Later Biolo et al. (1997) demonstrated that net muscle protein synthesis after exercise involved an interaction between exercise and nutrition, since exogenous amino acids were shown to enhance the metabolic effect of exercise on muscle protein. Hyperaminoacidemia increased muscle protein synthesis about 150% at rest and about 200% after resistance exercise suggesting that exogenous amino acid infusion had a more positive effect on protein balance after exercise than at rest. Furthermore, Tipton et al. (1999) showed that the shift from net muscle protein degradation to net muscle protein synthesis after resistance exercise can be caused by orally administered amino acids as well as by infused amino acids and by EAAs as well as by mixed amino acids (NEAAs + EAAs). There is only little information regarding free amino acid concentration in plasma following a resistance exercise session.

## 2.5 Training period and amino acid metabolism

The response provided by the bout of exercise is dictated, in addition to the type and intensity of exercise, by the level of training of the individual. Einspahr and Tharp (1989) has reported that plasma and muscle amino acid concentrations may be higher in endurance trained than in untrained individuals, but there is need for precise descriptions of the amino acid levels following a power (e.g. strength exercise, during which the levels of lactate change slightly) or anaerobic (e.g. sprint running period, during which the levels of lactate change strongly) training period. However, the detailed description of the nutrition has not been given in that particular study. During the training period the over all summation of protein balance is positive resulting in the accretion of muscle protein causing in many cases muscle hypertrophy. Muscle gain occurs due to several transient increases in net muscle protein balance following individual workout and not caused by an increase in the basal level of net protein balance (Tipton and Wolfe 2001). Therefore the changes in the levels of amino acids following a training period are worth investigating, especially under controlled nutritional conditions.

## 2.6 Amino acid and protein metabolism during recovery

Recovery period means the alteration from high to low energy demands compared with exercise. In addition, it means also the normalization of function and homeostatic equilibrium, restoration of energy reserves, elimination of accumulated metabolic intermediates and replenishment of water and ionic composition of the body. The actualisation of these functions occurs within minutes or in special cases within hours (Viru 1996). Post-exercise changes in amino acid metabolism have been examined in whole body techniques but there is poor agreement between studies (Tipton and Wolfe 1998). Following endurance exercise with whole body techniques protein breakdown has been shown to decrease (Rennie et al. 1981) or to be unchanged (Tipton et al. 1996), whereas protein synthesis was increased (Rennie et al. 1981). Following resistance exercise whole body techniques showed unchanged protein breakdown (Tipton et al. 1996) and unchanged protein synthesis (Tarnopolsky et al. 1991). After endurance exercise the adaptive protein synthesis occurs mainly in the mitocondrial proteins of oxidative or oxidative-glycolytic muscles, whereas after exercises for improved strength, the adaptive synthesis occurs in regard to the myofibril proteins of glycolytic fibres (Di Pasquale 1997). The studies with the modification of arterio-venous model and muscle biopsy showed the increase of leg protein breakdown of 50% (Biolo et al. 1995b), whereas the whole body protein breakdown increased only slightly simultaneously following an intense resistance exercise bout. The muscle protein synthesis was also increased (Biolo et al. 1995b). Post-exercise protein

breakdown has not been measured in contracting muscle following endurance exercise in humans, but protein synthesis has been shown to increase 4-hour post-exercise following long-term exercise (Carraro et al. 1990b). Literature still lacks the exact timetable for the changes in these parameters.

## 2.7 Leucine supplementation and amino acid metabolism

The essential branched chain amino acid LEU amounts to about 4.6% of all amino acids (Takala et al. 1980) and it accounts for many important roles in the body. It regulates protein metabolism by inhibiting degradation and stimulating synthesis (Nair et al. 1992), and supplies glycogenic precursors for the formation of ALA in muscle (Brooks 1987). LEU is interesting, since it can be oxidized in muscle in a higher rate than the other BCAAs, VAL and ILE, to asetylCoA (Wagenmakers 1998). Felig and Wahren (1971) have created the concept of glucose-alanine cycle, which accounts for the release of ALA from muscle and its use in gluconeogenesis in liver. Under catabolic conditions (for example fasting) the BCAAs are oxidised at increased rate suggesting that they serve as energy sources for muscle (Goldberg and Odessey 1972). Henderson et al. (1985) showed increased LEU oxidation in rats by both training and treadmill exercise. A significant decrease of 22% has been shown to occur following aerobic exercise in serum LEU level (Bergström et al. 1985) and in vastus lateralis muscle with reduced muscle glycogen stores (Blomstrand et al. 1996). Young and Bier (1987) have presented that the LEU requirement for adults is 14 mg/kg body weight /day, but it should be increased to 30 mg/kg/day in individuals who regularly participate in endurance type physical activities, since the recommended dietary intake of LEU is lower than measured whole body rates of LEU oxidation (Hood and Terjung 1990). The modulation of whole body protein by variation of dietary protein has been investigated by Bowtell et al. (1998). They found that LEU oxidation was increased by previous ingestion of a high protein diet due to increased LEU availability. Exogenous amino acids have also been shown to stimulate net muscle protein synthesis in the elderly as well as in the young (Volpi et al. 1999). Beta-hydroxy beta-methylbutyrate (HMB), a metabolite of LEU, is one of the latest dietary supplements used to enhance strength and increase lean body mass following resistance training (Slater and Jenkins 2000), by preventing exercise-induced proteolysis and/or muscle damage (Nissen et al. 1996). A low dose LEU supplementation had no effect on either blood or muscle lactate accumulation during exercise and the performance was not improved during intense exercise (Vukovich et al. 1997). However, LEU (270 mg) has been shown to stimulate muscle protein synthesis following exercise and further enhance post-exercise muscle recovery in rats (Anthony et al. 1999). It has been suggested that a mixture of BCAAs could improve both mental and physical performance and have a sparing effect on muscle glycogen degradation during

endurance exercise with low glycogen stores (Blomstrand and Newsholme 1992, Blomstrand et al. 1996), but there is only limited data concerning LEU supplementation alone (Mero 1999).

## 2.8 Age and amino acid metabolism

Aging is associated with a progressive decline in muscle mass and function (Lexell 1995) and it is characterized by decreased muscle strength and increased muscle fatigability (Hurley 1995). The cumulative decrease in skeletal muscle mass has been shown to amount to 35-40% between 20 and 80 years of age (Evans 1995), but the depletion of the muscle is compensated by the accumulation of body fat (Flegg and Lakatta 1988). Nair (1995) has suggested that the changes in body composition in the elderly, particularly a loss of fat free mass, most of which is skeletal muscle protein (Cohn et al. 1980), result from decreased levels of anabolic hormones, neuromuscular alterations and from a decrease in muscle protein turnover (Nair 1995). Volpi et al. (2001) investigated healthy men (age 28-70 years) and concluded that muscle loss with aging is not explicable by the differences in basal muscle protein turnover in the elderly. However, age-related decrease in muscle protein synthesis has been observed, probably associated with loss of muscle protein in the elderly (Welle et al. 1994). It is further unclear, whether regular physical activity and adequate protein intake can attenuate the loss of skeletal muscle with aging (Starling et al. 1999). Volpi et al. (1998a) has investigated the response of amino acid transport and protein synthesis. They found that despite decreased muscle mass in the elderly, muscle protein anabolism can be stimulated by exogenous amino acids, and thus the positive net balance of the muscle amino acids can be achieved.

It has been demonstrated that the intake of all nutrients is decreased with aging (Flynn et al. 1992). The decline in energy intake and consequently the reduced efficiency of dietary protein utilization may increase the protein need with aging (Young 1990). However, it must be taken into consideration that high protein intakes can have deleterious effects on renal function (Rowe 1980) and may enhance the risk of osteoporosis (Zhao 1994). The results, according to which high protein diet (67 g / day added with 40 g egg white protein) increased the calcium excretion and caused loss from the bone, based on the study in Chinese diet (Zhao 1994).

In conclusion, there is a need for longitudinal reference data on amino acid metabolism of elderly persons to describe the normal status and to develop valid prediction equations for estimating body composition of aging males and females (Going et al. 1995). The plasma levels of amino acids have been previously demonstrated in humans by Henry et al. (1974) with no reference with nutrition or physical activity. Therefore, there is need for data concerning

the levels associated with diet intake and daily physical activity and/or work loading.

## 2.9 Gender and amino acid metabolism

In recent years a great deal of research has been conducted in the area of protein metabolism, but there is paucity of data concerning females (Tipton 2001). Nutritional recommendations and exercise training prescriptions have been similar for both men and women, because only little is known for example about possible effects of gender on protein metabolism (Tarnopolsky 2000) despite clear differences in muscle mass and possibly in substrate metabolism between males and females (Tipton 2001). TE plays an important role in gender differences, since during puberty the levels of TE increase in the boys resulting in larger muscle mass (Ramos et al. 1998) due to increased protein synthesis and positive net muscle protein balance (Tipton 2001). Gender differences have been clearly shown in protein metabolism, but there is also evidence that whole body protein synthesis or breakdown is different between males and females (Tipton 2001). In addition Volpi et al. (1998b) have reported that in the basal post-absorptive state protein oxidation is lower in women than in men, which partly explains the difference in energy expenditure between males and females. Lamont et al. (2001) concluded that women oxidize more lipid than men during exercise. It is unclear, whether the gender-based differences in whole body fuel oxidation are related in differences in circulating substrate utilization (Tarnopolsky et al. 1990). However, some sex-related differences have been seen in the plasma concentrations of amino aids. Amino acids VAL, LEU, ILE, GLN, PHE and PRO have shown higher concentrations in men than in women (Bancel et al. 1994). Due to limited data, the exact quantification of the amino acid levels of women under controlled conditions (nutrition and exercise) is of great importance in determining gender base differences in amino acid metabolism.

# 3 PURPOSE OF THE STUDY

Based on the existing data in the literature it was hypothesised in this study that: 1) Since skeletal muscle is the major state of protein, the muscle loading by exercise or training affects the amino acid profile and there are differences in the responses between different kinds of exercise loading. 2) Ingestion of LEU supplement may play an important role in energy supply and may thus improve muscle performance. 3) A relationship exists between the three compartments (artery, vein, and muscle) of the free amino acid pool. 4) Since the skeletal muscle mass decreases with aging, changes are seen in the amino acid profile and there are also differences between males and females due to smaller muscle mass in females. Therefore, the purpose of the present study was to investigate the serum amino acid and hormonal responses to different exercise sessions and to a training period in male athletes with and without LEU supplementation. Protein synthesis, protein breakdown and protein net balance was evaluated after a resistance exercise session in physically active people. Finally, this study aimed to quantify the serum amino acid concentrations in aging men and women. The specific purposes were:

1. to compare the exercise-induced changes in the concentrations of serum amino acids between two lactic running exercise sessions and a strength exercise session in competitive male athletes.

2. to find out what kind of effects a training period has on serum amino acid concentrations and hormonal status in competitive male athletes.

3. to demonstrate whether LEU supplementation has effects on the response of serum amino acid concentrations or on physical performance during two different exercise sessions (MARE; maximal anaerobic running exercise, SES; strength exercise session) in competitive male athletes.

4. to demonstrate whether daily LEU supplementation has effects on the amino acid and hormonal responses during a 5-week training period in male athletes.

5. to find out serum and muscle concentrations of amino acids after a resistance exercise session with the simultaneous reference to net muscle protein balance in active male subjects.

6. to quantify serum amino acid concentrations in healthy untrained men and women (age range from 23 to 92 years) in the conditions where nutrition and free-time physical activity were strictly controlled.

# 4 MATERIAL AND METHODS

## 4.1 Subjects

A total of 118 men and 36 women volunteered to participate in these studies. They were healthy and their age varied between 19 and 92 years. The physical characteristics of the subjects in each experiment of the study are summarized in Table 2a. In experiments 1-4 the subjects were male Finnish national level track and field athletes (sprinters, jumpers and decathletes), in experiment 5 the subjects were healthy physically active men and in experiment 6 the subjects were non-athletic healthy (no medication, no oral contraception) men and women. The purpose and the potential risks of the studies were fully explained, and written informed consent was obtained from each participant. These studies were approved by the University Ethical Committee in Jyväskylä, Finland (experiments 1-5), by the Ethical Committee of the Central Finlands Central Hospital in Jyväskylä, Finland (experiment 5), and by Institutional Review Board of the Central Hospital of Satakunta in Pori, Finland (experiment 6).

## 4.2 Experimental design, testing procedures and analysis

### 4.2.1 Approach to the problem and experimental design

In general, protein metabolism has been under great interest for decades, but literature still lack data concerning the response of amino acid concentrations and metabolism to power-type exercise and power-type training. Despite the increased use of protein supplements among athletes, the available data about LEU alone as a possible ergogenic aid in exercise and training is scant, even though LEU has an important role in protein metabolism. In addition, more

TABLE 2a Physical characteristics of the subjects.

| Experiments | Status | Age (years) | | Height (cm) | | Weight (kg) | | Body fat (%) | | Original paper |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | |
| Exp. 1 | | | | | | | | | | |
| Men (n=11) | Competitive | 23.7 | 4.4 | 184.0 | 4.9 | 77.3 | 6.2 | 9.3 | 1.6 | I |
| Exp. 2 | | | | | | | | | | |
| Men (n=11) | Competitive | 25.4 | 5.3 | 183.2 | 4.3 | 76.0 | 5.5 | 9.1 | 1.5 | II |
| Exp. 3 | | | | | | | | | | |
| SES group, men (n=16) | Competitive | 26.0 | 4.0 | 184.2 | 4.8 | 77.0 | 6.0 | 9.3 | 1.6 | III |
| MARE group, men (n=12) | Competitive | 26.0 | 5.0 | 184.4 | 4.7 | 77.3 | 6.2 | 9.1 | 1.6 | III |
| Exp.4 | | | | | | | | | | |
| A group, men (n=10) | Competitive | 25.4 | 5.3 | 182.2 | 3.9 | 73.9 | 4.0 | 8.9 | 1.5 | IV |
| B group, men (n=10) | Competitive | 23.7 | 4.4 | 183.6 | 5.0 | 76.3 | 5.4 | 9.2 | 1.6 | IV |
| Exp. 5 | | | | | | | | | | |
| PG group, men (n=6) | Active | 26.2 | 4.7 | 181.3 | 3.2 | 81.3 | 9.2 | | | V |
| EG group, men (n=6) | Active | 25.9 | 5.1 | 182.5 | 8.4 | 86.8 | 21.7 | | | V |
| Exp. 6 | | | | | | | | | | |
| MALE 20-39 (n=12) | Untrained | 33.3 | 3.8 | 177.5 | 7.3 | 78.7 | 11.7 | 16.5 | 2.9 | VI |
| MALE 40-59 (n=12) | Untrained | 48.4 | 6.0 | 179.6 | 4.2 | 85.6 | 14.4 | 23.0 | 4.6 | VI |
| MALE >60 (n=12) | Untrained | 75.9 | 9.3 | 172.4 | 5.2 | 77.0 | 10.9 | 25.3 | 4.3 | VI |
| FEMALE 20-39 (n=12) | Untrained | 32.6 | 4.4 | 166.3 | 4.4 | 66.4 | 5.9 | 26.5 | 4.5 | VI |
| FEMALE 40-59 (n=12) | Untrained | 48.0 | 4.3 | 165.5 | 3.9 | 67.1 | 11.9 | 33.1 | 4.3 | VI |
| FEMALE >60 (n=12) | Untrained | 74.7 | 10.0 | 157.6 | 6.1 | 64.0 | 10.0 | 35.2 | 3.9 | VI |

information is needed about changes in protein metabolism with advanced age or differences between genders. This study included six separate experiments.

The first experiment compared the serum amino acid responses of the three different exercise sessions. All participants were designed to perform the short-run session (SRS), long-run session (LRS) and the strength exercise session (SES). The experiment was planned to reveal possible differences in serum amino acid responses following two different lactic exercise sessions as well as following a resistance exercise session. After the familiarization phase the subjects visited the laboratory, where the anthropometrical measurements were made and the jumping test was done after the warm-up. SRS and LRS were performed during consecutive days and SES four weeks later.

The second experiment investigated the serum amino acid responses following the high intensity running exercise sessions (SRS and LRS) before and after the 5-week training period. The hormonal changes were also included in this experiment. After the familiarization phase the subjects visited the laboratory as in experiment 1. The participants performed one SRS in the afternoon and LRS in the next morning.

Experiments 3 and 4 investigated the serum amino acid and hormonal (4) responses following exercise (3) and training (4) with LEU supplementation. The third experiment aimed to describe the effect of LEU on two different exercise sessions: an intensive SES and a maximal anaerobic running exercise session (MARE), which was performed to exhaustion. The subjects were randomised and divided in a placebo (P) or in a LEU (L) group, which consumed LEU either in two doses (50 mg/kg per body weight before and 50 mg/kg per body weight during SES) or in one dose (200 mg/kg per body weight before MARE). In addition, the physical performance was evaluated in this experiment. The fourth experiment was carried out as a randomised double-blind cross over study to investigate the effect of LEU supplementation on the amino acid concentrations and hormone profile during 5 weeks of training. The subjects consumed LEU 50 mg/kg per day or placebo in two doses: 25 mg/kg body weight in the morning and 25 mg/kg body weight in the evening immediately prior to the exercise session, which was completed twice per day (in the morning and in the evening).

The fifth experiment investigated protein metabolism during recovery. In addition to the amino acid concentrations, muscle protein kinetics was observed and the subjects were either at rest or they completed a resistance exercise session (RES). Blood samples were drawn from the femoral aretery (FA), femoral vein (FV) and antecubital vein (AV). Moreover, muscle biopsies were taken after the beginning of the infusion of stable isotopic tracer L-[*ring*-$^{2}H_{5}$] PHE. The enrichment was analysed by gas chromatography/mass spectrometry (GC/MS) and the calculations were based on the data obtained from blood samples and muscle biopsies. In experiments 1 and 3-5 one repetition maximum (1 RM; the maximum weight that can be lifted with one repetition) was measured in exercises and used during SES or RES.

The sixth experiment differed from the other experiments, since the subjects were ordinary people of different ages including both men and women. The participants were divided into six groups according to gender and age. The serum amino acid concentrations were investigated after an overnight fast at rest.

Generally, the subjects of the six experiments were familiarised with the exercise protocol for two months (1-4), for two weeks (5) or for 5 days (6) before the test/control period. The whole study was carried out under the conditions, where the pre-test physical activities and nutrition were carefully controlled and recorded with the diaries, which were later analysed. In experiments 1-4 the amount and intensity of training was controlled for one week before measurements and the subjects trained according to the given program. In experiment 5 the subjects were instructed to keep on their normal physical activity for 2 weeks before the measurements and in experiment 6 the subjects were asked to maintain their normal daily physical activity during 5 days.

The subjects recorded their food and beverage intakes for 5 days (1, 5, 6), for 1 week (3) or for 10 days (2, 4). In the experiments 1-4 the subjects were instructed to eat according to the principles of Nutrition Recommendations for Athletes in Finland (1990) and in experiments 5-6 to eat according to their normal dietary habits without any clinical complementary nutritional products. The diaries were later analysed by the researches. Table 2b summarises the experimental design and measurements of the original papers I-VI.

Table 2b Experimental design and measurements of the original papers I-VI.

| Original Paper | Analysis of Amino Acids | | Leucine Supplements | Analysis of Nutrition | Analysis of Training | Anthropometry |
|---|---|---|---|---|---|---|
| | Blood | Muscle | | | | |
| I | X | | | X | X | X |
| II | X | | | X | X | X |
| III | X | | X | X | X | X |
| IV | X | | X | X | X | X |
| V | X | X | | X | X | |
| VI | X | | | X | X | X |

### 4.2.2 Short run session (I, II), long run session (I, II) and maximal anaerobic running exercise (III)

SRS consisted of 3 x 4 x 60 m on an indoor track with recovery periods of 2 min between repetitions and 6 min between series. The speed in series gradually increased (91, 93 and 95% from the one repetition maximum).

LRS consisted of as many running repetitions of 20 s as possible on a treadmill with recoveries of 100 s between the runs. The initial treadmill speed

(4.08 m x $s^{-1}$, 4° slope) was increased by 0.38 m x $s^{-1}$ for each consecutive run until exhaustion. The speed ranged from 56 to 100% from the speed of the last run. MARE consisted of numerous bouts to exhaustion (total fatigue), i.e. n x 20 s on a treadmill with recoveries of 100 s between the runs. The initial treadmill speed and the speed range changed similarly than in LRS.

### 4.2.3 Strength exercise session (I, III) and resistance exercise session (V)

In experiments 1 and 3, SES consisted of jumps and heavy resistance exercises during 90 min (TABLE 3). The speed strength of leg extensor muscles was evaluated 5 min after SES using a counter movement jump (CMJ) on a contact mat (Newtest, Oulu, Finland) connected to a digital timer (∂0.001 s). The timer was triggered by the lift of feet from the mat and stopped at the touch-down. The flight time during the jump was thus recorded. The rise of the center of gravity in a jump was then calculated from the measured flight time (Komi and Bosco, 1978).

TABLE 3 Content of SES.

| Exercise order | Sets<br>Distance | Repetitions | Recovery between sets and exercise, min |
|---|---|---|---|
| Sprint coordination / speed | | | |
| Skipping | 25 m | 4 | 2 |
| Knee flexion | 25 m | 4 | 2 |
| Bounding | 25 m | 4 | 2 |
| Acceleration | 25 m | 4 | 2 |
| Speed Strength | | | |
| Hurdle jumps | 5 | 10 | 3 |
| 5 jumps | 6 | 5 | 3 |
| Heavy resistance | | | |
| Deep squat | 4 | 10 RM | 3 |
| Calf raises | 4 | 20 RM | 3 |
| Bench press | 4 | 10 RM | 3 |
| | | (RM = repetition maximum) | |

A heavy hypertrophic resistance exercise session (RES) of 50 minutes for lower extremities was performed in experiment 5 (TABLE 4).

TABLE 4 Content of RES.

| Exercise order | Sets | Repetitions | Recovery between sets, min | Recovery between exercises, min |
|---|---|---|---|---|
| Maximal isometric leg extension | 3 | 1 | 1 | 2 |
| Deep squat | 2 | 10 | 2 | 2 |
| Hip extension | 2 | 10 | 1.5 | 2 |
| Maximal isometric leg extension | 3 | 1 | 1 | 2 |
| One leg press | 2 | 10 | 0.5 | 2 |
| Deep squat | 2 | 10 | 2 | 2 |
| Hip extension | 2 | 10 | 1.5 | 2 |
| One leg press | 2 | 10 | 0.5 | 2 |
| Maximal isometric leg extension | 3 | 1 | 1 | |

### 4.2.4 Blood and muscle samples

#### 4.2.4.1 Analysis of blood samples (I, II, III, IV, V, VI)

In general, the use of plasma has been preferred over the use of serum in amino acid analysis (Perry and Hansen 1969). However, only minor changes in amino acid concentrations have been shown to occur during clotting (Armstrong and Stave 1973a,b,c; Katz and Keck 1977). They are significant only in the case of disulfide-containing amino acids, i.e., CYS, if the deproteinization of samples is delayed more than half an hour (Armstrong and Stave 1973a). Now this step was taken immediately after the collection of blood samples. All blood samples were treated identically and therefore their comparisons are totally valid and unaffected by the choice of serum. In the present experiments other analyses were also done from the same blood samples and then the use of serum was more favourable as a whole than the use of plasma. Moreover, there is a danger of slight hemolysis when heparin is used as an anticoagulant, and this could give rise to more serious errors, i.e., changes in the levels of ARG, ORN and CYS.

In experiments 1-4, the sample of 5 ml of blood was drawn from an AV 10 min before and 10 min after each session. In experiment 5 a catheter was inserted into left AV to take basal blood samples, and in experiment 6 the sample was taken after 10 hour of fasting. All blood samples were centrifuged for 10 min at 3500 rpm to separate cells from serum, which was immediately frozen and stored below at -20°C for the free amino acid analysis to be performed after two weeks. They were deproteinized with 5 % sulphosalicylic acid containing L-2,4-diaminobutyrate as an internal standard, mixed with lithium citrate buffer and subjected to ion-exchange chromatography using by automatic Pharmacia LKB Alpha Plus amino acid analyzer in experiments 1-4, by reversed phase high performance liquid chromatography (RPHPLC) in

experiment 5 and by Shimadzu chromatography in experiment 6. All samples were analysed in duplicate and the samples from one individual were run in the same assay to minimize interassay variability. Intrassay variation ranged from 1.7% to 2.8% for single amino acids.

TE, COR and GH were determined in duplicate by radioimmunoassay (RIA). Serum samples for TE were determined with a solid phase $^{125}$I RIA (Spectria Testosterone Coated Tube RIA Kit, Orion Diagnostica, Orion Corporation, Turku, Finland), serum concentrations of COR were determined with $^{125}$I RIA (Cortisol RIA Kit, Orion Diagnostica, Orion Corporation, Turku, Finland), and serum concentration of GH were determined with an $^{125}$I liquid-phase double-antibody procedure (Pharmacia hGH RIA, Pharmacia Diagnostigs AB, Uppsala, Sweden).

All of the samples from an individual for hormonal analysis were run in the same assay to avoid any changes in interassay variability. Intra-assay coefficients of variation were 2-5% and inter-assay coefficients of variation were 4-10% for variables.

#### 4.2.4.2 Analysis of serum free amino acid concentration in femoral artery and femoral vein (V)

Concentrations of free amino acids in serum were determined applying the procedure of Pfeifer et al. (1983) by RPHPLC (Waters 501 pumps, Waters 717 autosampler and Zorbax $C_{18}$ column). 18 essential amino acids and two internal standards (η-Abc and Nor-VAL) were detected by Perkin Elmer LS-4 fluorecent detector using wavelengths 338 nm (exitation) and 455 nm (emission). 100 σl of internal standard solution was added to the serum sample (50 σl) and acetonitrile (100 σl) was used to precipitate the proteins. 750 σl of distilled deionized water was added and the resulted sample was vortexed and allowed to stand on ice bath for 1 h. 200 σl of the sample was transferred to the ultraspin centrifuge filter and centrifuged. The clear mixture was transferred to the high performance liquid chromatography (HPLC) vial, derivatized with orto-pthaldehyde (OPA) derivatizing solution and analysed by Waters HPLC system using gradient two-buffer elution. The same researcher performed all measurements.

#### 4.2.4.3 Analysis of free amino acid concentration in muscle (V)

Concentrations of free amino acids in muscle were analysed using HPLC equipped with fluorescent detector similarly to blood samples (see above). Samples were prepared by taking at least 10 mg of muscle and adding 400 σl of 5 % perchloric acid and 100 σl of internal standard solution. After standing on an ice bath for 1 h the samples were grinded with pestles and centrifuged. Supernatants were transferred to a clean tubes and pH of the supernatants were adjusted to 5-7 by using kaliumhydroxide (KOH). After spinning 200 σl of the mixtures were transferred to the ultraspin centrifuge filter and centrifuged. The

residual matters were removed and the solutions were ready for the HPLC analysis. The same researcher performed all free muscle amino acid measurements.

### 4.2.5 Blood lactate analysis (I, II, III, IV)

Peak blood lactate was determined enzymatically (Roche Diagnostics GmbH, Mannheim, Germany) from fingertip blood samples (50 σl) taken 1 and 5 min after the sessions. The same researcher performed all blood lactate measurements and analyses in experiments 1-4.

### 4.2.6 Data collection and analysis in tracer stable isotopic measurements (V)

Subjects fasted overnight (total fasting time was 15±3 hours including overnight fasting time 10±3 hours and total experiment time of 5 hours) in order to assure as similar nutritional "baseline" as possible for each subject. They arrived the laboratory in the morning, where their body mass and height was measured. An 18-gauge polyethylene catheter was inserted into a left AV to take background blood samples. After taking background blood samples, a primed, continuous infusion of L-[*ring*-$^{2}H_5$] PHE was started and maintained throughout the day. The prime was 2 σmol/kg and the infusion rate was 0,05 σmol/kg/min. After exercise polyethylene catheters (20-gauge) were inserted into the right FA and FV, as well as an 18-gauge catheter was inserted into a right AV for drawing blood samples. The femoral arterial catheter was used for the infusion of indocyanine green (ICG) for measuring blood flow.

#### 4.2.6.1 Indocyanine green infusion (V)

The ICG infusion (0,5 mg/ml; 60 ml/h) was started at 125 minutes and blood samples for isotopic measurements and blood flow were drawn at 135, 145, 155 and 165 minutes (period 1) after the initiation of L-[*ring*-$^{2}H_5$] PHE infusion. Blood samples for free amino acid concentrations were taken at 135 and 165 minutes after the initiation of infusion. Blood flow samples were simultaneously drawn from a FV and a right AV. The ICG infusion was briefly halted to allow sampling from the FA for isotopic measurements. Blood for amino acid concentrations and enrichments was placed into preweighed tubes containing 2 ml sulfosalicylic acid and known amount of internal standard ($^{13}C_6$ PHE; 50 σmol/l). Samples were mixed carefully and stored in ice. Blood flow samples were stored at room temperature until analysis. After the last blood sample (165 minutes) a muscle biopsy was taken for isotopic measurements and for analysis of free amino acid concentration from the vastus lateralis muscle under local anesthesia. With the use of sterile technique, the skin and subcutaneous tissue were anesthetized and a 6-7 mm incision was made. A 4-mm biopsy needle was advanced 3-5 cm into the muscle with the closed cutting window. The cutting cylinder was opened and closed 2-4 times and a sample of 30-50 mg was obtained. Visible fat and connective tissue were removed and the

samples were rinsed with ice-cold saline before storing into tubes in liquid nitrogen. In period 2, ICG infusions were started 10 minutes before the first blood sample. Blood samples for isotopic measurements were taken 270, 280, 290 and 300 minutes (period 2) after the initiation of infusion. For free amino acid concentration analysis blood samples were drawn 270 and 300 minutes after the initiation of infusion. The second muscle biopsy was taken after the last blood sample.

#### 4.2.6.2 Enrichment of phenylalanine (V)

Enrichment and concentration of PHE in whole blood were measured by GC/MS (Hewlett Packard Agilent 5973N, GC 6890 Plus+, USA). In order to determine the enrichment of infused amino acid in whole blood, the *tertiary*-butyl dimethylsilyl derivative was made. Isotopic enrichments were expressed as a tracer-to-tracee ratio (TTR). Concentrations of PHE were determined with an internal standard solution as previously described. Because the tube weight and the amount of blood were known, the blood amino acid concentration was determined from the internal standard enrichment on the basis of the amount of blood and internal standard added. Appropriate corrections (overlapping) were made.

Muscle tissue samples were analyzed for intracellular amino acid enrichments. On thawing, the tissue was weighed and the protein was precipitated with 0,8 ml of 14 % percholoroacetic acid. The tissue was then homogenized and centrifuged, and the supernatant was collected. This procedure was repeated one more time and the collected supernatant was processed as blood samples.

Leg blood flow was determined from blood samples collected during a continuous infusion of ICG (Jorfeld and Wahren 1971). Serum from the blood samples was analysed in a spectrophotometer with absorbance set at 805 nm.

#### 4.2.6.3 Calculations of the three-compartment model (V)

The three-compartment model of leg muscle amino acid kinetics has been previously described by Biolo et al (1999). The use of this model allows to determine the rate of utilization of PHE for muscle protein synthesis and appearance from breakdown, because PHE is neither oxidized nor synthesized in muscle. The average values during several hours for blood flow, blood and muscle PHE concentrations, and enrichments were calculated from individual samples drawn during two periods. Net balance was determined by taking the difference between arterial and venous PHE concentration and multiplying by the blood flow.

The calculation of intracellular PHE utilization (protein synthesis) and appearance (protein breakdown) assumes that there is no de novo trace production or oxidation in the leg. Net serum balance and the muscle biopsy data assume that the muscle accounts for the leg metabolism of amino acids. It is also assumed that the tissue enrichment and amino acid concentrations are

representative of the intracellular space and that the intracellular free amino acid pool is homogenous. Also it is assumed that the free amino acid pool is the precursor for protein synthesis. The detailed model assumption has been described earlier (Biolo et al. 1992 and 1995a).

### 4.2.7 Analysis of nutrition (I, II, III, IV, V, VI)

In each experiment the subjects recorded their food and beverage intake, which was then analysed using Micro Nutrica software (version 1.0 in experiments 1-5 and 3.0 in experiment 6, Social Insurance Institution, Finland). The same researchers checked the food records and asked supplemental questions if necessary and analysed the food records in all experiments.

### 4.2.8 Anthropometrical measurements (I, II, III, IV,VI)

The bilateral skin fold measurements were done with a John Bull Skin fold caliper (British Indicators, LTD, England). Skin site readings were taken from four skin sites (subscapula, triceps brachii, biceps brachii and supra iliaca) from the upper body in experiments 1-6 and from four skin sites (calf, quadriceps femoris, hamstrings, gluteus maximus) from the lower extremities in experiments 1-4. The averaged (right and left) 8 readings together (experiments 1-4) with the reading from a trunk skin site (abdomen) formed the total sum of skin folds (Durnin and Rahaman 1967). In experiment 6, skin fold readings were taken from four skin fold points from the upper body. The same researcher performed all anthropometrical measurements (height, body weight and skin fold measurements just before the tapping of blood samples) in experiments 1-4 and 6.

### 4.2.9 Statistical methods

Statistical testing was based on the analysis of variance with repeated measures in experiments 1-5. However, the statistical models used in the analyses were based on the design of experiments and so the models in analyses differed between experiments. In experiment 6 the statistical analyses were based on analysis of variance too, but all factors in the model were perceived as between-subjects factors (no repeated measures effects). All results are presented in mean values ± SD, SE or SEM. The main idea was that the SD is a measure of the variability between individuals in the level of the factor being investigated and correspondingly SE or SEM is a measure of the uncertainty in sample statistics. In experiments 1-4 t-test type contrast examinations were used as post hoc test, correspondingly in experiment 5 Bonferoni approach and in experiment 6 Tukey HSD. In all experiments before the final analysis were performed, the assumptions of equality of group variances and multivariate

normality assumptions of errors were checked by diagnostic methods. If violations in assumptions of statistical analyses were found, data transformations were done to rectify violations. In these cases the final results of statistical tests were based on transformed variable (mostly log - transformations). In experiments 1-4 the statistical analysis was performed by means of SAS statistical package and in the experiments 5-6 by means on SPSS software package.

# 5 RESULTS

The main data from the experiments (1-6) as well as some unpublished results are presented in this section.

## 5.1 Nutrition

### 5.1.1 Nutrient intake

The mean nutrient intake in all experiments is summarised in table 5. There was a significant ($P$<0.001) difference in total energy between men and women.

TABLE 5 Daily nutrient intake in experiments I-VI.

| | Original Papers | | | | | | |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VI |
| | Male Competitive | Male Competitive | Male Competitive | Male Competitive | Male Active | Male Untrained | Female Untrained |
| Total energy (MJ) | 10.2±2.0 | 10.5±1.5 | 9.5±3.3 | 10.4±1.9 | 10.3±1.8 | 9.0±1.5 | 7.1±1.5 |
| Protein intake (g/kg BW) | 1.3±0.2 | 1.3±0.2 | 1.2±0.2 | 1.3±0.2 | 1.3±0.3 | 1.2±0.3 | 1.1±0.3 |
| Protein (%) | 15.8±1.9 | 15.3±2.0 | 16.1±4.1 | 15.6±1.9 | 18.1±4.3 | 17.7±3.3 | 18.0±3.0 |
| Fat (%) | 30.4±3.8 | 31.7±3.7 | 31.5±6.7 | 31.0±4.3 | 26.5±5.7 | 31.0±5.7 | 30.3±6.0 |
| Carbohydrate (%) | 54.0±4.7 | 53.2±4.3 | 52.3±7.7 | 53.5±4.9 | 54.2±7.0 | 48.0±8.3 | 50.3±7.0 |

Values are mean± SD
Values are average intake per day.
BW, body weight.
%, percent of total energy.

## 5.2 Amino acid responses to exercise sessions and a training period

### 5.2.1 Serum amino acid concentrations before and after different exercise sessions

The results of experiment 1 indicate that the serum concentrations of BCAAs (8.1%; $P<0.05$) and all EAAs (8.7%; $P<0.01$), except MET (3.5%; $P$ =ns), significantly decreased after SRS, whereas the concentrations of ALA (26.7%; $P<0.001$) and GLU (9.2%; $P<0.05$) increased. After LRS the sums of BCAAs (6.8%; $P<0.05$) and EAAs (8.7%; $P<0.01$) significantly decreased but only significant decreases in individual amino acids were in VAL (8.4 %; $P<0.01$), TRP (8.7%; $P<0.001$) and THR (13.9%; $P<0.001$). The sum of NEAAs significantly increased (6.9%; $P<0.05$) and ALA (25.3%), GLU (14.7%), TAU (16.0%) and ARG (20.9%) exhibited individually significant increases. SES induced remarkable decreases in total amino acids (TAAs; 14.8%; $P<0.01$), BCAAs (23.4%; $P<0.001$), EAAs (20.6%; $P<0.001$) and NEAAs (11.9%; $P<0.05$) (TABLE 6).

After a comparison between three exercise sessions, no differences were observed between SRS and LRS except in the case of ASP ($P<0.01$), whereas significant ($P<0.05$ – 0.001) differences were observed in TAAs, NEAAs, LEU, ILE, GLN, ALA, ASP, MET, GLU and HIS, when SRS and LRS were compared to SES. In addition to this, LRS versus SES differed significantly ($P<0.05$ – 0.001) in the case of BCAAs, TRP, TAU, LYS, PHE, ARG and ASP (TABLE 6).

The peak blood lactate concentration after SRS 13.8 ± 1.9 mmol/L and LRS 16.4 ± 1.3 mmol/L differed significantly ($P<0.001$) from the value of SES 2.5 ± 0.4 mmol/L (FIG. 3).

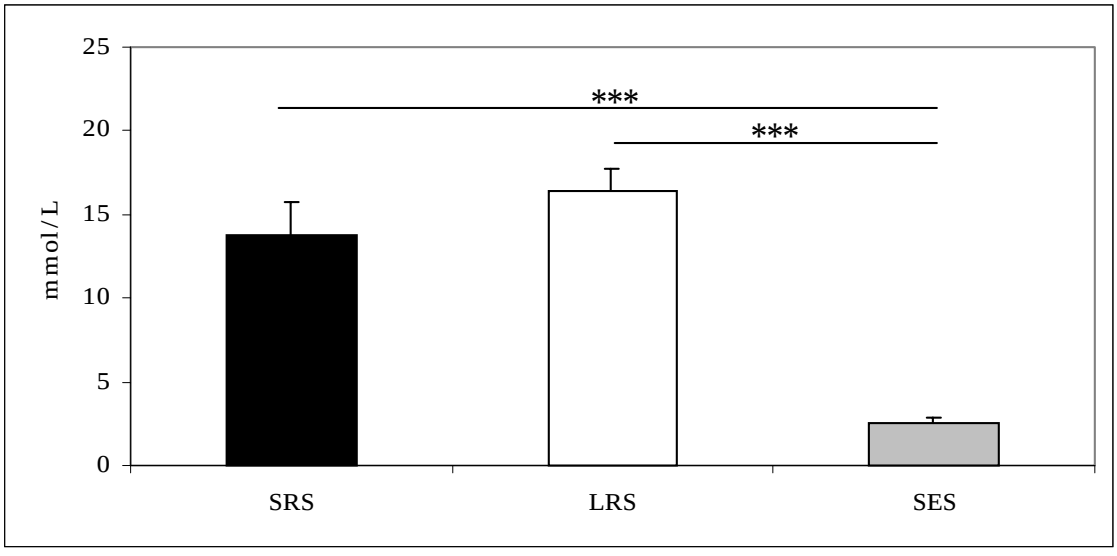


FIGURE 3 Peak blood lactate concentrations after various exercise sessions (Mean value ± SEM). ***$P<0.001$.

TABLE 6 Serum amino acid concentrations in various exercise sessions

| Amino | SRS | | LRS | | SES | |
|---|---|---|---|---|---|---|
| Acid | Before | After | Before | After | Before | After |
| TAAs | 3423± 97 | 3462± 103 | 3496± 97 | 3562± 97 | 2817± 147 | 2401± 147** |
| EAAs | 1194± 36 | 1090± 37** | 1197± 36 | 1093± 36** | 929± 53 | 738± 53*** |
| NEAAs | 2229± 72 | 2370± 75 | 2298± 72 | 2468± 72* | 1888± 106 | 1663± 106* |
| BCAAs | 518± 19 | 476± 19* | 512±19 | 477±19* | 397± 27 | 304± 27*** |
| ALA | 574± 35 | 783± 37*** | 582± 35 | 779± 35*** | 408± 50 | 412± 50 |
| ARG | 112± 8 | 103± 8 | 106± 8 | 134± 8** | 206± 11 | 174± 11* |
| ASN | 66± 4 | 61± 4 | 70± 4 | 62± 4** | 79± 6 | 64± 5** |
| ASP | 29± 1 | 26± 1* | 30± 1 | 31± 1 | 15± 2 | 10± 2** |
| CIT | 46± 3 | 44± 3 | 51± 3 | 48± 3 | 28± 4 | 24± 4 |
| GLN | 719± 23 | 723± 24 | 737± 23 | 738± 23 | 580± 33 | 495± 33* |
| GLU | 59± 3 | 65± 3* | 58± 3 | 68± 3*** | 42± 4 | 36± 4* |
| GLY | 260± 10 | 236± 10** | 279± 10 | 247± 10*** | 208± 14 | 184± 14* |
| HIS | 106± 3 | 109± 4 | 109± 3 | 114± 3 | 88± 5 | 77± 5* |
| ILE | 78± 4 | 71± 4* | 72± 4 | 68± 4 | 64± 5 | 44± 5*** |
| LEU | 155± 6 | 139± 6** | 153± 6 | 146± 6 | 128± 9 | 88± 9*** |
| LYS | 192± 9 | 176± 9* | 201± 9 | 195± 9 | 173± 13 | 134± 13*** |
| MET | 29± 1 | 28± 1 | 29± 1 | 28± 1 | 27± 2 | 22± 2*** |
| ORN | 95± 3 | 79± 3*** | 94± 3 | 79± 3*** | 90± 5 | 70± 5*** |
| PHE | 66± 2 | 61± 2* | 68± 2 | 65± 2 | 57± 3 | 46± 3*** |
| SER | 121± 5 | 109± 5** | 121± 5 | 116± 5 | 94± 7 | 77± 7* |
| TAU | 73± 6 | 71± 6 | 79± 6 | 94± 6** | 62± 9 | 53± 9 |
| THR | 153± 6 | 141± 6** | 151± 6 | 130± 6*** | 124± 8 | 106± 8** |
| TRP | 130± 5 | 99± 5*** | 128± 5 | 85± 5*** | 60± 7 | 46± 7** |
| TYR | 75± 3 | 72± 3 | 79± 3 | 74± 3* | 68± 4 | 60± 4** |
| VAL | 284± 10 | 265± 10* | 286± 10 | 262± 10** | 204± 15 | 171± 15** |

Values (μmol/L) are mean± SEM.
Significance for before-after comparisons is shown following the after values *$P$<0.05, **$P$<0.01, ***$P$<0.001.

In experiment 2 the exercise-induced changes were observed before and after the training period. There were no statistical differences in peak blood lactates when the values were compared before and after the training period. However, the peak blood lactate concentration (average of the two test occasions) was higher ($P$<0.01) following LRS (16.6±1.4 mmol/L) than following SRS (12.7±1.6 mmol/L). There were significant changes in the concentration of NEAAs and in 5 out of 21 single amino acids before the 5-week training period during SRS, whereas after training period the concentration of BCAAs, EAAs and 14 out of 21 amino acids changed significantly (TABLE 7). Following LRS the significant changes were seen in NEAAs and in 11 single amino acids before the training period and in NEAAs and in 7 out of 21 amino acids after the training period (TABLE 8).

TABLE 7 Changes in concentrations of amino acids following SRS before and after the 5-week training period (BI=before SRS and before the 5-week training period, AI=after SRS and before the 5-week training period, BII=before SRS and after the 5-week training period and AII=after SRS and after the 5-week training period).

| Amino Acid | BI Mean | SEM | AI Mean | SEM | BII Mean | SEM | AII Mean | SEM |
|---|---|---|---|---|---|---|---|---|
| EAAs | 1208 | 59 | 1130 | 59 | 1143 | 62 | 990 | 62** |
| NEAAs | 2210 | 101 | 2457 | 104* | 2102 | 106 | 2141 | 106 |
| BCAAs | 522 | 30 | 499 | 31 | 506 | 32 | 426 | 32** |
| ALA | 577 | 52 | 822 | 54*** | 555 | 55 | 718 | 55** |
| GLY | 261 | 11 | 242 | 11* | 228 | 11 | 199 | 11** |
| HIS | 106 | 6 | 114 | 6* | 106 | 6 | 106 | 6 |
| ORN | 96 | 4 | 82 | 4** | 91 | 4 | 70 | 4*** |
| TRP | 128 | 7 | 94 | 8** | 124 | 8 | 94 | 8** |

Values (µmol/L) are mean± SEM.
Significance for before-after comparisons is shown following the after values *$P$<0.05, **$P$<0.01, ***$P$<0.001.

TABLE 8 Changes in concentrations of amino acids following LRS before and after the 5-week training period (BI=before LRS and before the 5-week training period, AI=after LRS and before the 5-week training period, BII=before LRS and after the 5-week training period and AII=after LRS and after the 5-week training period).

| Amino Acid | BI Mean | SEM | AI Mean | SEM | BII Mean | SEM | AII Mean | SEM |
|---|---|---|---|---|---|---|---|---|
| NEAAs | 2190 | 73 | 2472 | 73** | 2052 | 75 | 2279 | 75* |
| ALA | 562 | 38 | 788 | 38*** | 514 | 39 | 748 | 39*** |
| ARG | 101 | 12 | 139 | 12* | 105 | 13 | 99 | 13 |
| ASN | 66 | 4 | 58 | 4* | 65 | 4 | 57 | 4* |
| GLU | 58 | 4 | 69 | 4** | 44 | 4 | 56 | 4*** |
| GLY | 267 | 8 | 248 | 8** | 252 | 9 | 221 | 9** |
| HIS | 107 | 5 | 115 | 5* | 104 | 5 | 112 | 5* |
| ORN | 93 | 3 | 79 | 3** | 75 | 3 | 67 | 3 |
| TAU | 76 | 8 | 94 | 8* | 62 | 8 | 78 | 8 |
| TRP | 120 | 8 | 79 | 8*** | 130 | 9 | 101 | 9** |
| THR | 143 | 7 | 128 | 7* | 135 | 7 | 121 | 7* |
| VAL | 285 | 16 | 264 | 16* | 268 | 17 | 253 | 17 |

Values (µmol/L) are mean± SEM.
Significance for before-after comparisons is shown following the after values *$P$<0.05, **$P$<0.01, ***$P$<0.001.

The second comparison was made in order to find out differences in the range of exercise session induced relative changes before and after the 5-week training period. Following SRS significant decreases were found in VAL from -7.60 ∂ 10.5 σmol/L to -38.2 ∂ 10.6 σmol/L ($P$=0.048) 95% confidence interval (CI) from -61.0 to -0.27 σmol/L, in ASP from -1.48 ∂ 2.42 σmol/L to -9.80 ∂ 2.43 σmol/L ($P$=0.029), 95% CI from -15.6 to -1.08 σmol/L and in TAU from 2.73 ∂ 6.62 σmol/L to -16.6 ∂ 6.68 σmol/L ($P$=0.030), 95% CI from -36.17 to -2.40 σmol/L. There were no significant changes in amino acids following LRS.

### 5.2.2 Fasting serum amino acid concentrations before and after a training period

When the fasting amino acid concentrations were compared before and after the 5-week training period (exp. 2) decreases were seen in the levels of TAAs from 3648 ∂ 870 σmol/L to 2941 ∂ 249 σmol/L ($P$<0.05; 19.4%), BCAAs from 598 ∂ 162 σmol/L to 491 ∂ 79.5 σmol/L ($P$<0.05; 17.9%), EAAs from 1364 ∂ 86.7 σmol/L to 1121 ∂ 72.0 σmol/L ($P$<0.01; 17.8%) and NEAAs from 2284 ∂ 254 σmol/L to 1820 ∂ 198 σmol/L ($P$<0.01; 20.3%) (FIG. 4). In addition, the decreases were observed in the fasting concentrations of the single amino acids, in 14 out of 21 amino acids the decrease was significant.

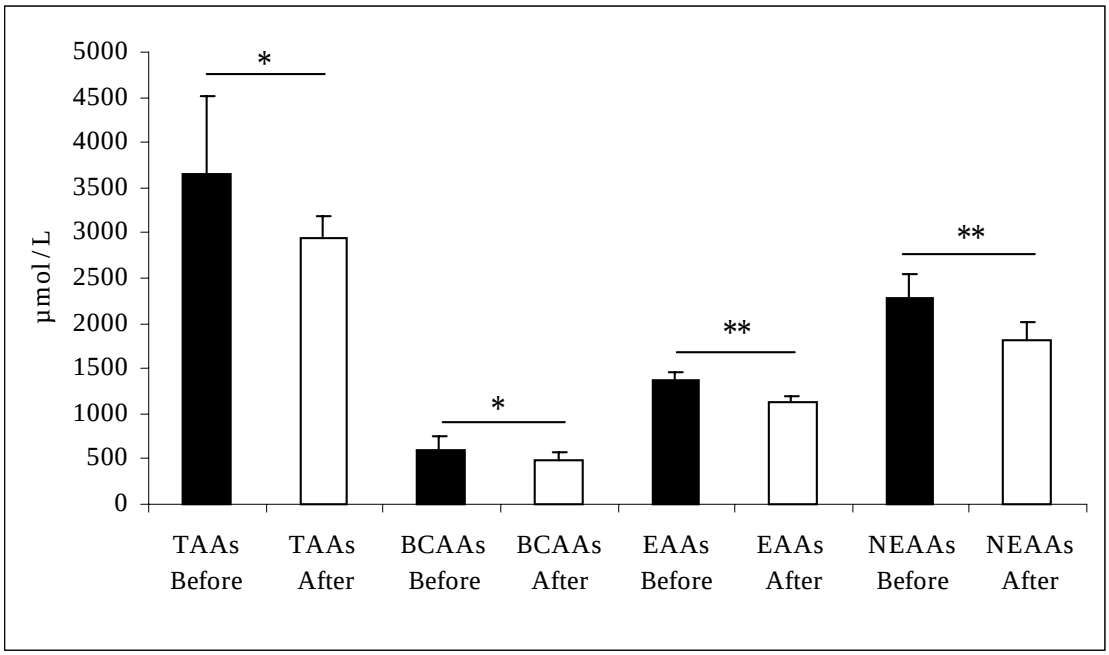


FIGURE 4 Fasting concentrations of serum amino acids during a 5-week training period (mean values± SEM). *$P$<0.05, **$P$<0.01.

## 5.3 Amino acid responses to exercise sessions and to a training period with leucine supplementation

### 5.3.1 Exercise-induced changes in serum amino acid concentrations with leucine supplementation

In experiment 3 during SES the treatment (LEU supplementation) had an effect on LEU ($P$=0.004), ILE ($P$=0.017), VAL ($P$=0.001) and TAU ($P$=0.037). The concentration of LEU, BCAAs and EAAs was greater in L than in P in both before (B; $P$<0.001) and after (A; $P$<0.001) samples. Following SES the concentration of LEU decreased with placebo ($P$=0.005) but not with LEU supplementation. The BCAAs decreased significantly only in P, but the EAAs and TAAs decreased significantly in both groups (TABLE 9).

TABLE 9 Serum amino acid concentrations (µmol / L) before and after SES.

| | Leucine group (L) | | | | | Placebo group (P) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Before | | After | | Diff. | Before | | After | | Diff. |
| | Mean | SEM | Mean | SEM | | Mean | SEM | Mean | SEM | |
| TAAs | 2932 | 140 | 2633 | 140 | $P$ =0.020 | 2820 | 137 | 2431 | 137 | $P$ =0.003 |
| EAAs | 1142 | 60 | 961 | 60 | $P$ =0.011 | 979 | 59 | 785 | 59 | $P$ =0.006 |
| NEAAs | 1788 | 90 | 1673 | 90 | $P$ =0.077 | 1841 | 88 | 1646 | 88 | $P$ =0.006 |
| BCAAs | 591 | 37 | 502 | 37 | $P$ =0.064 | 430 | 36 | 329 | 36 | $P$ =0.033 |
| ALA | 371 | 32 | 401 | 32 | $P$ =0.170 | 419 | 31 | 433 | 31 | $P$ =0.490 |
| ARG | 95 | 5 | 82 | 4 | $P$ =0.003 | 92 | 4 | 72 | 4 | $P$ <0.001 |
| ASN | 99 | 10 | 74 | 10 | $P$ =0.010 | 99 | 9 | 74 | 9 | $P$ =0.010 |
| ASP | 17 | 1 | 12 | 1 | $P$ <0.001 | 17 | 1 | 11 | 1 | $P$ <0.001 |
| CIT | 29 | 2 | 30 | 2 | $P$ =0.929 | 28 | 2 | 26 | 2 | $P$ =0.272 |
| GLN | 569 | 33 | 552 | 33 | $P$ =0.482 | 578 | 33 | 517 | 33 | $P$ =0.021 |
| GLU | 45 | 3 | 37 | 3 | $P$ =0.004 | 47 | 3 | 37 | 3 | $P$ =0.002 |
| GLY | 203 | 12 | 178 | 12 | $P$ =0.005 | 210 | 12 | 188 | 12 | $P$ =0.009 |
| HIS | 96 | 5 | 86 | 4 | $P$ =0.014 | 92 | 4 | 80 | 4 | $P$ =0.002 |
| ILE | 64 | 6 | 25 | 6 | $P$ <0.001 | 69 | 5 | 48 | 5 | $P$ =0.002 |
| LEU | 318 | 24 | 354 | 23 | $P$ =0.266 | 139 | 22 | 97 | 22 | $P$ =0.005 |
| LYS | 176 | 13 | 147 | 13 | $P$ =0.003 | 176 | 12 | 139 | 12 | $P$ <0.001 |
| MET | 29 | 2 | 22 | 2 | $P$ <0.001 | 29 | 2 | 24 | 2 | $P$ =0.002 |
| ORN | 112 | 7 | 93 | 7 | $P$ <0.003 | 102 | 7 | 79 | 7 | $P$ <0.001 |
| PHE | 63 | 4 | 48 | 3 | $P$ <0.001 | 61 | 3 | 50 | 3 | $P$ <0.001 |
| SER | 99 | 6 | 82 | 6 | $P$ =0.001 | 103 | 6 | 86 | 6 | $P$ =0.001 |
| TAU | 73 | 6 | 70 | 6 | $P$ =0.477 | 71 | 6 | 58 | 6 | $P$ =0.001 |
| THR | 125 | 8 | 105 | 8 | $P$ =0.001 | 132 | 7 | 113 | 7 | $P$ =0.002 |
| TRP | 59 | 5 | 51 | 5 | $P$ =0.090 | 60 | 4 | 50 | 4 | $P$ =0.036 |
| TYR | 79 | 5 | 63 | 5 | $P$ <0.001 | 77 | 5 | 66 | 5 | $P$ =0.004 |
| VAL | 209 | 15 | 122 | 15 | $P$ <0.001 | 222 | 14 | 184 | 14 | $P$ =0.006 |

Values (µmol / L) are mean ± SEM.

Interactions (FIG. 5) between treatment and exercise were as follows: LEU ($P$<0.001), ILE ($P$=0.017), VAL ($P$=0.006), and ARG ($P$=0.020).

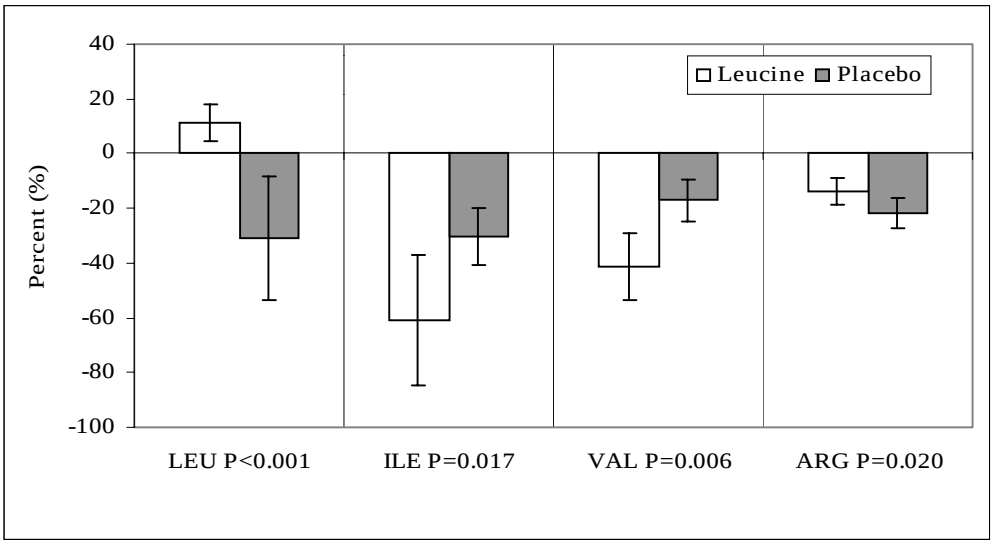


FIGURE 5 Relative changes in concentration of LEU, ILE, VAL and ARG in both groups following SES (before-after comparison between groups, mean values ± SEM).

There were no significant differences in peak blood lactate (2.5∂0.4 mmol/L in L and 2.4∂0.8 mmol/L in P) or in CMJ (0.55∂0.03 m in L and 0.56∂0.03 m in P) following SES.

During MARE the treatment (LEU supplementation) had an effect on LEU ($P$<0.001), BCAAs ($P$=0.005) and EAAs ($P$=0.009). The concentration of LEU, BCAAs and EAAs was greater in L than in P in both B and A samples as follows: LEU (B: $P$<0.001; A: $P$<0.001), BCAAs (B: $P$=0.003; A: $P$=0.001), EAAs (B: $P$=0.007; A: $P$=0.007). Following MARE the concentration of LEU increased in L (28%; $P$< 0.001) but not in P (TABLE 10).

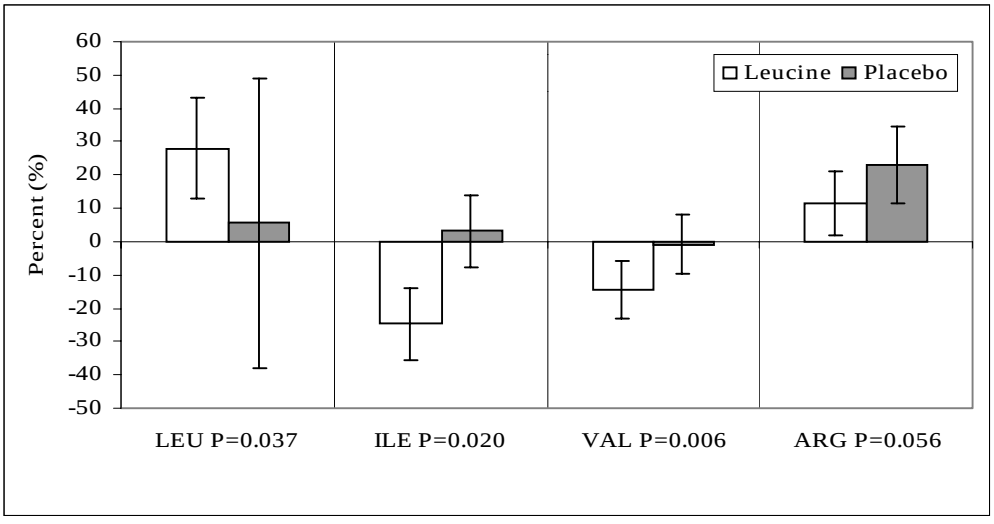


FIGURE 6 Relative changes in concentration of LEU, ILE, VAL and ARG in both groups following MARE (before-after comparison between groups, mean values ± SEM).

Interactions between treatment and exercise were as follows: LEU ($P$=0.037), ILE ($P$=0.020), VAL ($P$=0.006) and ARG ($P$ =0.056) (FIG. 6). There was a similar significant exercise-induced increase in both groups ($P$=0.002 in L and $P$<0.001 in P) in ALA following MARE. Running velocity and peak blood lactate at the end of MARE were similar in L and P (7.66∂0.28 m/s, 20.7∂2.2 mmol/L and 7.63∂0.29 m/s, 20.7∂2.4 mmol/L in L and P, respectively).

TABLE 10 Serum amino acid concentrations (µmol/L) before and after MARE.

| | Leucine group (L) | | | | | Placebo group (P) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Before | | After | | Diff. | Before | | After | | Diff. |
| | Mean | SEM | Mean | SEM | | Mean | SEM | Mean | SEM | |
| TAAs | 3560 | 305 | 3734 | 305 | $P$ =0.120 | 3221 | 305 | 3482 | 306 | $P$ =0.031 |
| EAAs | 1404 | 142 | 1457 | 142 | $P$ =0.340 | 1044 | 142 | 1037 | 142 | $P$ =0.890 |
| NEAAs | 2156 | 177 | 2277 | 177 | $P$ =0.120 | 2177 | 177 | 2417 | 178 | $P$ =0.006 |
| BCAAs | 837 | 93 | 934 | 93 | $P$ =0.019 | 484 | 93 | 494 | 93 | $P$ =0.660 |
| ALA | 429 | 40 | 526 | 40 | $P$ =0.002 | 462 | 40 | 579 | 40 | $P$ <0.001 |
| ARG | 104 | 10 | 116 | 10 | $P$ =0.070 | 87 | 10 | 107 | 10 | $P$ =0.006 |
| ASN | 88 | 8 | 75 | 8 | $P$ =0.003 | 95 | 8 | 88 | 8 | $P$ =0.063 |
| ASP | 19 | 2 | 19 | 2 | $P$ =0.920 | 19 | 2 | 21 | 2 | $P$ =0.046 |
| CIT | 37 | 5 | 39 | 5 | $P$ =0.046 | 41 | 5 | 43 | 5 | $P$ =0.230 |
| GLN | 666 | 55 | 702 | 55 | $P$ =0.120 | 654 | 55 | 707 | 56 | $P$ =0.028 |
| GLU | 70 | 6 | 82 | 6 | $P$ =0.022 | 67 | 6 | 81 | 6 | $P$ =0.006 |
| GLY | 236 | 22 | 215 | 22 | $P$ =0.016 | 238 | 22 | 236 | 22 | $P$ =0.820 |
| ILE | 65 | 7 | 49 | 7 | $P$ =0.006 | 64 | 7 | 66 | 7 | $P$ =0.700 |
| LEU | 528 | 79 | 676 | 79 | $P$ <0.001 | 181 | 79 | 191 | 79 | $P$ =0.790 |
| HIS | 101 | 9 | 107 | 9 | $P$ =0.120 | 98 | 9 | 110 | 9 | $P$ =0.003 |
| LYS | 244 | 36 | 251 | 36 | $P$ =0.300 | 241 | 36 | 253 | 36 | $P$ =0.110 |
| MET | 30 | 2 | 28 | 2 | $P$ =0.230 | 28 | 2 | 29 | 2 | $P$ =0.610 |
| ORN | 121 | 10 | 117 | 10 | $P$ =0.310 | 120 | 10 | 114 | 10 | $P$ =0.120 |
| PHE | 68 | 9 | 60 | 9 | $P$ =0.054 | 63 | 9 | 62 | 9 | $P$ =0.890 |
| SER | 107 | 9 | 95 | 9 | $P$ =0.017 | 111 | 9 | 109 | 9 | $P$ =0.590 |
| TAU | 93 | 11 | 105 | 11 | $P$ =0.270 | 97 | 11 | 131 | 11 | $P$ =0.027 |
| THR | 134 | 10 | 119 | 10 | $P$ =0.004 | 140 | 10 | 135 | 10 | $P$ =0.300 |
| TRP | 92 | 9 | 64 | 9 | $P$ =0.002 | 88 | 9 | 64 | 9 | $P$ =0.007 |
| TYR | 84 | 11 | 79 | 11 | $P$ =0.150 | 89 | 11 | 88 | 11 | $P$ =0.820 |
| VAL | 245 | 21 | 210 | 21 | $P$ =0.008 | 240 | 21 | 238 | 21 | $P$ =0.900 |

Values (µmol/L) are mean ± SEM.

Summary of acute changes of amino acid concentrations before and after LRS or MARE and SES with or without LEU supplements during experiments 1 and 3 are presented in tables 11 and 12.

TABLE 11 Summary of acute changes of TAA, EAA, NEAA and BCAA concentrations (µmol/L) before and after LRS or MARE and SES with or without LEU supplements.

| | | LRS/MARE | | | | | SES | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Original | | Before | | | After | | Before | | | After | |
| Paper | | Mean | SE | Change | Mean | SE | Mean | SE | Change | Mean | SE |
| I | TAAs | 3496 | 100 | | 3562 | 100 | 2817 | 147 | ▼ | 2401 | 147** |
| | EAAs | 1197 | 36 | ▼ | 1093 | 36** | 929 | 53 | ▼ | 738 | 53*** |
| | NEAAs | 2298 | 72 | ▲ | 2468 | 72* | 1888 | 106 | ▼ | 1663 | 106* |
| | BCAAs | 512 | 19 | ▼ | 477 | 19* | 397 | 27 | ▼ | 304 | 27*** |
| III | TAAs | 3221 | 305 | ▲ | 3482 | 306* | 2820 | 137 | ▼ | 2431 | 137** |
| (Placebo) | EAAs | 1044 | 142 | | 1037 | 142 | 979 | 59 | ▼ | 785 | 59** |
| | NEAAs | 2177 | 177 | ▲ | 2417 | 178** | 1841 | 88 | ▼ | 1646 | 88** |
| | BCAAs | 484 | 93 | | 494 | 93 | 430 | 36 | ▼ | 329 | 36* |
| III | TAAs | 3560 | 305 | | 3734 | 305 | 2932 | 140 | ▼ | 2633 | 140* |
| (Leucine) | EAAs | 1404 | 142 | | 1457 | 142 | 1142 | 60 | ▼ | 961 | 60* |
| | NEAAs | 2156 | 177 | | 2277 | 177 | 1788 | 90 | | 1673 | 90 |
| | BCAAs | 837 | 93 | ▲ | 934 | 93* | 591 | 37 | | 502 | 37 |

Values (µmol/L) are mean ± SE.
Significance for before-after comparisons is shown following the after values *$P$<0.05, **$P$<0.01 ***$P$<0.001
LRS or MARE; both exercises consisted of numerous bouts to exhaustion (total fatigue), i.e., n x 20 seconds on a treadmill with recoveries of 100 seconds between the runs
^ Increase
ˆ Decrease

TABLE 12 Summary of acute changes of ALA, ILE, LEU and VAL concentrations (µmol/L) before and after LRS or MARE and SES with or without LEU supplements.

| | | LRS or MARE | | | | | SES | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Original | | Before | | | After | | Before | | | After | |
| paper | | Mean | SE | Change | Mean | SE | Mean | SE | Change | Mean | SE |
| I | ALA | 582 | 35 | ▲ | 779 | 35*** | 408 | 50 | | 412 | 50 |
| | ILE | 72 | 4 | | 68 | 4 | 64 | 5 | ▼ | 44 | 5*** |
| | LEU | 153 | 6 | | 146 | 6 | 128 | 9 | ▼ | 88 | 9*** |
| | VAL | 286 | 10 | ▼ | 262 | 10** | 204 | 15 | ▼ | 171 | 15** |
| III | ALA | 462 | 40 | ▲ | 579 | 40*** | 419 | 31 | | 433 | 31 |
| (Placebo) | ILE | 64 | 7 | | 66 | 7 | 69 | 5 | ▼ | 48 | 5*** |
| | LEU | 181 | 79 | | 191 | 79 | 139 | 22 | ▼ | 97 | 22** |
| | VAL | 240 | 21 | | 238 | 21 | 222 | 14 | ▼ | 184 | 14** |
| III | ALA | 429 | 40 | ▲ | 526 | 40** | 371 | 32 | | 401 | 32 |
| (Leucine) | ILE | 65 | 7 | ▼ | 49 | 7** | 64 | 6 | ▼ | 25 | 6*** |
| | LEU | 528 | 79 | ▲ | 676 | 79*** | 318 | 24 | | 354 | 23 |
| | VAL | 245 | 21 | ▼ | 210 | 21** | 209 | 15 | ▼ | 122 | 15*** |

Values (µmol/L) are mean ± SE.
Significance for before-after comparisons is shown following the after values *$P$<0.05, **$P$<0.01 ***$P$<0.001
LRS or MARE; both exercises consisted of numerous bouts to exhaustion (total fatigue), i.e., n x 20 seconds on a treadmill with recoveries of 100 seconds between the runs
^ Increase
ˆ Decrease

### 5.3.2 Training-induced changes in fasting serum amino acid concentrations without and with leucine supplementation

In experiment 4 there were initially no significant differences in EAAs, NEAAs and BCAAs between the two test groups. During the first 5 weeks of training the sum of BCAAs decreased ($P$<0.05; 15.6%), including decreases in ILE ($P$<0.001; 25.3%), LEU ($P$=NS; 1.1%) and VAL ($P$<0.001; 21.4%) in L group, whereas P group showed decreases in BCAAs ($P$<0.05; 20.6%), ILE ($P$<0.05; 20.1%), LEU ($P$<0.05; 17.5%) and VAL ($P$<0.05; 17.5%), respectively. During the next 5 weeks no statistical differences were seen in BCAAs between L and P groups. However, after the 10-week training period decreases were seen in TAAs ($P$<0.01; 21.2%), EAAs ($P$<0.01; 19.1%), NEAAs ($P$<0.05; 22.4%) and BCAAs ($P$<0.01; 19.5%) in all subjects. The decreases were more pronounced during the first 5 weeks, since 14 out of 21 amino acids decreased significantly, whereas during the second 5 weeks 12 out of 21 amino decreased and 4 out of 21 amino acids increased significantly. Summary of significant changes in fasting amino acid concentrations during experiments 2 and 4 are completed in table 13 during the first 5 weeks.

TABLE 13 Summary of significant changes in fasting serum amino acid concentrations (µmol/L) during a 5-week training period without (II) or with (IV) leucine supplementation.

| Original paper | Amino Acid | Change | P-values | Original paper | Amino Acid | Change | *P* -values |
|---|---|---|---|---|---|---|---|
| II | ALA | ▼ | *P* <0.05 | IV | ALA | ▼ | *P* <0.05 |
| | ARG | ▼ | *P* <0.01 | | ARG | ▼ | *P* <0.05 |
| | CIT | ▼ | *P* <0.01 | | CIT | ▼ | *P* <0.05 |
| | GLN | ▼ | *P* <0.05 | | GLN | ▼ | *P* <0.01 |
| | GLU | ▼ | *P* <0.001 | | HIS | ▼ | *P* <0.05 |
| | GLY | ▼ | *P* <0.05 | | ILE | ▼ | *P* <0.01 |
| | HIS | ▼ | *P* <0.01 | | LYS | ▼ | *P* <0.05 |
| | ILE | ▼ | *P* <0.05 | | MET | ▼ | *P* <0.05 |
| | LEU | ▼ | *P* <0.05 | | ORN | ▼ | *P* <0.01 |
| | MET | ▼ | *P* <0.05 | | SER | ▼ | *P* <0.05 |
| | ORN | ▼ | *P* <0.01 | | THR | ▼ | *P* <0.01 |
| | THR | ▼ | *P* <0.05 | | VAL | ▼ | *P* <0.001 |
| | TRP | ▼ | *P* <0.01 | | | | |
| | VAL | ▼ | *P* <0.01 | | | | |

ˆ Decrease

## 5.4 Hormone concentrations before and after exercise sessions and a training period

### 5.4.1 Exercise-induced changes in hormone concentrations

In experiment 2 the hormonal status was measured before and after a 5-week training period. The comparison during the first and the second test occasion (mean values of the exercise session induced changes) revealed no significant changes following SRS, but following LRS there were significant increases in TE 7.98 ∂ 1.86 nmol/L ($P$=0.002; 30.4%), 95% CI from 3.76 to 12.19 nmol/L, COR 103 ∂ 28.6 σmol/L ($P$=0.006; 12.0%), 95% CI from 37.9 to167 σmol/L and testosterone/corticol –ratio (TE/COR –ratio) 0.006 ∂ 0.003 ($P$=0.047; 21.0%), 95% CI from 0.000 to 0.013. No significant changes were observed in the concentration of GH following SRS or LRS during the 5-week training period. No differences were either detected in hormonal responses following SRS and LRS, when the range of the relative changes was compared before and after the 5-week training period.

### 5.4.2 Training-induced changes in fasting hormone concentrations

In experiment 2 the fasting level of TE increased from 17.6 ∂ 3.5 nmol/L to 23.3 ∂ 5.2 nmol/L ($P$<0.01; 24.5%) after the 5-week period. There were no significant changes in the fasting levels of COR, TE/COR –ratio and GH.

### 5.4.3 Training-induced changes in fasting hormone concentrations with leucine supplementation

In experiment 4 the fasting level of TE changed significantly ($P$<0.01) in both groups during 10 weeks. At first the level of TE increased during the first 5 weeks and then decreased during the second 5-week period. However, there was a significant difference between the test groups on all test occasions. In addition, during the first 5 weeks there was seen an increase in the serum level of COR, but no significant change in TE/COR –ratio or in the serum level of GH.

## 5.5 Protein and amino acid metabolism after a resistance exercise session

### 5.5.1 Serum amino acid concentrations

The statistical significances of main effect of group and the interactions (exp. 5) are presented in Table 14. The amino acid concentrations during all measurements in all subjects were as follows (mean ± SE): ALA in FV 157±8 µmol/L and in FA 127±8 µmol/L (*P*{0.001), ASN in FV 24±1 µmol/L and in FA 21±1 µmol/L (*P*{0.001), ASP in FV 7±1 µmol/L and in FA 8±1 µmol/L, LEU in FV 66±3 µmol/L and in FA 65±3 µmol/L, MET in FV 15±1 µmol/L and in FA 13±1 µmol/L (*P*{0.001) and THR in FV 49±3 µmol/L and in FA 46±3 µmol/L (*P*{0.05). The results revealed that compared with control group (CG) (FV: 5±2 µmol/L; FA: 6±2 µmol/L), exercise group (EG) had the significantly (*P*{0.05) higher concentration (FV: 10±2 µmol/L; FA: 11±2 µmol/L) in ASP. The concentration in LEU was lower (*P*<0.05) in EG (FV: 59±4 µmol/L; FA: 59±4 µmol/L) than in CG (FV: 73±4 µmol/L; FA: 71±4 µmol/L) (TABLE 11). FV concentrations of ASN and THR were significantly (*P*<0.05) higher in CG than in EG compared to FA. The concentrations of ALA and MET were significantly higher (*P*<0.05) in EG than in CG during both periods and in both vessels. In addition, there was a significant interaction between group x vessel x sample (*P*{0.05) in ASP.

TABLE 14 Significant differences of main effect of group (G) and interactions of vessel (V) and samples (S) in serum.

| Amino Acid | G | G*V | G*S | G*V*S |
|---|---|---|---|---|
| ALA | | | P<0.05 | |
| ASN | | P<0.05 | | |
| ASP | P<0.05 | | | P<0.05 |
| LEU | P<0.05 | | | |
| MET | | | P<0.05 | |
| THR | | P<0.05 | | |

In addition, there were no significant differences between the groups in fasting free amino acid concentrations in AV (0 min samples before study period; TABLE 15). At 300 min the sum of free BCAAs in FV was significantly (*P*<0.01) lower in EG than in CG (TABLE 15). There was a significantly higher

concentration of ALA at 135 min ($P$<0.01) and 165 min ($P$<0.05) in FA and at 135 min ($P$<0.01) in FV in EG than in CG (TABLE 16).

TABLE 15 Concentrations of free amino acids in the AV, FA and FV, and in muscle of the EG and CG.

| | EG | | | | CG | | | |
|---|---|---|---|---|---|---|---|---|
| Amino Acid | AV (µmol/L) | FA (µmol/L) | FV (µmol/L) | Muscle (µmol/kg) | AV (µmol/L) | FA (µmol/L) | FV (µmol/L) | Muscle (µmol/kg) |
| TAAs | | | | | | | | |
| 0 min | 1319 ± 64 | | | | 1357 ± 64 | | | |
| 135 min | | 1318 ± 69 | 1328 ± 74 | | | 1195 ± 69 | 1259 ± 74 | |
| 165 min | | 1211 ± 57 | 1264 ± 63 | 78 ± 6 | | 1209 ± 57 | 1262 ± 63 | 68 ± 6 |
| 270 min | | 1104 ± 70 | 1148 ± 65 | | | 1122 ± 70 | 1159 ± 65 | |
| 300 min | | 1123 ± 63 | 1186 ± 92 | 78 ± 8 | | 1110 ± 63 | 1256 ± 92 | 62 ± 9 |
| EAAs | | | | | | | | |
| 0 min | 644 ± 36 | | | | 650 ± 36 | | | |
| 135 min | | 549 ± 35 | 571 ± 34 | | | 584 ± 35 | 620 ± 36 | |
| 165 min | | 540 ± 31 | 548 ± 31 | 12 ± 2 | | 575 ± 31 | 602 ± 31 | 13 ± 2 |
| 270 min | | 515 ± 38 | 512 ± 31 | | | 541 ± 38 | 534 ± 31 | |
| 300 min | | 505 ± 30 | 508 ± 37 | 12 ± 1 | | 522 ± 30 | 586 ± 37 | 11 ± 1 |
| NEAAs | | | | | | | | |
| 0 min | 675 ± 34 | | | | 707 ± 34 | | | |
| 135 min | | 769 ± 52 | 758 ± 59 | | | 611 ± 52 | 639 ± 59 | |
| 165 min | | 671 ± 34 | 716 ± 49 | 65 ± 5 | | 635 ± 34 | 660 ± 49 | 55 ± 5 |
| 270 min | | 589 ± 45 | 636 ± 43 | | | 580 ± 45 | 625 ± 43 | |
| 300 min | | 617 ± 44 | 678 ± 63 | 66 ± 7 | | 588 ± 44 | 670 ± 63 | 51 ± 8 |
| BCAAs | | | | | | | | |
| 0 min | 254 ± 14 | | | | 237 ± 14 | | | |
| 135 min | | 203 ± 15 | 214 ± 14 | | | 219 ± 15 | 230 ± 14 | |
| 165 min | | 196 ± 12 | 195 ± 12 | 2.6 ± 0.3 | | 224 ± 12 | 226 ± 12 | 2.9 ± 0.3 |
| 270 min | | 199 ± 11 | 195 ± 12 | | | 218 ± 11 | 209 ± 12 | |
| 300 min | | 195 ± 12 | 184 ± 9** | 2.4 ± 0.3 | | 217 ± 12 | 230 ± 9** | 2.7 ± 0.4 |

Values (µmol/L) are mean ± SEM. Significant difference between EG and CG (**$P$<0.01).

### 5.5.2 Muscle amino acid concentrations

There was a significantly higher ($P$<0.001) concentration of ALA in EG than in CG after RES at 165 min (TABLE 16). In addition, a significant ($P$<0.05) interaction in group x sample (time effect: period 1; 165 min versus period 2; 300 min) was observed for ALA. All results in the concentrations of ALA and in the sums of muscle free amino acids are presented in TABLE 15 and 16.

TABLE 16 Concentrations of ALA in the AV, FA and FV, and in muscle of the EG and CG.

| | EG | | | | CG | | | |
|---|---|---|---|---|---|---|---|---|
| Amino Acid | AV (µmol/L) | FA (µmol/L) | FV (µmol/L) | Muscle (µmol/kg) | AV (µmol/L) | FA (µmol/L) | FV (µmol/L) | Muscle (µmol/kg) |
| ALA | | | | | | | | |
| 0 min | 146 ± 12 | | | | 170 ± 12 | | | |
| 135 min | | 207 ± 17** | 218 ± 15** | | | 118 ± 17 | 151 ± 15 | |
| 165 min | | 171 ± 13* | 196 ± 16 | 20 ± 1*** | | 119 ± 13 | 154 ± 16 | 12 ± 1 |
| 270 min | | 101 ± 10 | 133 ± 11 | | | 99 ± 10 | 133 ± 11 | |
| 300 min | | 100 ± 11 | 126 ± 16 | 14 ± 2 | | 104 ± 11 | 149 ± 16 | 11 ± 2 |

Values (µmol/L) are mean ± SEM. Significant difference between EG and CG (*$P$<0.05, **$P$<0.01,***$P$<0.001).

### 5.5.3 Correlation coefficients between serum free amino acid and muscle free amino acid concentrations

There was no significant correlation in the amino acid concentrations between blood and muscle the coefficiens beeing as follows: in TAAs r was -0.39 (FV vs. muscle) and -0.23 (FA vs. muscle) during period 1 and -0.39 and –0.27 during period 2, in EAAs r was -0.41 and -0.14 during period 1 and –0.07 and –0.33 during period 2, in NEAAs r was -0.09 and -0.04 during period 1 and 0.45 and –0.10 during period 2 and in BCAAs r was -0.11 and 0.13 during period 1 and –0.14 and –0.03 during period 2, respectively. There were no significant correlation coefficients either in ALA concentrations between blood and muscle, since in period 1, r was 0.30 (FV vs. muscle) and 0.53 (FA vs. muscle) and in period 2, r was -0.23 and –0.12, respectively.

### 5.5.4 Leg blood flow

Mean blood flow in leg was significantly ($P$<0.01) higher in EG (period 1: 6.10 ∂ 0.48 ml ⌠min$^{-1}$ ⌠100 ml leg volume$^{-1}$ and period 2: and 5.58 ∂ 0.18 ml ⌠min$^{-1}$ ⌠100 ml leg volume$^{-1}$) than in CG (period 1: 3.62 ∂ 0.17 ml ⌠min$^{-1}$ ⌠100 ml leg volume$^{-1}$ and period 2: 3.96 ∂ 0.22 ml ⌠min$^{-1}$ ⌠100 ml leg volume$^{-1}$) during both periods.

### 5.5.5 Muscle protein synthesis, breakdown and net balance

In period 1 there were no significant differences between the groups in muscle protein synthesis, breakdown or net balance. However, muscle protein synthesis and protein breakdown were significantly ($P$<0.05) increased in EG compared to CG during period 2 (FIG. 7).

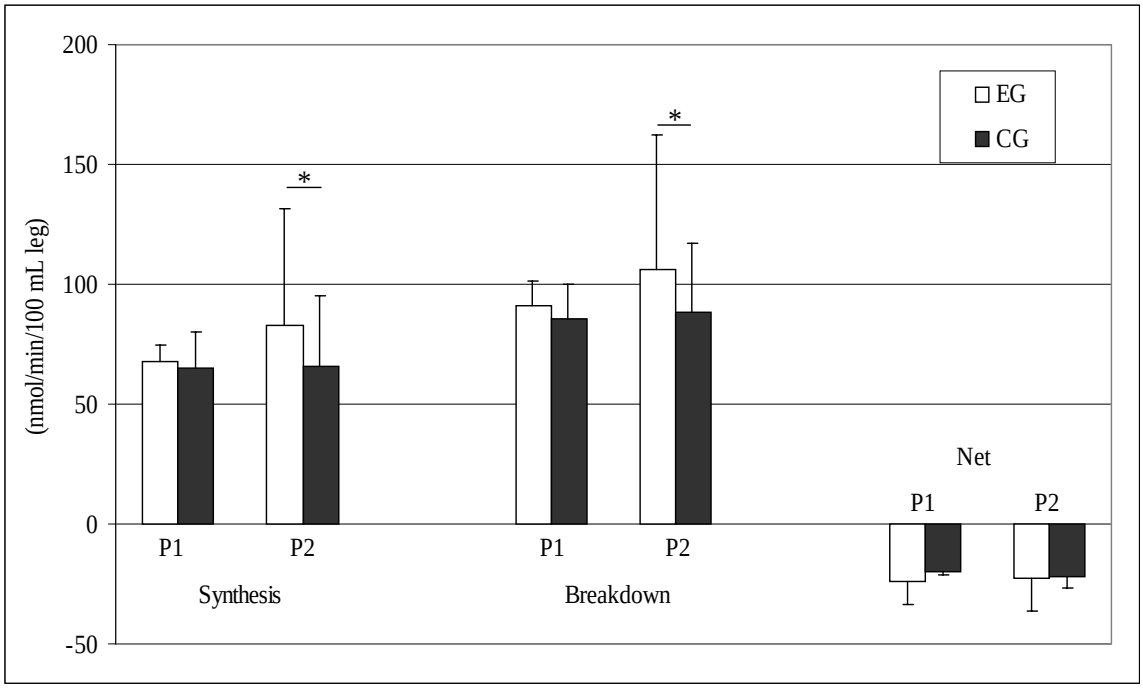


FIGURE 7 Comparison of the response of muscle protein synthesis, muscle protein breakdown and net protein balance in the exercise group (EG) and the control group (CG) during period 1 (P1) and period 2 (P2). Data are presented mean ± SE. A significant difference (*$P$<0.05) between EG and CG.

## 5.6 Serum amino acid concentrations with aging

### 5.6.1 Anthropometry, nutrition and amino acid concentrations

The body height and weight were greater in men and height decreased in both genders with age, whereas body fat percentage was lower in men and increased in both genders with age ($P${0.001). There were no significant differences in the free-time physical activity between the age groups. Men had greater total energy intake ($P${0.001), greater protein intake ($P${0.001) and total sodium intake ($P${0.001) than women. The nutrient intake of total energy ($P$<0.001), protein ($P$<0.001), alcohol ($P${0.05), water ($P$<0.01), sodium ($P$<0.001) and fibre ($P$<0.001) decreased significantly with age (TABLE 17). In addition, the consumption of alcohol, especially in 20-39 and 40-59-year-old males and 40-59 year-old females, was markedly high, because more than 27% of the subjects reported to consume alcohol 3.0% or more of the total energy intake.

TABLE 17 Macronutrient intakes of the subjects and statistical significances of main effect of gender (G) and age (A) and their interactions.

| | MALE 20-39 | | MALE 40-59 | | MALE >60 | | FEMALE 20-39 | | FEMALE 40-59 | | FEMALE >60 | | EFFECT OF | EFFECT OF | EFFECT OF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | G | A | G * A |
| Total Energy (kJ) | 10946 | 1862 | 9565 | 1491 | 6448 | 1085 | 8485 | 1812 | 6860 | 1124 | 5861 | 1450 | P<0.001 | P<0.001 | P<0.05 |
| Protein (g) | 116 | 28 | 106 | 21 | 59 | 12 | 91 | 28 | 72 | 14 | 65 | 14 | P<0.001 | P<0.001 | P<0.05 |
| Protein (g/kg BW) | 1.5 | 0.4 | 1.3 | 0.4 | 0.8 | 0.2 | 1.4 | 0.5 | 1.1 | 0.3 | 0.9 | 0.2 | P=0.546 | P<0.001 | P=0.146 |
| Protein (%) | 18 | 3 | 19 | 4 | 16 | 3 | 18 | 3 | 18 | 3 | 18 | 3 | P=0.624 | P=0.155 | P=0.216 |
| Fat (%) | 29 | 5 | 33 | 6 | 31 | 6 | 31 | 8 | 28 | 6 | 32 | 4 | P=0.678 | P=0.738 | P=0.192 |
| Carbohydrate (%) | 49 | 8 | 44 | 10 | 51 | 7 | 50 | 7 | 51 | 7 | 50 | 7 | P=0.217 | P=0.347 | P=0.180 |
| Alcohol (%) | 4.3 | 4.6 | 4.8 | 4.5 | 1.9 | 3.9 | 0.8 | 1.0 | 3.0 | 3.6 | 0.8 | 2.4 | P<0.05 | P<0.05 | P=0.499 |
| Total Water (g) | 3059 | 839 | 2647 | 332 | 2100 | 522 | 3020 | 896 | 2483 | 967 | 2292 | 703 | P=0.985 | P<0.01 | P=0.703 |
| Total Sodium (g) | 3915 | 864 | 3969 | 832 | 2710 | 567 | 3198 | 782 | 2674 | 592 | 2471 | 396 | P<0.001 | P<0.001 | P<0.05 |
| Total Fiber (g) | 25 | 6 | 26 | 7 | 20 | 6 | 25 | 7 | 22 | 4 | 17 | 5 | P=0.115 | P<0.001 | P=0.488 |

Values are mean± SD
Values are average intake per day
BW, body weight
%, percent of total energy

TABLE 18 Serum amino acid concentrations (μmol/L) of the subjects and statistical significances of main effect of gender (G) and age (A) and their interactions.

| Amino Acid | MALE 20-39 | | MALE 40-59 | | MALE >60 | | FEMALE 20-39 | | FEMALE 40-59 | | FEMALE >60 | | EFFECT OF G | EFFECT OF A | EFFECT OF G * A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | | | |
| TAAs | 3085 | 272 | 3121 | 336 | 2832 | 286 | 3050 | 238 | 2889 | 365 | 2706 | 271 | P=0.066 | P<0.01 | P=0.521 |
| EAAs | 1259 | 139 | 1260 | 130 | 1097 | 168 | 1115 | 112 | 1060 | 134 | 966 | 122 | P<0.001 | P<0.001 | P=0.648 |
| NEAAs | 1826 | 162 | 1861 | 215 | 1736 | 154 | 1935 | 183 | 1829 | 262 | 1740 | 180 | P=0.559 | P<0.05 | P=0.435 |
| BCAAs | 495 | 69 | 492 | 77 | 430 | 92 | 386 | 58 | 383 | 57 | 340 | 62 | P<0.001 | P<0.05 | P=0.873 |
| ALA | 379 | 65 | 406 | 102 | 418 | 87 | 391 | 60 | 347 | 67 | 359 | 64 | P=0.053 | P=0.851 | P=0.176 |
| ARG | 131 | 26 | 128 | 22 | 118 | 22 | 110 | 20 | 99 | 12 | 106 | 11 | P<0.001 | P=0.288 | P=0.349 |
| ASN | 60 | 8 | 65 | 10 | 54 | 9 | 68 | 12 | 58 | 10 | 53 | 6 | P=0.972 | P<0.01 | P<0.05 |
| ASP | 21 | 8 | 17 | 7 | 13 | 3 | 29 | 12 | 18 | 6 | 16 | 4 | P<0.05 | P<0.001 | P=0.199 |
| CIT | 39 | 6 | 39 | 5 | 48 | 11 | 35 | 6 | 37 | 7 | 44 | 11 | P=0.097 | P<0.001 | P=0.899 |
| CYS | 9 | 8 | 4 | 2 | 15 | 7 | 2 | 3 | 7 | 3 | 16 | 9 | P=0.493 | P<0.001 | P<0.05 |
| GLU | 48 | 18 | 66 | 26 | 44 | 15 | 67 | 23 | 45 | 19 | 41 | 9 | P=0.699 | P<0.05 | P<0.01 |
| GLN | 611 | 75 | 561 | 68 | 515 | 41 | 579 | 54 | 556 | 58 | 525 | 82 | P=0.559 | P<0.01 | P=0.529 |
| GLY | 250 | 35 | 262 | 47 | 244 | 44 | 306 | 67 | 315 | 100 | 279 | 107 | P<0.01 | P=0.457 | P=0.860 |
| HIS | 112 | 15 | 107 | 13 | 88 | 13 | 98 | 12 | 96 | 8 | 81 | 14 | P<0.01 | P<0.001 | P=0.627 |
| ILE | 79 | 10 | 74 | 17 | 69 | 14 | 53 | 7 | 58 | 9 | 51 | 11 | P<0.001 | P=0.148 | P=0.275 |
| LEU | 166 | 22 | 151 | 25 | 130 | 32 | 117 | 14 | 121 | 17 | 103 | 17 | P<0.001 | P<0.001 | P=0.174 |
| LYS | 211 | 33 | 232 | 24 | 191 | 31 | 187 | 25 | 203 | 33 | 181 | 25 | P<0.01 | P<0.01 | P=0.497 |
| MET | 31 | 5 | 33 | 6 | 27 | 4 | 24 | 3 | 26 | 4 | 21 | 3 | P<0.001 | P<0.001 | P=0.750 |
| ORN | 77 | 25 | 80 | 14 | 78 | 13 | 64 | 18 | 74 | 18 | 75 | 14 | P=0.073 | P=0.340 | P=0.623 |
| PHE | 76 | 10 | 67 | 7 | 61 | 9 | 60 | 8 | 60 | 7 | 58 | 9 | P<0.001 | P<0.01 | P<0.05 |
| SER | 131 | 22 | 132 | 21 | 127 | 18 | 167 | 23 | 155 | 22 | 129 | 21 | P<0.001 | P<0.01 | P<0.05 |
| TAU | 130 | 33 | 150 | 36 | 108 | 31 | 169 | 39 | 155 | 26 | 137 | 33 | P<0.01 | P<0.01 | P=0.195 |
| THR | 137 | 15 | 148 | 26 | 128 | 28 | 200 | 33 | 142 | 29 | 134 | 27 | P<0.01 | P<0.001 | P<0.001 |
| TRP | 65 | 11 | 54 | 4 | 55 | 8 | 50 | 8 | 51 | 7 | 46 | 9 | P<0.001 | P<0.05 | P<0.05 |
| TYR | 71 | 14 | 80 | 14 | 73 | 16 | 58 | 11 | 62 | 11 | 67 | 16 | P<0.001 | P=0.228 | P=0.330 |
| VAL | 250 | 39 | 267 | 39 | 231 | 46 | 216 | 40 | 204 | 33 | 186 | 36 | P<0.001 | P<0.05 | P=0.442 |

Values (μmol/L) are mean±SD

The concentrations of serum amino acids are shown in TABLE 18. Compared with women, men had a significantly higher level of EAAs ($P$<0.001), BCAAs ($P$<0.001), and 10 out of 22 individual amino acids ($P$<0.01). On the other hand, women had significantly higher levels of ASP ($P$<0.05), GLY ($P$<0.01), SER ($P$<0.001) and TAU ($P$<0.01). The TAAs ($P$<0.01), EAAs ($P$<0.001), NEAAs ($P$<0.05) and BCAAs ($P$<0.05) decreased with age. CIT and CYS were the only single amino acids to be higher ($P$<0.001) in the older than in the younger groups. In addition, significant interactions between gender and age were observed in 7 out of 22 single amino acids.

The Tukey HSD *post hoc* test revealed significant differences in the pairwise comparisons of TAAs, EAAs, NEAAs and BCAAs (FIG. 8) and in 8 out of 22 single amino acids (FIG. 9) between the age groups. In addition, significant differences were obtained in 5 single amino acids in men (FIG. 10) and also in 5 single amino acids in women (FIG. 11).

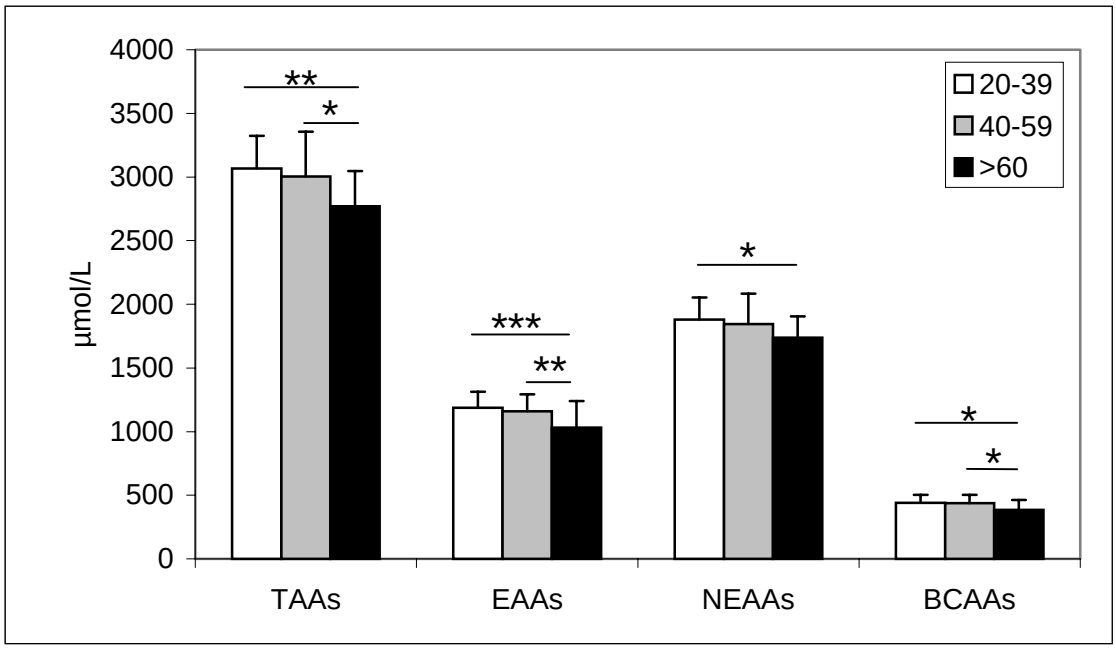


FIGURE 8 Comparison in concentrations of TAAs, EAAs, NEAAs and BCAAs between age groups (N=24 per age groups; 20-39, 40-59 and >60 years). Data are presented as mean ∂ SD. Tukey HSD test significance; *$P$<0.05, **$P$<0.01, ***$P$<0.001.

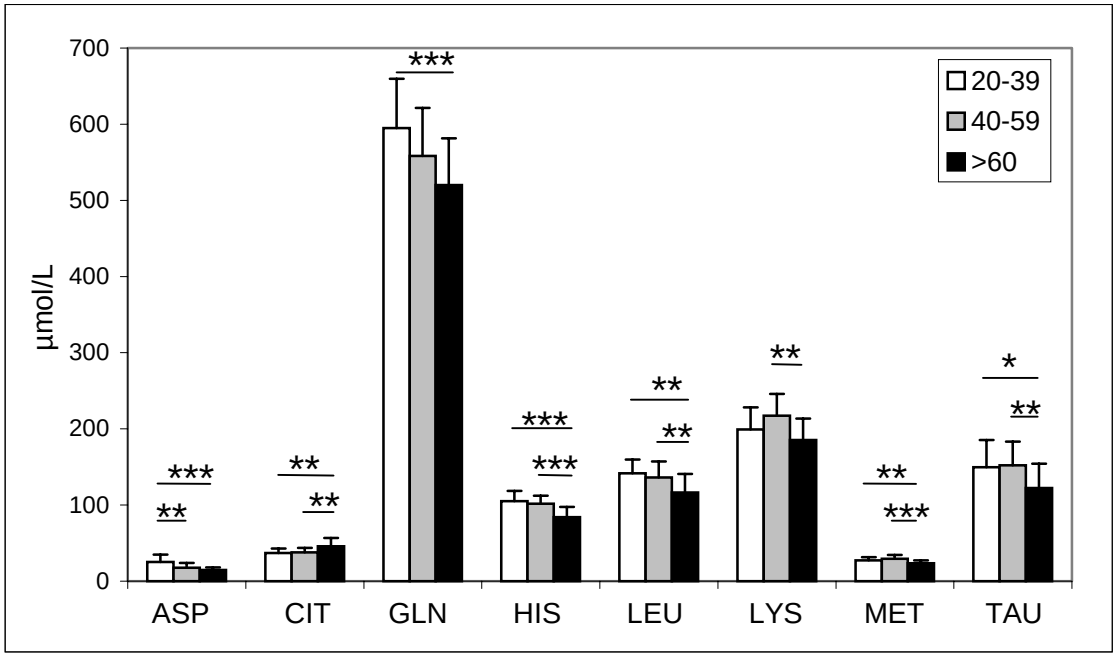


FIGURE 9 Comparison in concentrations of ASP, CIT, GLN, HIS, LEU, LYS, MET and TAU between age groups (N=24 per age groups; 20-39, 40-59 and >60 years). Data are presented as mean ∂ SD. Tukey HSD test significance; *$P$<0.05, **$P$<0.01, ***$P$<0.001.

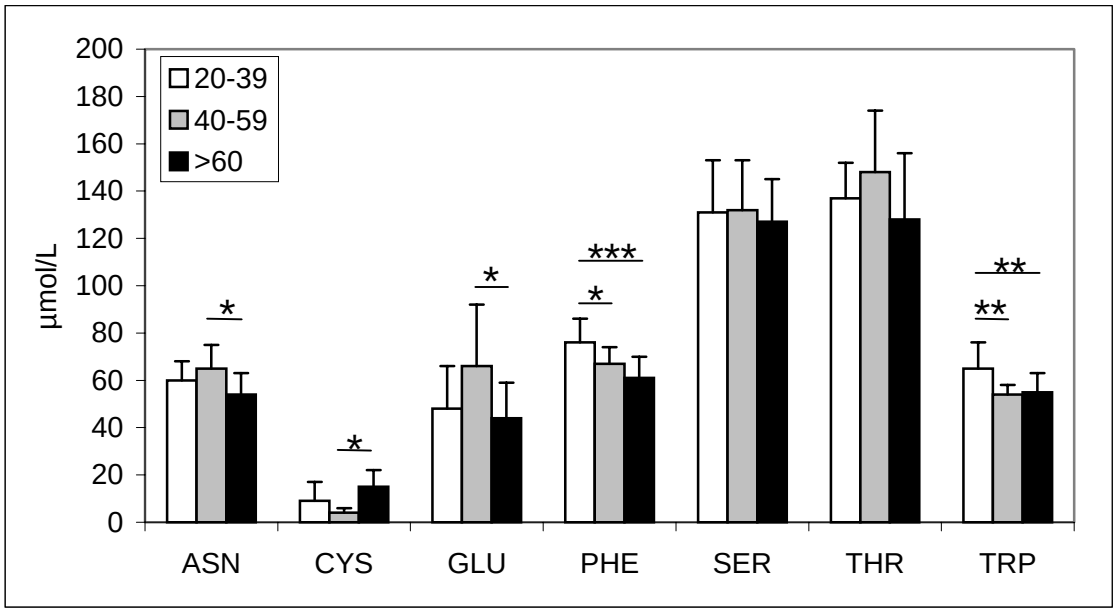


FIGURE 10 Comparison in concentrations of ASN, CYS, GLU, PHE, SER, THR and TRP between age groups of male (N=12 per age groups; 20-39, 40-59 and >60 years). Data are presented as mean ∂ SD. Tukey HSD test significance; *$P$<0.05, **$P$<0.01, ***$P$<0.001.

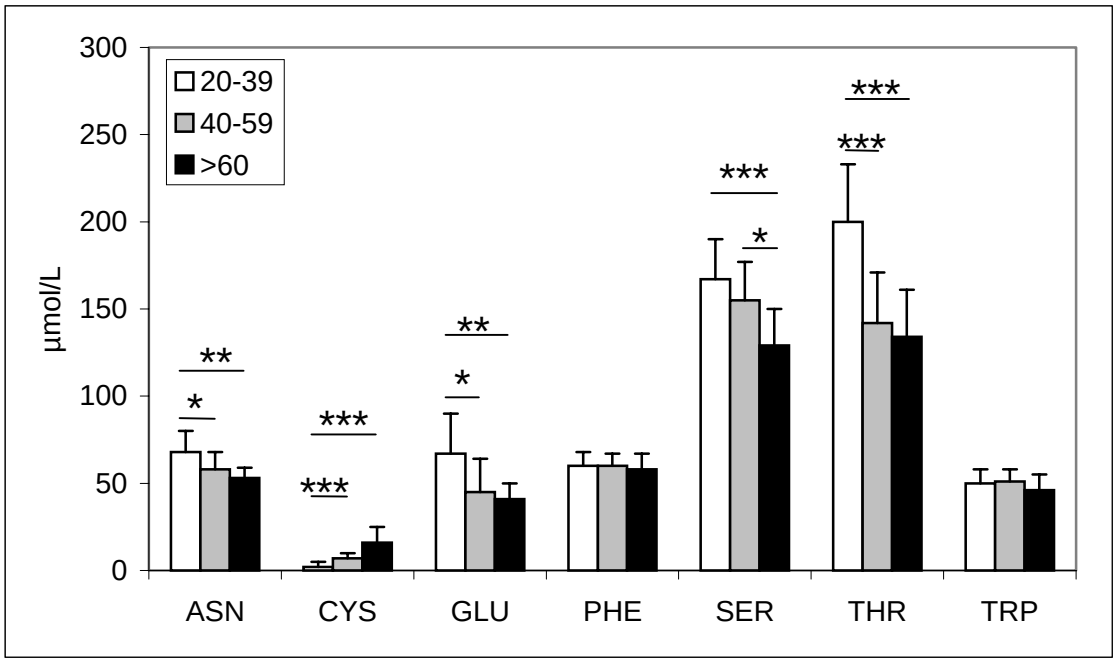


FIGURE 11 Comparison in concentrations of ASN, CYS, GLU, PHE, SER, THR and TRP between age groups of female (N=12 per age groups; 20-39, 40-59 and >60 years). Data are presented as mean ∂ SD. Tukey HSD test significance; *$P$<0.05, **$P$<0.01, ***$P$<0.001.

# 6 DISCUSSION

## 6.1 Serum amino acid concentrations following anaerobic lactic exercise sessions and a strength exercise session

Although a lot of studies have examined the response of the whole body protein metabolism to exercise (e.g. Lamont et al. 1987 and Tarnopolsky et al. 1991), there is a lack of data concerning the changes in serum amino acid concentrations in power-type exercise. Previous studies have described changes in plasma amino acid concentrations due to acute and prolonged exercise (Poortmans 1984, Bergström et al. 1985, Henriksson 1991), but the comparison- and interpretation of the results is difficult, since the previous dietary intake has been poorly controlled (Forslund et al. 2000). Since the changes in the amino acid concentrations are brought about by a complexity of factors that interact, for example by diet or exercise (Forslund et al. 2000), the present study carefully recorded and analysed the previous dietary intake in order to identify the exercise-induced changes per se. The exercise-induced changes in the amino acid concentrations were different between the running exercise sessions (SRS and LRS) and the strength exercise session (SES). There were decreases in the concentrations of EAAs and BCAAs in all sessions, but ALA increased during SRS and LRS. Since ALA did not increase in SES as it did strongly in the other exercises, SES resulted in a decrease in the sum of all amino acids. ALA is released from muscle during exercise and taken up in liver (Felig 1973). The increased level of ALA that was seen in blood during SRS and LRS is in agreement with the finding of Felig (1977), according to whom the uptake of ALA is unchanged during a short-term exercise and the concentration of ALA increases in blood. Instead in SES the uptake of ALA in liver must have been increased, since the concentration in blood was almost unchanged during the exercise. The decrease in BCAAs and the increase in ALA supports the theory of Felig and Wahren (1971), according to which the BCAAs are oxidased in

muscle for energy or served as precursors (-$NH_2$) for ALA in the glucose-alanine cycle.

## 6.2 Serum amino acid concentrations following a training period

Endurance trained subjects have been shown to have higher plasma levels of LEU, ILE and TYR at rest compared to untrained subjects (Einspahr and Tharp 1989), but nutrition and protein intake were not measured. In the present study (exp. 2) among power trained athletes decreases in the fasting levels of all amino acids were seen after the 5-week training period. Simultaneously we observed changes in hormone profile, which showed a good anabolic balance. We chose the anabolic hormones TE and GH and the catabolic hormone COR to evaluate the anabolic state after the training period. Due to an increase in TE level and the unchanged level of COR, the TE/COR –ratio increased, reflecting a suitable physiological loading during training. The increase in TE accelerates protein synthesis (Hedge et al. 1987), which may explain the decrease in the fasting amino acid concentrations during training. The regular protein intake of 1.26 g/kg body weight /day might not have been sufficient for accelerated protein synthesis and the decrease in fasting amino acid concentrations (reflecting the decrease in the free amino acid pool) has not been compensated by tissue degradation during the 5-week training period. On the other hand, the amino acid pool may have been decreased through the amino acid use for fuel supply in glucose-alanine cycle. Babij et al. (1983) demonstrated a near-linear increase in amino acid oxidation due to increase in exercise intensity. In our study the intensifying training during the 5-week period may have accelerated the glucose-alanine cycle. The strong decreases in fasting amino acid concentrations due to training must be taken into consideration in sport nutrition, when aiming at optimal exercise performance.

## 6.3 Serum amino acid concentrations following anaerobic lactic exercise sessions and a strength exercise session with leucine supplementation

In experiment 3 the amino acid responses were investigated during LEU ingestion, since the BCAA supplementation is reported to help to maintain balance in the free amino acid pool (May and Buse 1989). At the same time the possible effect of the timing of the LEU dose was investigated. MARE and SES were chosen, since these two exercise sessions differ from each other (e.g. the lactate increase is stronger in MARE, whereas the duration is longer in SES). Moreover they differ from an aerobic-type endurance exercise session, on which the previous data are mainly available. In both MARE and SES the LEU

supplementation increased the serum concentration of LEU, as expected, but it simultaneously decreased the levels of other BCAAs (ILE and VAL) possibly due to an intensified effect of LEU on the transport system, since Oxender and Christensen (1963) have suggested that these three amino acids may have the same transport system. Later Nair et al. (1992) have concluded that the increase of LEU by infusion may decrease protein degradation and further decrease the level of ILE and VAL. It has been shown that muscle protein synthesis is stimulated after exercise (Anthony et al. 1999) by the increased availability of exogenous oral amino acids (Volpi et al. 1998a and Volpi et al. 1999). Thus the increase seen in LEU concentration in the present study (exp. 3) increased the availability of amino acids for protein synthesis during short-term recoveries within the session or after the exercise session. The increased LEU concentration due to supplementation could have intensified the glucose-alanine cycle possibly resulting in extra fuel, since the amino group ($-NH_2$) of leucine has an important role in the formation of ALA, which can further be used in gluconeogenesis. However, the physical performance was not enhanced in the present study. The long-term effects of LEU supplementation must be noted, since the increased protein synthesis through consecutive muscle hypertrophy can later affect performance capability. In addition, Blomstrand and Saltin (2001) have examined the effect of BCAA intake on muscle protein metabolism during and after one hour of ergometer cycle exercise. They suggested that protein synthesis is stimulated and/or protein degradation decreased with BCAA intake during recovery, but not during exercise. The smaller release of aromatic amino acids (PHE, TYR, TRP) from muscle after exercise shows possibly some protein sparing effect of BCAA intake in the recovery period. Thus the administration of exogenous amino acids after exercise may improve the net muscle protein balance by increasing protein synthesis and decreasing protein breakdown (Tipton and Wolfe 1998). Unfortunately, protein kinetics was not quantified in this experiment.

## 6.4 Serum amino acid concentrations following a training period with leucine supplementation

In experiment 4 the effects of oral LEU supplementation were investigated during a heavy power-type training period. Previously regular resistance training has been shown to cause muscle fibre hypertrophy (Staron et al. 1994) and after resistance exercise an abundant supply of amino acids by infusion has been suggested to enhance the exercise-induced metabolic effect on muscle proteins leading to muscle hypertrophy (Biolo et al. 1997). Moreover BCAA supplementation has been shown to decrease protein degradation and muscle enzyme release, which probably indicates muscle damage (Carli et al. 1992). Thus long-term supplementation can lead to greater gains in fat-free mass (Kreider 1998). Consequently, a theoretical basis exists for expecting some

beneficial effects of protein supplement in those who are involved with regular training. The current protein recommendation for adult men is 0.8 g/kg body weight /day (Lemon 1998) with no additional need for those involved in regular exercise. In this study the total sum of amino acids decreased with the protein intake of 1.26 g/kg body weight / day during the intensive strength power training in experienced power athletes. The decrease in the serum LEU concentration that was seen during the intensive training period was prevented by the LEU supplement. We speculated that some extra LEU might have been oxidised in skeletal muscle as a result of the aerobic/anaerobic type of training. On the other hand, the extra LEU may have been incorporated into protein or accumulated in the intracellular free amino acid pool. In any case, the LEU metabolism was probably not optimal, since without LEU supplementation the serum LEU decreased markedly, despite the regular protein intake over RDA. No changes were observed in anthropometrical measurements and in performance possibly due to too short a training period. The higher level of LEU may be valuable in the recovery phases during the training period, when the post-exercise protein synthesis occurs.

## 6.5 Amino acid and protein metabolism following a resistance exercise session

The protocol of experiment 5 was designed to simultaneously assess the serum amino acid concentrations and the intracellular amino acid concentrations of muscle. In addition, there was an attempt to demonstrate the muscle protein kinetics following RES using a continuous infusion of L-[*ring*-$^{2}H_{5}$] PHE, femoral arterio-venous catheterization and muscle biopsies. Previously, it has been reported that muscle amino acid transport and protein synthesis as well as protein degradation are accelerated following resistance exercise in a post-absorptive state, but the net balance is improved only little (Biolo et al. 1995b). Since amino acid availability has an important role in muscle protein kinetics (e.g. Tipton and Wolfe 2001), the purpose of the present experiment was to determine the amino acid profile in arterial and venous blood and in vastus lateralis muscle after a resistance exercise session. The use of labelled tracers in the metabolic studies allows the estimation of protein synthesis and protein breakdown simultaneously (Wagenmakers 1999). PHE was chosen to demonstrate muscle amino acid and protein kinetics after RES.

RES induced a significant increase in muscle protein synthesis and muscle protein breakdown at 195 minutes, but not yet at 60 minutes during recovery in fasting condition. At 60 min a slight decrease was seen in the intracellular concentration of ALA and a slight increase in protein synthesis, whereas later (at 195 minutes recovery) the concentration of ALA was low and the rate of protein synthesis had increased significantly. Simultaneously with the increase of protein synthesis protein breakdown also increased, even though the

decrease of ALA and the subsequent increase in GLN would be expected to decrease degradation, since GLN is shown to be a direct regulator of muscle protein synthesis and degradation (Viru 1996). One explanation may be hormonal factors and especially insulin. Insulin has been shown to modulate protein turnover by decreasing muscle protein degradation (Di Pasquale 1997). Thus in fasting conditions, when insulin was low, muscle protein breakdown was not depressed. Secondly it is possible that in the later recovery the catabolic responses might have taken place in the most active muscle fibers that had not contributed to the mobilization of protein resources during intense exercise. When compared to earlier studies, the changes in muscle protein synthesis and in muscle protein breakdown were smaller in our study. In the present study protein synthesis increased significantly by 21% and protein breakdown by 17% in the second period after the recovery of 195 min following RES. The mean values of protein synthesis and protein breakdown in our study may underestimate the real effect of exercise on these parameters, since the deviation in the amount of protein synthesis and breakdown was remarkable among the EG subjects especially in the second period, possibly due to a different individual response to the exercise workload. The diminished availability of the free amino acids due to fasting state may be another explanation to the low absolute protein synthesis. After resistance exercise protein synthesis has been reported to rebound for 48 h, whereas protein degradation remains slightly elevated resulting in positive net balance only if amino acid availability is increased (Rennie and Tipton 2000). These findings are in line with our results, in which net balance remained negative, both in EG and in CG, in fasting conditions, when the low availability of free amino acids was further diminished following exercise.

The arterial concentration of ALA was 43% higher in the EG than in CG at 30 minutes of recovery. Simultaneously, the venous concentration of ASN was lower in EG, which may be due to its role as a donor of the amino group ($-NH_2$) in the synthesis of ALA (Di Pasquale 1997). After exercise the splanchnic uptake of gluconeogenic substrates has been shown to increase (Brooks 1986, Johnson and Bagdy 1988, Varrick et al. 1992), and since ALA is utilized in hepatic gluconeogenesis (Favier et al. 1987), the concentration of ALA decreases. Thus the difference between EG and CG had almost disappeared at 60 min of recovery. The changes in the concentration of THR, which is one of the glucogenic amino acids that are released from muscles during fasting (Pozefsky et al. 1976), were similar as in ASN, since both of these amino acids had higher concentrations in FV than in FA and, moreover, non-significantly lower in EG than in CG. The smaller increase in THR concentration in EG than in CG in FV may result from the increased gluconeogenesis due to both fasting and exercise. MET is also a glycogenic essential amino acid, which is released from muscle during fasting (Pozefsky et al. 1976) and which has a limiting role in the maintenance of body protein and nitrogen balance under conditions of greater protein turnover (Kien et al. 1978). In this study the concentration of MET remained at the same level through the recovery at rest, but in EG the

concentration was significantly higher in the post-exercise situation. Then the concentration started to decrease and was similar to that at rest at 195 minutes of recovery. In EG the level of BCAAs decreased during both periods, but significantly only at 195 min of recovery after RES in FV, whereas the level in CG remained almost unchanged through the recovery. This may reflect the point, where the amino acid's need for energy "turns off" and the metabolism of protein synthesis "turns on". The base level of BCAAs, measured from AV before exercise, was similar in EG and CG. The BCAA concentration remained lower in EG than in CG throughout the recovery, but the difference between the groups was only significant at 195 min of recovery after RES, when the concentration was 20% lower in EG than in CG in FV. The present results indicate that serum free amino acids may have been utilized in energy metabolism after RES in fasting conditions, which confirms the previous study (Lehmann et al. 1996), suggesting that increased muscle protein metabolism results in a decrease in blood BCAAs. The results also indicated that the concentration of LEU decreased, whereas that of ASP increased. ASP plays an important role in the purine nucleotide cycle in maintaining the pool of ATP in muscle (Wagenmakers 1992). The observed inverse relationship between these two amino acids may reflect the degradation of LEU and the consequent synthesis of ASP. BCAA degradation in muscle results in the formation of GLU, which can further provide the amino group ($-NH_2$) with oxaloacetate to synthesize ASP (Wagenmakers 1992). The changes in serum amino acids concentrations following an intensive RES may be associated with the energy demands.

Our results indicate that the differences in the concentrations of free amino acids in muscle between CG and EG are scant, except in ALA. ALA was significantly increased after RES, but then it decreased more slowly in muscle than in blood. The fasting conditions (at least 12 hours before the first samples) in both groups may partly explain small differences via the decreased availability of the free amino acids. In addition, no correlative relationships between muscle and blood amino acid concentrations were seen. In fasting conditions following RES the changes occur in a few glycogenic single amino acids in blood, whereas in muscle the changes occur only in ALA.

In conclusion, this study provided evidence that RES induced a significant increase in muscle protein synthesis and muscle protein breakdown at 195 minutes, but not yet at 60 minutes during recovery in fasting conditions. This was associated with the simultaneous decrease in the serum and muscle concentration of ALA at the end of recovery. However, the protein net balance remained negative.

## 6.6 Age and gender related serum amino acid concentrations

The goal of experiment 6 was to determine the contribution of age and gender to serum fasting amino acid concentrations at rest. The available data in the

literature are scant and the lack of information concerning gender and age related changes in serum amino acid concentrations may partly be due to methodological difficulties and the inadequate control of several factors including diet composition, total energy intake, physical activity and due to limitations imposed by the need for a sample comprising a sufficiently large size of subjects. Hence, it was desirable to examine in some detail the pattern and degree of change of serum amino acid concentrations in healthy subjects when supplying a normal diet and every-day physical activity.

When both men and women were grouped, age-related changes could be observed. In agreement with previous findings (Elahi et al. 1983, Flynn et al. 1992) this experiment showed significant declines in energy and protein intake with aging. Consequently, we observed decreases in all amino acids except CIT and CYS, which was controversial with the results of Bancel et al. (1994), who found the concentrations of many amino acids to be higher in the elderly compared with younger adults. It is difficult to interpret or compare the results of this study with earlier findings on plasma amino acid changes in relation to exercise and diet, since the subjects studied had not been strictly controlled for previous dietary protein intake or daily free-time activity. In addition, most studies have analysed the amino acid levels in plasma and not in serum. The decreased level of amino acids seen in this study might have been insufficient for the amino acid supply needed for protein synthesis. In addition, it can be speculated that the increased mobilization of CIT and CYS observed in this study, is due to the normal physiological process of aging. Declines in the levels and actions of anabolic hormones and reduced synthetic rates of contractile and metabolic measures are shown to be associated with aging (Proctor et al. 1998) and the decrease in muscle mass and in protein content in the elderly is associated with the decreased synthesis of muscle protein (Nair 1995).

The current experiment demonstrated also gender-based differences in the concentrations of many amino acids. The higher levels of EAAs and BCAAs seen in men are in line with the studies of Caballero et al. (1991) and Bancel et al. (1994). Previously, Galante et al. (1978) had investigated young adults and found the amount of ILE, LEU, VAL, TYR, PHE, GLU and ORN to be significantly greater in men than in women. The observed higher concentrations of BCAAs of the present study may be associated with higher LEU oxidation rate in men (Lamont et al. 2001). However, there is only little evidence for differences in whole body protein breakdown or protein synthesis between men and women (Tipton 2001). Given the clear differences in musculature there is no doubt that differences in protein metabolism exist between genders due to the contribution of sex hormones (Tipton 2001). The larger (6-9%) blood volume of men than women (Guyton 1986) may be one factor in interpreting the observed differences in serum amino acid concentrations. Consequently, the amino acid concentration differences between genders would be even bigger in equal plasma volume, since the amino acid concentration in blood is more diluted in men than in women.

In conclusion, the observed decreases in the energy, protein and water intakes and in amino acid concentrations in serum confirm earlier findings with aging, except CIT and CYS, the concentration of which increased in the present study. Since the protein intake, which was greater than the recommended dietary allowances (RDA) in all age groups, seemed not to suffice to maintain the levels of serum amino acids with aging, an additional allowance should be considered in the elderly, at least for those who are involved in regular physical activities. Furthermore, the concentration of EAAs and BCAAs is greater in men than in women. This information may be beneficial for the planning of nutritional support, rehabilitation and the pharmacological treatment of elderly women and men.

# 7 SUMMARY AND CONCLUSIONS

1. The results of the present study indicated decreases in EAAs and BCAAs both following SRS and LRS (average lactate values: 13.8±1.9 mmol/L in SRS and 16.4±1.3 mmol/L in LRS, respectively). However, the TAAs were unchanged, since NEAAs, mainly ALA and GLU, strongly increased. SES (average lactate value 2.5±0.4 mmol/L) differed from these two other exercise types, since all the groups of amino acids decreased markedly. In the present study the mean protein intake ranged from 1.2 to 1.3 g/kg body weight/day.

2. The fasting concentrations of all amino acids in serum decreased significantly during the 5-week speed and strength training period among power trained athletes showing that there are strong decreases in the blood amino acid pool with a daily protein intake of 1.3 g/kg body weight. However, according to the hormonal measurements it was suggested that the subjects were in an anabolic state during the 5-week period.

3. The ingestion of LEU before (200 mg/kg/body weight in one dose in MARE) or before and during (50 mg/kg body weight twice in SES) the exercise session increased the serum LEU concentration, whereas VAL and ILE were simultaneously decreased. However, the supplementation did not affect physical performance following SES or in MARE when the daily protein intake was 1.1 – 1.3 g/kg body weight.

4. During 10 weeks of power-type training the serum concentrations of amino acids were lowered considerably and the decrease occurred earlier than in the serum TE concentration with the daily protein intake of 1.3 g/kg body weight. Daily supplementation with LEU (50 mg/kg body weight) seemed to prevent the decrease in the serum LEU concentration during intensive training.

5. In fasting conditions following RES the changes occurred in a few glycogenic single amino acids (ALA and ASP increased, LEU decreased) in blood, whereas in muscle the changes occurred only in ALA (increased). In addition, the changes were smaller in muscle than in blood. Furthermore, a significant increase was seen in muscle protein synthesis and in muscle protein breakdown at 195 minutes, but not yet at 60 minutes during recovery. However, protein net balance was not changed.

6. The results of this study indicated that all amino acids, except CIT and CYS, decreased with aging. However, the serum amino acid concentrations exceeded the plasma reference values of Henry et al. (1974) in all age groups. Furthermore, despite the observed decrease in protein intake with aging, all age groups had a daily protein intake over the recommended dietary allowances (RDA; 0.8 g/kg/day body weight). Compared with women, men had higher concentrations of EAAs, BCAAs and 10 out of 22 single amino acids.

The results of the present study indicate that decreases occur in the free amino acid pool due to different exercise sessions, the training period and finally due to aging, although the mean protein intake (0.8 – 1.5 g/kg body weight) is above RDA. LEU supplementation prevents the decrease in LEU concentration, but has no effects on acute physical performance. The data supports the suggestion by Lemon (1998) that it could be good to have also RDA values for athletes. The recommended protein intake of 0.8 g/kg body weight/day for adult sedentary men is inadequate to meet the LEU requirement, depending on the type of protein habitually ingested. For active individuals, a protein intake of 1.2 to 1.7 g/kg body weight/day appears to be generally adequate (Lemon 1998). Consequently, this means that the RDA for LEU in active individuals should be increased from the recommended values for sedentary people. Furthermore, during fasting of 12-24 hours remarkable decreases occurred in the amino acid concentrations. This must be taken into consideration in the busy every day life when diet protein intake may be limited for hours. In future, the amino acid profile may be helpful in planning nutrition, training programs and the rehabilitation of healthy, ill, injured or aging individuals.

# TIIVISTELMÄ

Ihmisen elämälle välttämätön ravintoaine, proteiini, jota on noin 15 % kehon painosta, koostuu aminohapoista, joista välttämättömät aminohapot on saatava ravinnosta, kun taas ei-välttämättömiä aminohappoja voidaan syntesoida elimistössä. Vain noin 0.5-1.0 % aminohapoista on vapaana veressä ja lihaksen sisällä ns. vapaana aminohappoaltaana (noin 200 g). Aminohappoja tulee altaaseen ravinnosta, proteiinien hajoamisesta ja hiilihydraatti- ja rasvaaineenvaihdunnan välituotteita transaminoimalla. Altaan aminohapot ovat nopeasti mobilisoitavissa proteiinien päätehtävään rakennusaineeksi (proteiinisynteesiin) ja pieneltä osin energiaksi. Altaan tasapainotilaan voidaan vaikuttaa muuttamalla proteiinin saantia ravinnosta tai muuttamalla fyysistä aktiviteettia. Aminohappoallas on hyvin aktiivinen; se vaihtuu noin kuusi kertaa vuorokaudessa, joten sen rooli erityyppisissä suorituksissa ja harjoittelun yhteydessä on mielenkiintoinen ja merkittävä. Pitkään on keskusteltu siitä, kuinka suuri proteiinin saanti on riittävä optimaaliseen fyysiseen suoritukseen, mutta edelleenkään yksimielisiä, tutkimuksiin perustuvia suosituksia eri urheilutyyppien harrastajille ei ole saatavissa. Suurin osa tähänastisista tutkimuksista on keskittynyt proteiinimetaboliaan kestävyyslajien miesurheilijoilla. Sen sijaan nopeus- ja voimalajien urheilijoita on tutkittu vain vähän ja lisäksi tietoa puuttuu sukupuolien välisistä eroista sekä ikääntymiseen liittyvistä muutoksista aminohappometaboliassa.

Tämän väitöskirjan tarkoituksena oli tutkia veren ja lihaksen aminohappokonsentraatioissa tapahtuvia muutoksia erilaisissa anaerobisissa kuormitustilanteissa ja harjoittelujakson yhteydessä ravinnon ja harjoittelun ollessa tarkoin kontrolloituja. Pyrittiin myös selvittämään sitä, voidaanko leusiinilisällä parantaa suorituskykyä tällaisten harjoitteiden yhteydessä. Lisäksi tutkittiin sukupuolen ja ikääntymisen vaikutusta veren aminohappotasoihin eri ikäryhmissä. Koehenkilöt olivat 19-92 –vuotiaita terveitä urheilijoita, aktiivisia liikunnan harrastajia ja työikäisiä sekä eläkkeellä olevia suomalaisia miehiä ja naisia. Koehenkilöryhmiin kuului yhteensä 118 miestä ja 36 naista. Aminohappopitoisuuksia määritettiin kyynärlaskimosta, reisilaskimosta ja reisivaltimosta otetuista verinäytteistä. Ns. kolmiallasmenetelmässä analysoitiin verinäytteiden lisäksi lihasnäytteitä ulommasta reisilihaksesta. Saatujen tulosten avulla laskettiin proteiinisynteesin, proteiininhajoamisen ja nettotasapainon aktiivisuus. Tutkimuksen tulokset osoittavat, seuraavaa:

1. Välttämättömien ja haaraketjuisten aminohappojen pitoisuudet laskivat sekä lyhyiden että pitkien juoksusarjojen muodostamassa harjoituksessa veren laktaattipitoisuuksien keskiarvojen vaihdellessa 13.8±1.9 – 16.4±1.3 mmol/L välillä. Kuitenkin aminohappojen totaalisumma pysyi muuttumattomana, koska ei-välttämättömissä aminohapoissa todettiin samanaikaisesti nousua pääosin alaniini- ja glutamaattikonsentraatioiden osalta. Voimaharjoitus erosi näistä kahdesta em. harjoitustyypistä, sillä huomat-

tavaa laskua tapahtui kaikkien aminohapporyhmien summissa, erityisesti haaraketjuisissa aminohapoissa (leusiini ja isoleusiini), kun veren laktaattitaso suorituksen aikana oli keskimäärin 2.5±0.4 mmol/L. Proteiinin saanti oli tutkimuksessa keskimäärin 1.2 – 1.3 g kehon painokiloa kohti vuorokaudessa.

2. Teholajien (pikajuoksut, hypyt, ottelut) urheilijoilla nopeusvoimapohjainen viiden viikon harjoittelujakso laski kaikkien aminohapposummien paastopitoisuutta seerumissa. Veren vapaassa aminohappoaltaassa tapahtui laskua vuorokautisen proteiinin saannin ollessa keskimäärin 1.3 g kehon painokiloa kohti, vaikka hormonaalisen tilanteen oli arvioitu olevan anabolinen testosteroni/kortisoli –suhteen avulla mitattuna.

3. Ravinnon leusiinilisän (200 mg yhtenä annoksena ennen juoksuharjoitusta tai 50 mg kahtena annoksena ennen voimaharjoitusta ja sen aikana) nauttiminen ennen yksittäistä suoritusta ja/tai sen aikana nosti seerumin leusiinitasoa ja laski samanaikaisesti valiinin ja isoleusiinin konsentraatiota seerumissa. Leusiinilisän ei kuitenkaan osoitettu parantavan anaerobista suorituskykyä tai voimantuottokykyä, kun keskimääräinen proteiinin saanti oli 1.1 – 1.3 g kehon painokiloa kohti vuorokaudessa.

4. Teholajien urheilijoilla aminohappopitoisuuksien todettiin laskevan jo ennen testosteronin laskua intensiivisen nopeus-voimatyyppisen harjoittelujakson (10 viikkoa) aikana. Seerumin leusiinitason laskua pystyttiin estämään ravinnon päivittäisellä leusiinilisällä (50 mg kehon painokiloa kohti vuorokaudessa).

5. Paastotilassa voimaharjoitus lisäsi lihaksen proteiinisynteesiä (21%) ja hajoamista (17%) vasta 195 minuuttia kuormituksen jälkeen, kun 60 minuutin kohdalla ei vielä havaittu merkitsevää muutosta. Kuitenkin lihaksen netto proteiinitasapaino pysyi negatiivisena koko 195 minuutin palautumisjakson ajan. Seerumin aspartaattikonsentraatio oli suurempi voimaharjoituksen tehneellä ryhmällä ja leusiinikonsentratio kontrolliryhmällä sekä valtimossa että laskimossa. Kontrolliryhmään verrattuna haaraketjuisten aminohappojen taso oli matalampi voimaharjoituksen suorittaneella ryhmällä 195 minuutin palautusjakson lopulla. Lisäksi suorituksesta aiheutunut kohonnut alaniinitaso lähti nopeasti laskemaan palautumisen aikana sekä veressä että lihaksessa voimaryhmällä ja ero ryhmien välillä oli tasoittunut jo 60 minuutin kuluttua palautumisen alkamisesta.

6. Vuorokautisen energian ja proteiinin saannin todettiin laskevan ikääntymisen myötä ja huolimatta suositukset ylittävästä ravinnon proteiinin saannista (≥0.8 g/kehon painokiloa kohti vuorokaudessa), seerumin kokonaisaminohappopitoisuudet laskivat voimakkaasti. Poikkeuksena havaittiin sitrulliini- ja kysteiinikonsentraatioiden nousua ikääntymisen myötä.

Kuitenkin seerumin aminohappokonsentraatiot ylittivät kaikissa ikäryhmissä Henryn ja tutkimusryhmän (1974) plasmapitoisuuksille asettamat viitearvot. Lisäksi välttämättömien ja haaraketjuisten aminohapporyhmien sekä 10 yksittäisen aminohapon konsentraatiot olivat merkittävästi korkeammat miehillä kuin naisilla kaikilla tutkituilla ikäryhmillä.

Tämän työn päätulos oli se, että vapaassa aminohappoaltaassa tapahtuu muutoksia sekä erilaisten yksittäisten harjoitusten, harjoittelujaksojen että myös ikääntymisen seurauksena. Leusiinilisällä pystytään estämään leusiinikonsentraation laskua, mutta ei parantamaan fyysistä suorituskykyä. Tutkimuksessa saadut tulokset tukevat Lemonin (1998) ehdotusta, jonka mukaan urheilijoille pitäisi olla omat RDA-arvot. Tavallista istumatyötä tekeville proteiinin saannin vuorokautiseksi suositusarvoksi esitetty 0.8 g/kehon painokiloa kohti ei ole riittävä leusiinitarpeen tyydyttämiseen. Lemon (1998) on suositellut päivittäiseksi proteiinin saanniksi 1.2-1.7 g/painokiloa kohti vuorokaudessa aktiivisille henkilöille, joten myös leusiinin RDA pitäisi olla suurempi. Tutkimus osoitti, että pelkkä 12-24 tunnin paasto laskee aminohappokonsentraatioita voimakkaasti, mikä pitäisi ottaa huomioon jokapäiväisessä kiireellisessä elämässä, jolloin proteiinin saanti voi olla rajoittunutta useiden tuntien ajan. Aminohappojen tärkeä rooli sekä urheilijoiden että tavallisten eri sukupuolta olevien eri ikäisten aikuisten metaboliassa on otettava huomioon vuorokautisessa ravinnossa. Tulevaisuudessa aminohappoprofiilin selvittäminen voi olla hyödyllistä suunniteltaessa eri ikäisten terveiden, sairaiden tai vammautuneiden henkilöiden ravitsemusta, harjoitusohjelmia tai kuntoutusta.

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