EFFECT OF EXERCISE ON AMINO ACID CONCENTRATIONS IN SKELETAL MUSCLE AND PLASMA

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Summary

Protein is not normally an important energy fuel for exercising muscle. In spite of this, there is a significant increase in the rate of amino acid catabolism during exercise. This is secondary to the exercise-induced increase in several metabolic processes, such as hepatic gluconeogenesis and the citric acid cycle, where amino acid carbon is utilized. The suppression of protein synthesis during an exercise bout leaves amino acids available for catabolism. There is some evidence that basal amino acid concentrations in plasma and muscle may be higher in trained than in untrained individuals. In the rat, the concentration of free amino acids is higher in slow-twitch than in fast-twitch muscles. With short-term exercise, the transamination of glutamate by alanine aminotransferase leads to increased levels of alanine in muscle and plasma, and an increased release of alanine from the muscle. At the same time, the muscle and plasma glutamate concentrations are markedly decreased. The plasma glutamine level is elevated with short-term exercise, but changes in muscle glutamine concentration are more variable. With prolonged exercise, there is a depletion of the plasma amino acid pool, which may be explained by an increased consumption in organs other than muscle. With the exception of alanine, we found, however, that the muscle levels of free amino acids are kept stable throughout a 3.5-h exercise period. There is a significant activation of branched-chain amino acid metabolism with prolonged exercise, and the current data indicate that this is more pronounced in endurance-trained subjects than in untrained controls.

Introduction

Skeletal muscle protein makes up more than 60% of the whole-body protein mass (Ballard and Tomas, 1983), and protein metabolism in skeletal muscle, which accounts for more than 25% of the whole-body protein turnover (Young and Torun, 1981), has thus long been an important field of research. Different types of protein constitute the vital basis of muscular function, and the contractile activity, in turn, exerts influence on muscle protein turnover and amino acid metabolism. Protein is, however, not normally an important energy fuel for the exercising muscle. This was shown more than a century ago by Pettenkofer and Voit (1866) who, in groundbreaking experiments, estimated protein breakdown by measuring the urinary nitrogen output. Protein (including that derived from muscle tissue

itself) will constitute an important fuel in the energy metabolism of muscle only situations where the body's energy supply is compromised, such as in long-term starvation. It is possible that severe glycogen depletion may be somewhat analogous to starvation. Thus, Lemon and Mullin (1980) observed significantly increased serum and sweat urea excretion rates during bicycle ergometer exercise (1 h at 61 % of the maximal rate of oxygen uptake, $\dot{V}_{O_2max}$) when the subjects had been severely depleted of their muscle glycogen stores before the exercise bout.

More recent investigations suggest, however, that, although protein could contribute only a minor portion in terms of energy expenditure (Wolfe et al. 1984), there is a significant increase in the rate of amino acid catabolism with exercise (Rennie et al. 1981). This is secondary to the exercise-induced increase in several metabolic processes, such as hepatic gluconeogenesis and the citric acid cycle, where amino acid carbon is utilized. Hepatic gluconeogenesis may be the limiting pathway during prolonged exercise, since the brain and the nervous system are restricted to blood glucose as their sole energy-yielding substrate. It is well established that, during exercise, liver gluconeogenesis is increased in proportion to both the intensity and the duration of exercise (Ahlborg and Felig, 1982; Ahlborg et al. 1974) and that alanine, the release of which from muscle is markedly increased by exercise, is an important gluconeogenic precursor during exercise. Alanine is formed from pyruvate and glutamate in the alanine aminotransferase reaction. The 2-oxoglutarate formed from glutamate in this reaction is, further-more, important for the function of the citric acid cycle (Chang and Goldberg, 1978). In addition to alanine, there is a substantial release of glutamine from skeletal muscle. Glutamine is believed to be indispensable for normal function of the immune system and other rapidly dividing cells (Newsholme and Leech, 1983).

There is limited information, however, concerning the influence of different patterns of exercise on muscular production and release of glutamine. Further-more, there is ample evidence to suggest that branched-chain amino acid (BCAA) metabolism is activated, at least during prolonged exhaustive exercise (Wagen-makers et al. 1990; Kasperek, 1989; Dohm et al. 1985).

It is generally accepted that during exercise protein synthesis in skeletal muscle is depressed. This has been found both in humans and in other species (Arvill, 1967; Bazulko and Rogozkin, 1973; Dohm et al. 1985; Millward et al. 1982; Rennie et al. 1980; Zimmer et al. 1970), whereas it is still controversial whether exercise increases the overall rate of muscle protein breakdown (see reviews by Viru, 1987, and Wolfe, 1987). Irrespective of this, the depressed protein synthesis will leave amino acids available for catabolic processes. In general, amino acids in the free pool of skeletal muscle tissue have three ways of entry into the pool: (a) degrada-tion of muscle proteins; (b) conversion from other amino acids and intermediates; and (c) uptake from the extracellular fluid, where b applies to non-essential amino acids. Simultaneously, amino acids within the pool have three ways of leaving the pool: (a) release into the extracellular fluid; (b) reincorporation into protein and (c) metabolism in the muscle. Although the response to a single bout of exercise is mainly catabolic, it is evident that longer periods of exercise training often lead

▌h anabolic response, at least with respect to muscle. One reason is that, in contrast to the exercise period *per se*, whole-body protein synthesis is significantly increased after exercise (Devlin *et al.* 1990). In hospitalized patients on intravenous feeding only, Albert *et al.* (1989) recently presented evidence that 1 h of daily exercise was effective in providing systemic as well as limb-specific enhancement of amino acid balance in muscle.

Fibre type differences

The rates of protein synthesis and breakdown in skeletal muscle are known to be higher in slow-twitch than in fast-twitch fibres (Garlick *et al.* 1989). In rat skeletal muscle, the intracellular concentration of free amino acids has also been shown to be dependent on muscle fibre population; Turinsky and Long (1990) reported that the total concentration of 23 free amino acids was twice as high in slow-twitch as in fast-twitch muscles. With respect to glutamine and glutamate, the concentrations were threefold and fourfold greater in slow-twitch than in fast-twitch muscles, respectively. BCAA levels were 20–30 % higher in slow-twitch than in fast-twitch muscle. The only amino acids that displayed higher (approx. 30 %) concentrations in fast-twitch muscle were alanine and glycine. There are no corresponding human data, but it is to be expected that fibre-type differences would be smaller in human skeletal muscle. This has been shown for most other variables, the probable reason being that, in most experimental animals, the different fibre types are largely located in different muscles or in different parts of one muscle, whereas most human muscles are mixed muscles, with all fibre types intermingled in a mosaic pattern.

The effect of training state

We have found some evidence that trained individuals may have higher basal amino acid concentrations in their plasma than untrained individuals (J. Henriksson, H. Rosdahl, Å. Agius and K. Carlin, unpublished observations). We compared seven endurance-trained individuals and eight sedentary controls (see legend to Fig. 1 for maximal oxygen uptake values) and found that 10 out of 11 measured amino acids (all except serine) displayed higher plasma levels in the trained subjects. However, the differences were quite small (16.1±5.5 %, mean±s.D.) and reached statistical significance only in the case of plasma phenylalanine (15 % higher in the trained group, $P<0.05$). The average amino acid concentrations in skeletal muscle were higher in the trained group for eight of the eleven amino acids, but there was a statistically significant difference only for glutamate (+39 %, $P<0.05$) and taurine (+36 %, $P<0.05$). In the literature, there is only one reference to a possible difference in this direction (Einspahr and Tharp, 1989). These investigators compared 12 members of a major university track team who ran an average of 110 km per week with 13 controls who ran less than 5 km per ▌eek and found that the trained subjects had significantly higher plasma

concentrations of leucine (41 %), isoleucine (27 %) and tyrosine (23 %). In a study by Holm *et al.* (1978), seven obese subjects and seven controls performed aerobic training three times weekly for 6 weeks. After the training period, the plasma concentration of leucine decreased and the concentration of alanine increased in the obese subjects, but no changes were observed with training in the controls. The obese subjects had elevated plasma levels of the BCAAs, tyrosine and phenylalanine before as well as after physical training.

Effects of short-term exercise

Here data are available mainly from studies in man. The characteristic findings, i.e. increased muscle alanine and glutamine concentrations and a decreased concentration of glutamate, are, however, observed also with electrical muscle stimulation in the rat (Goodman and Lowenstein, 1977; Meyer and Terjung, 1979).

Plasma amino acids

During 10–20 min of exercise at 70 % of the maximal oxygen uptake, Bergström *et al.* (1985) observed increased concentrations of alanine (30–50 %), glutamine (30–35 %) and arginine (35–45 %) in plasma. Plasma glutamate and aspartate levels were decreased (−30 %) by the exercise. Similarly, Eriksson *et al.* (1985), who studied the effect of 15 min of exercise at 33 %, 55 % and 80 % of the maximal oxygen uptake, observed increases in arterial glutamine (15 %, 15 % and 25 % at the respective exercise intensities) and alanine (45 %, 65 % and 120 %) as well as small (<20 %) increases in lysine, histidine and arginine concentrations. It should be pointed out that part of the increase in plasma amino acid levels as a result of exercise is attributable to the haemoconcentration that accompanies exercise; in the study by Eriksson *et al.* (1985) this effect was maximally 9 %. Sahlin *et al.* (1990), who studied their exercising subjects until fatigue (approx. 75 min) at 75 % of the maximal oxygen uptake, showed that the 40 % decrease in plasma glutamate concentration occurred after only 5 min of exercise. The plasma glutamate level then remained low during the entire exercise bout. The same time course held for the doubling of plasma alanine concentration, whereas the 25 % increase in plasma glutamine concentration occurred more gradually. The effect of higher exercise intensities was studied by Katz *et al.* (1986a) and by Babij *et al.* (1982). The latter authors observed changes in plasma amino acid levels at work loads demanding between 25 % and 100 % of the maximal oxygen uptake. They found that the plasma alanine concentration tended to increase exponentially with increasing work load, whereas the relationship between plasma glutamine concentration and exercise intensity was linear. An interesting observation was made by Katz *et al.* (1986a). They found a 60 % decrease in plasma glutamate concentration with maximal exercise, similar to that seen in other studies, but no significant change when glutamate was determined in whole blood. This was

tributed to the fact that most of the glutamate is confined to the red blood cell; at rest, the whole-blood glutamate concentration is twice the plasma concentration.

Muscle amino acids

With respect to the amino acid concentrations in skeletal muscle, alanine (increase) and glutamate (decrease) changed in the same way as they did in plasma. The behaviour of the muscle glutamine concentration was, however, more variable. 10–20 min of exercise at 70 % of the maximal oxygen uptake decreased muscle glutamate by 65 % and increased muscle alanine and glutamine levels by 60 % and 25 %, respectively (Bergström *et al.* 1985). At a similar exercise intensity, Sahlin *et al.* (1990) recorded a doubling of the muscle alanine concentration, a substantial decrease in muscle glutamate (-70 %), and a small decrease (-10 %) in muscle glutamine concentration. With 4 min of fatiguing exercise at an intensity corresponding to the maximal oxygen uptake, there was no change in the muscle glutamine concentration (Katz *et al.* 1986*a*), whereas muscle alanine and glutamate concentrations changed exactly as they did in the study by Sahlin *et al.* (1990). With isometric exercise to fatigue at two-thirds of the maximal contraction force (1 min) muscle glutamine concentration did not change, whereas there was an increase in muscle alanine ($+30$ %) and a decrease in muscle glutamate (-30 %) concentrations (Katz *et al.* 1986*b*). In addition to these amino acids, Bergström *et al.* (1985) observed significant increases in muscle aspartate concentration ($+55$ %). The studies of Eriksson *et al.* (1985), Katz *et al.* (1986*a*) and Sahlin *et al.* (1990) also included measurements of the exchange of the different amino acids during exercise in leg muscle. In the basal state, there was a net output of 16 amino acids from the leg tissues. The rate of release of alanine and glutamine (17 and 14 μmol min^{-1}, respectively) exceeded that of all the other amino acids and accounted for 60 % of the total output. This is a well-established finding (for references, see Felig, 1975). Leg alanine release increased significantly during exercise at 55 % (threefold, Eriksson *et al.* 1985), 75 % (2.5-fold, Sahlin *et al.* 1990), 80 % (ninefold, Eriksson *et al.* 1985) and 97 % (sixfold, Katz *et al.* 1986*a*) of the maximal oxygen uptake. An accelerated alanine release from working skeletal muscle was also demonstrated by Felig and Wahren (1971) and Ahlborg *et al.* (1974). Average values for glutamine release also increased substantially with exercise (two- to sevenfold at 75–80 % of the $\dot{V}_{O_2max}$), but the interindividual variability was very high and the changes were not statistically significant. For glutamate, a net uptake (9–13 μmol min^{-1}) was found in the basal state, which was unchanged (Sahlin *et al.* 1990) or doubled to tripled (Eriksson *et al.* 1985; Katz *et al.* 1986*a*) with exercise. The latter authors point out, however, that there is likely to be an influx of glutamate into the red blood cells during the passage of blood through the muscle. This suggests that, when based on measurements of the arteriovenous difference, muscle uptake of glutamate may be overestimated at rest as well as during exercise. The exchange of the other amino acids seems to be uninfluenced by exercise in leg muscle.

The branched-chain amino acids (BCAA)

These amino acids (leucine, isoleucine and valine) are of special interest since they have been reported to be preferentially degraded in skeletal muscle (Abumrad *et al.* 1982; Khatra *et al.* 1977). During short-term exercise, the BCAAs in plasma were found to be slightly increased (10 %) (Sahlin *et al.* 1990), decreased (−20 %) (Bergström *et al.* 1985) or unchanged (Eriksson *et al.* 1985) during the exercise period, but slightly decreased in the first hour post-exercise (Eriksson *et al.* 1985). Sahlin *et al.* (1990) attempted to determine arteriovenous differences for the BCAAs; however, these were not significantly different from zero, either at rest or during exercise. In contrast, Felig and Wahren (1971) have previously reported that aerobic exercise is associated with an accelerated muscle uptake of leucine. Muscle BCAA concentrations have been found to be unchanged with short-term exercise at intensities corresponding to 50–70 % of the $\dot{V}_{O_2max}$ (Bergström *et al.* 1985; J. Henriksson, unpublished data). The lack of changes in the muscle BCAA concentration with short-term exercise is notable since it has been clearly demonstrated, using an isotopic approach (Wolfe *et al.* 1982), that the oxidation of leucine is significantly increased during exercise in humans. Using an *in vitro* approach, we have never found a tendency for the BCAA content to be lower in stimulated muscle than control values (Nie and Henriksson, 1989). These results therefore suggest that the increased metabolism of the BCAAs in skeletal muscle during energy-demanding situations can be achieved only when there is a supply of exogenous branched-chain amino acids. The *in vitro* data furthermore suggest that a protective mechanism exists in muscle, which maintains the endogenous BCAA levels even when very energy-demanding conditions coincide with an insufficient supply of external BCAAs (Nie and Henriksson, 1989).

Fielding *et al.* (1986) studied muscle and blood levels of alpha-ketoisocaproic acid (KIC), the first metabolic intermediate in leucine degradation, in seven male volunteers performing bicycle exercise to exhaustion. Muscle KIC concentration was found to be increased by 50 % during the exercise period. Plasma KIC concentration remained unchanged during exercise, but following the exercise bout there was a marked increase, with peak values (an approximate doubling) occurring 30 min post-exercise. A similar pattern of change in plasma KIC concentration was also found with prolonged low-intensity (50 % of the maximal oxygen uptake) exercise. The data suggest that high-intensity brief exercise is associated with an increased transamination of leucine. The pattern of changes in muscle and plasma KIC levels during exercise and recovery may be explained by a relatively low leucine oxidation rate in muscle during the exercise bout in combination with a deficiency of glutamate as the reaminating substrate. This would lead to diffusion of KIC out of the muscle (leading to the high post-exercise plasma values) for transamination or oxidation elsewhere.

Phenylalanine, tyrosine and 3-methylhistidine (3-MH)

These amino acids are treated separately, since they can be neither synthesize

or degraded by muscle and thus provide a measure of the net rate of protein degradation (i.e. the rate of degradation minus the rate of synthesis). 3-MH may yield even more specific information since, once separated from protein, 3-MH is neither metabolized nor reincorporated into protein. 3-MH occurs solely in actin and myosin and is formed by the post-translational methylation of histidine, as there is no tRNA for 3-MH (Young *et al.* 1972). An increased muscle and plasma content of 3-methylhistidine is, therefore, often taken to indicate an elevated myofibrillar (contractile) protein degradation (Young *et al.* 1972; Young and Munro, 1978). A drawback with 3-MH is that a considerable portion of its plasma concentration originates from the degradation of intestinal proteins, which may have a greater turnover rate than skeletal muscle protein (Rennie and Millward, 1983). Sahlin *et al.* (1990) found that plasma phenylalanine and tyrosine levels increased by approximately 40 % as a result of the exercise; in the study by Bergström *et al.* (1985), the corresponding figure was 50–90 %, and in that of Eriksson *et al.* (1985) around 20 %. Bergström *et al.* (1985) recorded small, but statistically significant, decreases (approx. 25 %) in the muscle content of these amino acids. With 20 min and 1 h of exercise at 50 % of the maximal oxygen uptake, we could not detect any changes in muscle or plasma tyrosine. Muscle and plasma phenylalanine concentrations increased, however, by 15–20 %, but only in trained subjects (see below). Interestingly, the muscle concentration of 3-MH increased by 25 % in both trained and untrained subjects, with unchanged levels in plasma in accord with Rennie *et al.* (1981).

Effect of prolonged exercise

Studies in the rat

Dohm *et al.* (1981) studied levels of free amino acids in muscle and plasma in rats that either swam (1 or 2 h) or ran (until exhaustion). Exercise was found to lower the plasma levels of alanine and of the acidic amino acids and their amides. Alanine (15–20 %), glutamate (15–40 %) and glutamine (15–20 %) levels were depressed in the gastrocnemius muscle of the exercised rats. In contrast, muscle and plasma concentrations of the BCAAs were generally elevated by exercise, as were tyrosine, phenylalanine and (plasma) 3-methylhistidine levels. The changes in the concentrations of the latter three amino acids support the previous findings (see above) that the balance of protein synthesis/degradation is shifted towards degradation.

The increased muscle concentrations of the BCAAs in the study by Dohm *et al.* (1981) are in agreement with the results of Kasperek (1989), who investigated the effect of 2 h of treadmill running in the rat. Kasperek observed that the muscle levels of the branched-chain oxoacids were either unchanged or slightly reduced by exercise, but were elevated 10 min post-exercise. This pattern might be explained by the marked activation of the branched-chain 2-oxoacid dehydrogenase induced by exercise, but which had returned to the control value 10 min post-exercise. Regarding the increase in the plasma concentration of the BCAAs, the

results of Dohm *et al.* (1981) and of Kasperek (1989) are in general agreement with each other as well as with the results of Okamura *et al.* (1987). In contrast, Ji *et al.* (1987) found significantly decreased concentrations of the BCAAs in plasma following 1 h of treadmill running in the rat. It is possible that this difference is secondary to the fact that the rats in the latter study had undergone an 8-week endurance training programme before the study was performed. It has been shown that trained muscle possesses an increased enzymatic capacity to degrade BCAAs oxidatively (Dohm *et al.* 1977). This apparent variability in the effect of exercise on the plasma levels of the BCAAs is of particular interest, since decreased plasma BCAA concentrations have been proposed to be one factor explaining fatigue in prolonged exercise (Parry-Billings *et al.* 1990) (for further discussion, see below). Interestingly, Ji *et al.* (1987) obtained evidence that the BCAAs may be only partially metabolized within muscle to form branched-chain ketoacids and short-chain acylcarnitines, which are released into the circulation and transported to the liver for further oxidation. The amino group thereby liberated may be released from muscle in the form of glutamine or alanine.

The decreases in muscle and plasma levels of alanine, glutamine and glutamate with prolonged exercise are in agreement with results in man (see below). Changes in muscle glutamine concentration with exercise are of particular interest since changes in muscle glutamine with different physiological and pathological states have been found to correlate closely to the muscle protein synthesis rate (Jepson *et al.* 1988). This has been claimed to be unique to glutamine, and recent evidence suggests that glutamine could influence protein synthesis; in the perfused rat hindlimb, acute changes in glutamine concentration appeared directly to induce changes in the rate of muscle protein synthesis (MacLennan *et al.* 1987).

Results in man

Décombaz *et al.* (1979) investigated the concentration of free amino acids in venous blood of 11 trained male subjects before and after a 100 km running competition. They found the serum concentrations of most free amino acids, including the BCAAs and alanine, to be reduced to 35–85 % of the pre-race value. The production of 3-methylhistidine remained unchanged. The authors concluded that the metabolism of nitrogenous substances plays a part in the body economy during long-lasting exercise and that the utilization of endogenous amino acids, though of no quantitative importance as a direct source of energy, could be of some significance for gluconeogenesis. These data were, thus, quite similar to those of Dohm *et al.* (1977) in the rat. The results suggest an increased metabolic use of these amino acids with exercise. Changes in alanine and glutamine concentrations are, however, likely to be largely explained by an increased consumption in organs other than muscle. In man, Ahlborg *et al.* (1974) found that the arterial concentration of alanine was elevated after 40 min of exercise but was near control levels after 4 h of exercise, despite the fact that the release of alanine from exercising muscle was greater at 4 h than at 40 min. These authors could, furthermore, show that exercise of longer duration resulted in a greater uptake of

alanine by the liver and presumably increased gluconeogenesis. Of particular interest in the study by Décombaz *et al.* (1979) was the marked drop in the plasma content of all the BCAAs. This would tend to support the notion from the study of Ji *et al.* (1987) that this occurs in trained individuals only.

In another investigation of prolonged exercise, Rennie *et al.* (1981) studied four healthy men exercising at 50 % of the $\dot{V}_{O_2max}$ for 3.5 h. In this study, glutamine, glutamate, the BCAAs, alanine, phenylalanine and tyrosine concentrations were elevated in plasma at 40 and 90 min of exercise, but as exercise progressed all amino acid concentrations fell progressively. At the end of exercise large decreases were observed for almost all amino acids, but particularly for alanine, glutamine, glycine and the BCAAs. These findings were consistent with those of Décombaz *et al.* (1979). The training state of the subjects of Rennie *et al.* (1981) was not indicated, but only one of the four subjects had a high maximal oxygen uptake. We have repeated the design used by Rennie *et al.* (1981), but with muscle biopsy as well as blood sampling several times during the exercise bout (at rest and after 20 min, 1 h, 2 h and at 3.5 h of the bicycle exercise at 50 % of the $\dot{V}_{O_2max}$) (Figs 1–5). We found the plasma concentration of most amino acids to be either stable or increased up to 2 h of exercise; thereafter, the plasma concentration of several of the amino acids displayed a decrease. This latter group included glutamate (Fig. 2), glutamine (Fig. 3), alanine (Fig. 4), serine and taurine, but interestingly none of the three amino acids that are normally used as indicators of net muscle protein balance (i.e. phenylalanine, tyrosine and 3-methylhistidine, see above).

That long-term exercise may be needed for a significant decrease in plasma amino acid concentration is supported by the findings of Parry-Billings *et al.* (1990). They found that although the concentration of glutamine in plasma was
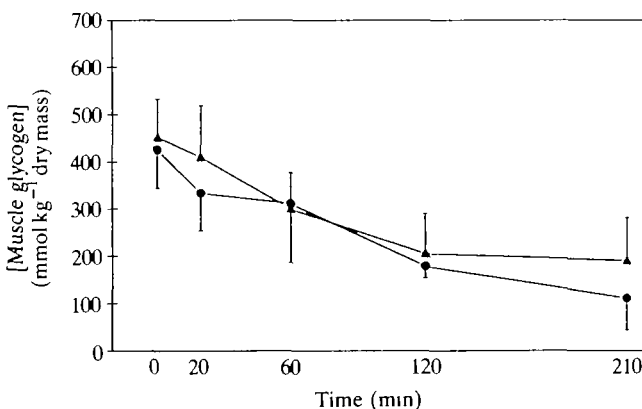

Fig. 1. Changes in the muscle glycogen concentration during 3.5 h of bicycle ergometer exercise at an intensity corresponding to 50 % of the maximal oxygen uptake. Repeated biopsies were obtained from the quadriceps femoris muscle, vastus lateralis, in seven well-trained subjects (▲) ($\dot{V}_{O_2max}$ 59.4±2.5 ml kg^{-1} min^{-1}) and eight untrained controls ($\dot{V}_{O_2max}$ 38.7±1.4 ml kg^{-1} min^{-1}). Values are mean±S.E.M.
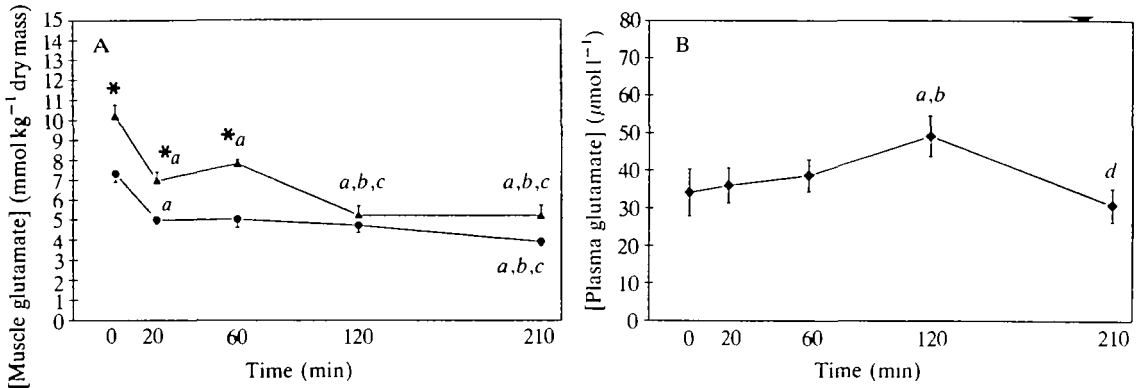
Fig. 2. Changes in the muscle (A) and plasma (B) glutamate concentrations during 3.5 h of bicycle ergometer exercise at an intensity corresponding to 50 % of the maximal oxygen uptake. For plasma glutamate, values from trained subjects and controls have been pooled (◆), since there were no significant differences between the groups. An asterisk indicates a significant difference between the trained (▲) and the untrained (●) groups ($P<0.05$), while a, b, c, or d indicates a significant difference from rest (0 min), 20 min, 60 min and 120 min of exercise, respectively ($P<0.05$). Statistical analysis by a two-way analysis of variance (ANOVA). Values are mean ±S.E.M. For further information, see the legend to Fig. 1.

decreased after a 42-km marathon it was unaffected by a 30-km treadmill run. Somewhat similar results were observed in rats; plasma glutamine levels were decreased after exercise to exhaustion in trained rats, but were unchanged following exercise in sedentary rats, which ran for a significantly shorter time (Parry-Billings *et al.* 1988). The response of the plasma concentrations of the BCAAs in our own study deserves mentioning, since these displayed a decrease in the seven subjects who were endurance trained [$\dot{V}_{O_2max}$: 59.4±2.5 ml kg^{-1} min^{-1}
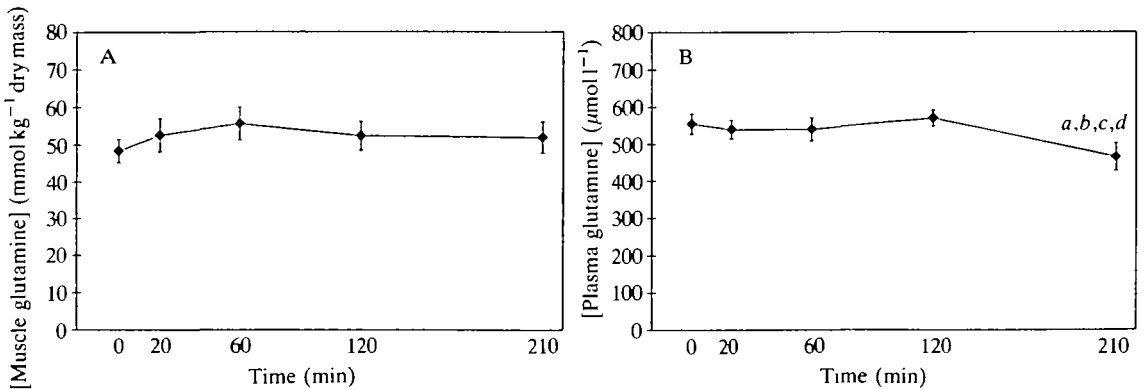

Fig. 3. Changes in the muscle (A) and plasma (B) glutamine concentration during 3.5 h of bicycle ergometer exercise at an intensity corresponding to 50 % of the maximal oxygen uptake. Values are mean±S.E.M. For further information see the legends to Figs 1 and 2.
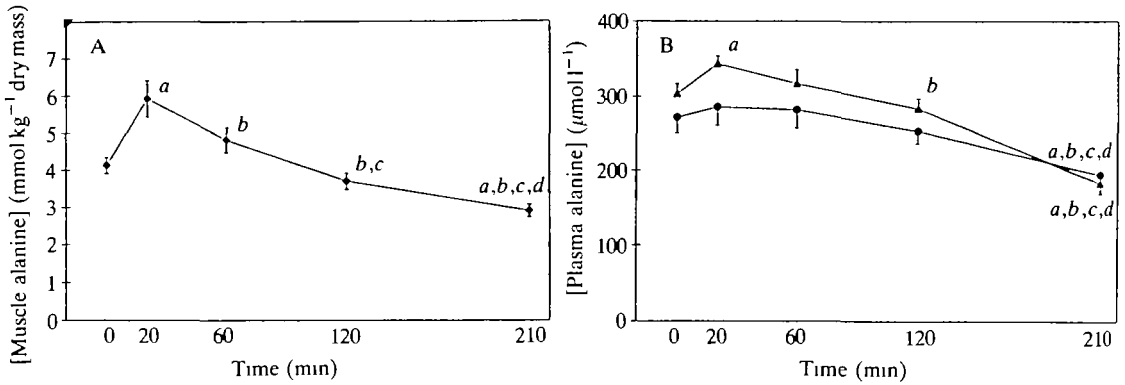
Fig. 4. Changes in the muscle (A) and plasma (B) alanine concentration during 3.5 h of bicycle ergometer exercise at an intensity corresponding to 50 % of the maximal oxygen uptake. Muscle alanine values have been pooled since there were no significant differences between the trained subjects and the controls. Values are mean±s.e.m. For further information see the legends to Figs 1 and 2.

(mean±s.e.m.)] but not in the untrained controls ($\dot{V}_{O_2max}$: $38.7\pm1.4\,\text{ml kg}^{-1}$ min^{-1}) (Fig. 5). Previously, exercise-induced changes in the levels of the branched-chain amino acids in man have been mainly limited to short-term exercise, but there is some information from measurements in conjunction with the Stockholm marathon (see Blomstrand *et al.* 1988). These data tend to support the hypothesis from our own study, as well as from the studies in the rat (see above), suggesting that a decreased plasma level of the branched-chain amino acids with prolonged exercise is most likely to occur in well-trained individuals.

Newsholme and colleagues (see Parry-Billings *et al.* 1990) have proposed a role
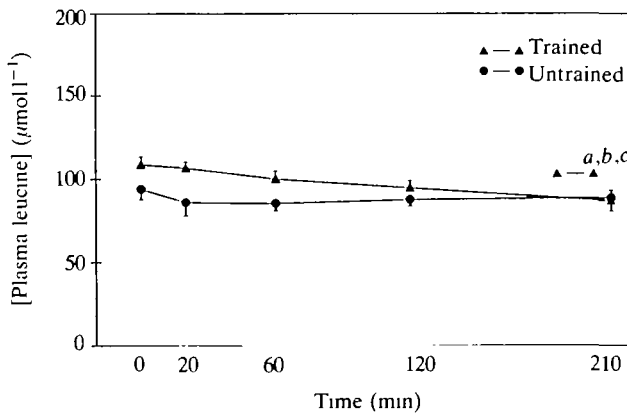

Fig. 5. Changes in the plasma concentration of the branched-chain amino acid leucine during 3.5 h of bicycle ergometer exercise at an intensity corresponding to 50 % of the maximal oxygen uptake. Values are mean±s.e.m. For further information see the legends to Figs 1 and 2.

for these specific amino acids in the etiology of central fatigue in athletes. The theory is based on evidence that the neurotransmitter 5-hydroxytryptamine (5-HT) may be responsible for causing a state of tiredness and sleep in both man and experimental animals as well as on the evidence that none of the reactions in the pathway for formation of 5-HT in the brain approaches saturation with substrate (see references in Parry-Billings *et al.* 1990). The authors speculate that, since the transport process responsible for transporting tryptophan (the precursor of 5-HT) over the blood–brain barrier is the same as that transporting the BCAAs, a decreased plasma concentration of the BCCAs would, through decreased competition, lead to an increased entry of tryptophan into the brain and thus to increased levels of 5-HT. The increase in the ratio of free tryptophan to branched-chain amino acids in plasma may, however, also be influenced by the marked increase in plasma free tryptophan concentration that occurs as a result of the increased plasma fatty acid concentration with endurance exercise (Curzon *et al.* 1973). Our own results cannot be used either to confirm or to negate this hypothesis, but do not indicate that the decrease in the concentrations of plasma branched-chain amino acids were important in this situation. Thus, although decreasing in the trained subjects with exercise, the levels never decreased below those of the untrained control subjects, who had constant plasma concentrations of the branched-chain amino acids throughout the 3.5 h of exercise. The decrease in plasma glutamine concentration with prolonged exercise is of considerable interest, since this amino acid has been reported to be an important fuel for macrophages and lymphocytes; the rate of glutamine utilization in these cells is either similar to or greater than that of glucose (Ardawi and Newsholme, 1983; Newsholme and Newsholme, 1989). Therefore, any significant decrease in the rate of glutamine utilization by these cells would be expected to decrease their rate of proliferation. Interestingly, a decreased plasma concentration of the branched-chain amino acids with exercise might further ameliorate the plasma glutamine depletion, since the BCAAs are likely to be an important source of nitrogen for the synthesis of glutamine in skeletal muscle, the body's major tissue involved in glutamine production in the post-absorptive state.

Muscle amino acids

In our study we also obtained data on amino acid concentrations in skeletal muscle during prolonged exercise at 50 % of the $\dot{V}_{O_2max}$ (Figs 2–4). Muscle glutamate concentration decreased on average by 32 % ($P<0.05$) during the first 20 min of exercise. This decreased level was maintained at a relatively stable level from 20 min to 3.5 h of exercise (Fig. 2). Muscle glutamine content showed no tendency towards a decrease during the 3.5 h of exercise (Fig. 3). Muscle alanine concentration displayed a different pattern, with an average 44 % increase ($P<0.05$) during the initial 20 min of exercise, but a decrease thereafter. At 3.5 h of exercise, the level was 30 % below basal values ($P<0.05$) (Fig. 4). Muscle 3-MH content increased with exercise by approximately 25 % ($P<0.05$). Similar changes were observed in the muscle contents of tyrosine and phenylalanine. In accord-

ance with the *in vitro* data (see above), muscle contents of the BCAAs were not influenced by the 3.5-h exercise session. It is noteworthy that this occurred in spite of a 70 % decrease in muscle glycogen concentration (Fig. 1). The literature contains very little information on amino acid changes in skeletal muscle during long-term exercise. Rennie *et al.* (1981) measured muscle free amino acid concentrations before and after 3.5 h of exercise at 50 % of $\dot{V}_{O_2max}$. They observed a slight decrease during exercise for most of the amino acids but, in contrast to our own study (Fig. 3), muscle glutamine concentration fell by 34 %.

Regulation

The concentration of glutamate in muscle and plasma consistently decreases during short-term exercise. This is for the most part balanced by an increase in alanine concentration. Muscle alanine, and in all probability also glutamine, is released to a greater extent during exercise than at rest (see above). Muscle contraction leads to an increased concentration of pyruvate, and the transamination of glutamate yielding alanine can be explained by an increased availability of pyruvate for the alanine aminotransferase reaction. This reaction also involves the formation of alpha-ketoglutarate, making it a major anaplerotic reaction with respect to the citric acid cycle. Most of the pyruvate formed in muscle originates from the glycolytic pathway, but the carbon chains of several amino acids are also converted to pyruvate. It is known that skeletal muscle produces ammonia (NH_3) during intense contractions (Meyer and Terjung, 1979), the major source being AMP, the deamination of which is catalyzed by adenylate deaminase. A portion of this NH_3 pool may be used in glutamine formation *via* the glutamine synthetase pathway and therefore contributes to the glutamate depletion with exhaustive exercise. Another possible source of glutamate removal is the glutamate dehydrogenase reaction, in which ammonia and alpha-ketoglutarate are formed. This could also be an important anaplerotic reaction, but the *in vivo* activity of this enzyme is considered to be low (see Sahlin *et al.* 1990).

There is evidence that high-intensity brief exercise leads to an increased transamination of leucine, but its oxidation rate in muscle appears to be low (see above). Leucine transamination also leads to glutamate formation for further conversion to alanine or glutamine, the release of which is increased at higher exercise intensities. The increased muscle concentration of 3-MH suggests that the changes in amino acid concentrations with exercise recorded in these studies are also influenced by an increased muscle proteolysis.

With prolonged exercise, there is a depletion of the plasma amino acid pool, which may be explained by an increased consumption in organs other than muscle. With respect to alanine, the muscle concentration is also decreased during the later stages of prolonged exercise (Fig. 4); therefore, a reduced muscle production of this amino acid seems to have contributed to the low plasma values. In our investigation, muscle glycogen was depleted by 70 % by the end of exercise (Fig. 1). This would result in a reduced pyruvate concentration and, subsequently,

a reduced flux through the alanine aminotransferase reaction, leading to a reduced
rate of alanine formation. The current data furthermore suggest that muscle levels
of free amino acids are more stable than those of plasma since, with the exception
of alanine, there were no corresponding decreases in muscle amino acids during
the later stages of exercise. There have been reports of a continuous rise in blood
ammonia concentration with prolonged exercise, and a considerable portion of
this increase is believed to originate from amino acid metabolism (see Wagen-
makers *et al.* 1990). This is based upon the following: (1) muscle ammonia
formation is greater than can be accounted for by the adenylate deaminase
reaction (see above); and (2) there is a significant activation of the muscle
branched-chain 2-oxoacid dehydrogenase complex in long-term exercise. The
latter would lead to a drainage of citric acid cycle intermediates (*via* BCAA
transamination) and possibly the formation of glutamine *via* the glutamine
synthetase reaction (Wagenmakers *et al.* 1990). The drainage of citric acid cycle
carbon by branched-chain amino acid transamination in combination with a
reduced inflow from glycolysis has been suggested to be a factor limiting the rate of
fatty acid oxidation during prolonged exhaustive exercise.

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