Changes in Serum Amino Acid Concentrations during Prolonged Endurance Running

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Abstract Eight endurance trained distance runners (5 male and 3 female) ran on a treadmill at 60% of their $\dot{V}O_{2\text{max}}$ for 3 h. Blood samples (about 12 ml) were taken immediately prior to the exercise, and thereafter every 30 min during and at the end of exercise. Urinary urea excretion was determined for the day of exercise, and for a control day a week after the exercise. Blood samples were analyzed for glucose, free fatty acids, urea and free amino acids concentrations. As the exercise was continued, there was a gradual decrease in the concentrations in serum of alanine and proline such that the concentrations of both the amino acids were significantly less at the end of exercise than the resting values ($p<0.01$). These changes appeared to be highly correlated ($r=0.981$, $p<0.05$). The serum glucose concentration increased significantly ($p<0.05$) after 30 min of exercise, remained significantly elevated until 150 min of exercise and then decreased to reach the resting value at the completion of the exercise. Serum free fatty acid (FFA) concentration was significantly higher at the end of exercise compared to the resting value. The increase in serum FFA concentration and the decreases in the concentrations in serum of alanine and proline were also found to be highly correlated (alanine versus FFA: $r=-0.986$, $p<0.05$; proline versus FFA: $r=-0.961$, $p<0.05$). The total urinary urea excretion ($332\pm43\text{mmol}$ versus $424\pm46\text{mmol}$) and urine volume ($978\pm187\text{ml}$ versus $1,480\pm245\text{ml}$) were less on the day of exercise than on the control day. The decrease in urine volume was found to be significant ($p<0.05$).

Key words: exercise, serum, amino acids concentration, free fatty acids level, glucose level.

Exercise is known to promote marked alterations in carbohydrate and lipid metabolism, enabling the organism to meet the increased energy demands for exercise [1]. In the last century, von Liebig [2] advanced the idea that amino acids derived from proteins served as the major fuel for exercise. In more recent times

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the overwhelming evidence indicates that carbohydrate and fat are the major energy sources of exercise, and this led to the belief that amino acids are not used to sustain the energy requirements of exercise [3]. Recent studies however indicate that protein catabolism and amino acid oxidation may be increased in strenuous exercise [4–8]. When exercise is performed in the presence of low glycogen levels [9] or if it is prolonged [10, 11] the extent of protein degradation may become greater. Calculations by Lemon and Nagle [6] and Dohm et al. [5] indicate that protein may provide as much as 10 to 18% of the energy when exercise is performed in the presence of low glycogen levels or if the exercise is prolonged.

When amino acids are metabolized, the amino group is first removed by transamination with α-ketoglutarate and the resultant glutamate is oxidatively deaminated. The carbon skeletons are converted to metabolites that are common to the pathways of carbohydrate and lipid metabolism. Convergence of metabolic pathways has made it difficult to quantify the involvement of amino acids as fuels in exercise. The ammonium ions produced are mostly converted into urea in the liver, which is then excreted. Since increased amino acid metabolism should lead to increased nitrogen excretion in the form of urea, many researchers measured nitrogen loss in the form of urea as an index of amino acid metabolism in exercise. A substantial number of experiments report increased urea excretion after exercise [5, 7, 12]. However, there have also been a significant number of studies in which nitrogen excretion did not change as a result of exercise [9, 13, 14]. Careful analysis shows that experiments in which increased nitrogen excretion has been reported involved a very strenuous single bout of exercise, and urea excretion was measured over a brief period of time. Experiments which did not show an increase in the urea excretion in urine involved measuring nitrogen balance over 10- to 18-day periods for subjects who were on a regular training schedule that did not involve a single strenuous exercise bout on any particular day. Some authors examined the serum urea concentration as a measure of amino acid metabolism. In short-term intense exercise, serum urea concentration remains fairly constant, but several authors observed that during long-lasting exercise, beyond 60–70 min, serum urea concentration increases [7, 10, 12].

Haralambie and Berg [10] have shown a significant correlation ($r = -0.905$) between the increase in serum urea concentration and the decrease in the serum total amino acid concentration. Lemon and co-workers [9] observed that in prolonged swimming involving eight interscholastic swimmers, serum urea concentration was increased to 252% of the pre-exercise value. The decrease in urine and sweat production could account for only 38% of the observed increase in serum urea concentration. Thus, the authors concluded that the swim caused an increase in urea production i.e. amino acid oxidation.

Several authors studied changes in total amino acid contents of blood as a function of exercise and others studied the changes in the serum concentration of individual amino acids such as alanine, leucine, lysine or tyrosine. When exercise exceeds 2 h in duration, a drop in the total concentration of free plasma amino acids

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and alanine occurs during the activity period [8, 10, 12]. Babij and co-workers [15], Felig and Wahren [16] and Poortmans and Delisse [17] observed that in humans, intense exercise of less than 2 h duration consistently increases plasma alanine concentrations. Einspahr and Tharp [18] investigated the effect of endurance training on plasma amino acid concentrations at rest and after short-duration intense exercise. Their results indicate that alanine, glycine, leucine, isoleucine, phenylalanine, tyrosine and glutamate concentrations in plasma increased significantly from pre- to post-exercise. Rennie and co-workers [8], Wolfe and co-workers [19], Hagg and co-workers [20] performed experiments in which they measured plasma leucine concentrations and leucine oxidation rates. They found that in exercise, leucine oxidation was increased but leucine concentration in serum was not changed, indicating that the only source for leucine oxidized was from the net breakdown of body protein since leucine is an essential amino acid. However, different results were observed when different labeled amino acids were used. For example, the increase in leucine oxidation due to exercise was much greater than that of lysine [21], indicating that the rate of oxidation of a particular essential amino acid cannot be representative of that of all essential amino acids and hence that of whole-body protein degradation. In view of the confusing nature of the literature regarding amino acids as fuels in exercise, we decided to study changes in the serum concentration of free amino acids, urea, glucose and free fatty acids and urinary urea excretion in a prolonged exercise event. Specifically, the aim of the study was to determine the evolution of some plasma amino acids during prolonged endurance running involving highly trained endurance runners.

METHODS

Informed written consent was obtained from eight volunteers (5 men, 3 women), as approved by Cumberland College of Health Sciences Human Ethics Committee, before their participation. Subjects were healthy highly trained endurance runners. Each subject initially undertook an incremental treadmill exercise test to volitional fatigue to determine $\dot{V}_{\text{O}_{2}\text{max}}$. Respiratory gases were measured using Ametek O$_2$ Analyser S-3A/1 and Ametek CO$_2$ CD-3A Analyser interfaced with an on-line respiratory gas analysis system developed at the Faculty of Health Sciences ("Extress"). Heart rate was monitored using a heart rate monitor (Philips) with electrodes attached in a CM5 configuration. Each subject was tested to establish an individual regression equation of the form:

$$Y = MX + B$$

where

$Y = \dot{V}_{\text{O}_2}$ ($l \text{ min}^{-1}$)  
$X =$ treadmill velocity  
$M =$ slope  
$B =$ $Y$ intercept
Rewriting the equation in the form \( X = (Y - B)/M \) allows the prediction of a treadmill speed for a given oxygen consumption. Data for this linear regression was obtained by running the subject for 3–4 min bouts of exercise at 12.0, 14.0, 16.0 km h\(^{-1}\) with the treadmill at zero gradient. Expired ventilation \( \dot{V}_{\text{E}} \), oxygen consumption \( \dot{V}_{O_2} \) and carbon dioxide production \( \dot{V}_{CO_2} \) were measured throughout each 4 min exercise period and recorded for the last minute i.e. third to fourth minute.

On the final visit to the laboratory, each subject undertook a continuous treadmill running bout of exercise of 3-h duration at 60% \( \dot{V}_{O_2}\text{max} \) in a post-absorptive but glycogen loaded state. The environmental conditions were maintained at 21 ± 2°C with relative humidity below 50% and a large electric fan was used to maintain convective airflow adequate to evaporate sweat. The subject voided bladder contents just prior to commencing the prolonged exercise. A venous catheter was inserted in a forearm vein for the purpose of obtaining serial blood samples throughout the prolonged exercise period. The catheter line was kept patent with 0.154 mol l\(^{-1}\) heparinized saline solution. Blood samples were taken just before the commencement of exercise and thereafter every 30 min until the conclusion of exercise.

After collection, blood was placed into chilled phlebotomy tubes. The clot tube was left on ice for an hour, then centrifuged at 4°C for 10 min at 2,000 × g. The serum was removed and an aliquot used for urea analysis. The remainder was frozen at −85°C for later amino acid analysis. Whole blood collected in an EDTA tube was used for hematocrit and hemoglobin determination. The fluoride/oxalate tube was centrifuged immediately after collection and the plasma frozen at −20°C for later analysis of free fatty acids and glucose.

**Urine.** One 24-h collection was made starting from the time the prolonged exercise began. The second control collection was made about a week after the prolonged exercise, on a day when the subjects were not engaged in any strenuous physical activity.

**Control experiment.** One subject remained standing for 3 h, and blood was taken every 30 min and analyzed for free fatty acids, hemoglobin and hematocrit.

1. **Analytical methodology.** Free amino acid concentrations in serum were determined by reverse phase HPLC. The amino acids were derivatized before injection with 4-dimethylaminoazobenzene-4′-sulfonyl chloride (dabsyl chloride) [22]. A 4 mmol l\(^{-1}\) solution in acetone was stored at −20°C. All amino acids were purchased from Sigma Chemical Co. and a mixture was prepared in 10 mmol l\(^{-1}\) HCl. The standard mixture was 10 mmol l\(^{-1}\) with respect to each of the amino acids: threonine, serine, glutamine, proline, glycine, alanine, valine, leucine and lysine, 2.5 mmol l\(^{-1}\) with respect to each of the amino acids: aspartic acid, asparagine, glutamic acid, methionine, isoleucine, phenylalanine, histidine, tryptophan, arginine, hydroxyproline, and 1 mmol l\(^{-1}\) with respect to tyrosine. The solution was aliquoted and frozen at −20°C. The chromatographic system consisted of two Waters (Milford, MA, USA) 510 pumps, and a 484 Waters

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absorbance detector. Pump control and data analysis were effected using a Maxima 820 chromatography workstation. Injections were made using a WISP 712 (Waters) with cooled sample compartment. A 30 cm \times 3.9 mm reversed phase C18 column (Novapak, Waters) with a Novapak guardpak was used. A gradient system consisting of acetate buffer (60 mmol \ l^{-1}, \text{pH} 4.18) plus 4\% dimethylformamide (A) and acetonitrile plus 4\% dimethylformamide (B) was used. The flow rate was set at 1.2 ml min^{-1}. The detection wavelength was 436 nm.

2. **Sample treatment.** In an icebath, 50 \mu l of plasma or standard was pipetted into an Eppendorf tube and 10 \mu l of standard mixture (diluted 1/10 with water) or 10 \mu l of 1 mmol \ l^{-1} HCl was added to the tube. 0.5 ml of cold ethanol (AR grade) was added to the mixture which was then vortexed and left on ice for 10 min. In was vortexed again and then centrifuged. The supernatant was removed and dried under nitrogen overnight at room temperature. The residue was dissolved in 100 \mu l of NaHCO\_3 (0.1 mmol \ l^{-1}, \text{pH} 9.1). To 20 \mu l of the resulting solution was added 40 \mu l of dabsyl chloride solution and mixed. After 10 min at 70°C, 140 \mu l of 70\% ethanol was added, followed by mixing and centrifuging; 40 \mu l of this solution in ethanol was injected into the chromatographic system.

3. **Quantitation.** A standard curve was constructed for each amino acid to check linearity. For each serum sample a known amount of each amino acid was added before the ethanol extraction. This sample was used for quantitation together with the serum sample without externally added amino acid. This method has been adapted from a report by Turpeinen and Pomoell [23].

4. **Other analysis.** Hemoglobin was measured using Drabkins reagent [24], total protein by biuret. Serum and urine urea were measured enzymatically using reagent kit cat no. 12012 from Trace Scientific, Melbourne, Australia. Plasma free fatty acids were also measured enzymatically using kit no. 990-75401 (Wako Pure Chemical Industries Ltd., Osaka, Japan). Serum glucose concentrations were measured enzymatically using Trace reagent kit cat no. 15012.

5. **Statistical analysis.** All data are expressed as mean \pm SE. Pooled data were tested using one-way ANOVA with repeated measures followed by a post hoc test where appropriate. A p value of less than 0.05 was considered to be significant.

**RESULTS**

Characteristics of the subjects are given in Table 1. The changes in serum concentrations of glucose, free fatty acids (FFA), hematocrit (Hct), hemoglobin (Hb) and free amino acids are given in Table 2. As the exercise was continued, the serum glucose concentration first increased, and then decreased gradually such that the level at the completion of the exercise was the same as pre-exercise value, and it remained significantly elevated from 30 min to 150 min of exercise compared to rest (p < 0.05).

The serum FFA concentration increased progressively from an initial value of 423 \pm 95 to 1,380 \pm 174 \mu mol \ l^{-1} at the completion of the exercise. This is illustrated

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Table 1. Physical characteristics of subjects.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Body mass (kg)</th>
<th>Type of athlete</th>
<th>$\dot{V}_{O_2,max}$ (l kg$^{-1}$ min$^{-1}$)</th>
<th>Maximum heart rate (beat min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>45</td>
<td>173.0</td>
<td>70.50</td>
<td>Distance runner</td>
<td>57.0</td>
<td>125</td>
</tr>
<tr>
<td>M</td>
<td>37</td>
<td>171.0</td>
<td>63.15</td>
<td>Distance runner</td>
<td>62.3</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&amp; triathlete</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>38</td>
<td>173.5</td>
<td>65.01</td>
<td>Distance runner</td>
<td>61.5</td>
<td>145</td>
</tr>
<tr>
<td>M</td>
<td>42</td>
<td>171.0</td>
<td>60.15</td>
<td>Distance runner</td>
<td>60.7</td>
<td>138</td>
</tr>
<tr>
<td>M</td>
<td>35</td>
<td>184.0</td>
<td>69.75</td>
<td>Distance runner</td>
<td>60.1</td>
<td>137</td>
</tr>
<tr>
<td>F</td>
<td>37</td>
<td>163.2</td>
<td>57.50</td>
<td>Distance runner</td>
<td>57.3</td>
<td>148</td>
</tr>
<tr>
<td>F</td>
<td>38</td>
<td>172.9</td>
<td>62.40</td>
<td>Distance runner</td>
<td>51.2</td>
<td>199</td>
</tr>
<tr>
<td>F</td>
<td>34</td>
<td>149.5</td>
<td>43.82</td>
<td>Distance runner</td>
<td>56.4</td>
<td>149</td>
</tr>
</tbody>
</table>

![Graph](image)

Fig. 1. Plot of serum concentrations (μmol L$^{-1}$) of FFA, alanine and proline against exercise duration (min).

in Fig. 1. The increase in serum FFA concentration was highly significant ($p < 0.01$ for post-exercise serum FFA concentration compared to pre-exercise serum FFA concentration). In the control experiment where no exercise was performed, the serum FFA concentration remained constant at 300 μmol L$^{-1}$ for the first 2 h, increasing to 500 μmol L$^{-1}$ after 3 h standing. The same person showed an increase.
Table 2. Changes in concentrations of glucose, FFA, Hct, Hb and different amino acids in serum as a function of exercise duration (mean±SE).*

<table>
<thead>
<tr>
<th>Substance</th>
<th>Time of exercise (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Glucose (mmol l⁻¹)</td>
<td>5.3±0.2</td>
</tr>
<tr>
<td>FFA (μmol l⁻¹)</td>
<td>423±95</td>
</tr>
<tr>
<td>Hct %</td>
<td>43.7±1.4</td>
</tr>
<tr>
<td>Hb (g dl⁻¹)</td>
<td>15.3±0.5</td>
</tr>
<tr>
<td>Urea (mmol l⁻¹)</td>
<td>5.0±0.5</td>
</tr>
<tr>
<td>Alanine (μmol l⁻¹)</td>
<td>363±21</td>
</tr>
<tr>
<td>Arginine (μmol l⁻¹)</td>
<td>76.8±7.0</td>
</tr>
<tr>
<td>Glycine (μmol l⁻¹)</td>
<td>227±15</td>
</tr>
<tr>
<td>Histidine (μmol l⁻¹)</td>
<td>77.7±6.5</td>
</tr>
<tr>
<td>Lysine (μmol l⁻¹)</td>
<td>138±13</td>
</tr>
<tr>
<td>Methionine (μmol l⁻¹)</td>
<td>20.6±1.2</td>
</tr>
<tr>
<td>OH-proline (μmol l⁻¹)</td>
<td>11.7±2.2</td>
</tr>
<tr>
<td>Proline (μmol l⁻¹)</td>
<td>222±21</td>
</tr>
<tr>
<td>Serine (μmol l⁻¹)</td>
<td>100±10</td>
</tr>
<tr>
<td>Threonine (μmol l⁻¹)</td>
<td>126±10</td>
</tr>
<tr>
<td>Tyrosine (μmol l⁻¹)</td>
<td>42.3±4.2</td>
</tr>
<tr>
<td>Valine (μmol l⁻¹)</td>
<td>180±8</td>
</tr>
</tbody>
</table>

*The changes in plasma volume did not show any systematic trend and in any case was less than 5%. Because of poor inter assay variation or poor resolution, changes in concentration of asparagine, glutamine, aspartic acid, leucine and isoleucine are not listed.

*Significantly different from resting value at p < 0.05.
in the serum FFA concentration from 250 \( \mu \text{mol l}^{-1} \) at rest to 1,080 \( \mu \text{mol l}^{-1} \) after 3 h of prolonged exercise.

The serum concentrations of two amino acids alanine and proline decreased progressively with the duration of exercise. For alanine, it decreased from an initial value of 363\( \pm \)21 to 253\( \pm \)19 \( \mu \text{mol l}^{-1} \) and for proline it decreased from 222\( \pm \)21 to 147\( \pm \)15 \( \mu \text{mol l}^{-1} \). These are also illustrated in Fig. 1. Both of these changes were found to be significant \((p<0.01)\) for both alanine and proline when pre-exercise values were compared to post-exercise values.

The changes in the concentration of alanine and proline appeared to be highly correlated \((r=0.981, p<0.01)\). There was also a significant decrease in the serum concentration of lysine at 2, 2.5 and 3 h of exercise compared to rest \((p<0.05)\). The decrease in serum alanine and proline levels together accounted for about 83\% of the total change in serum level of the 12 amino acids studied. The serum urea level increased from pre-exercise value of 5.0 to 5.3 mmol l\(^{-1}\) at the completion of the prolonged running. However, this was not found to be statistically significant.

There was no significant change in the concentration in blood of hematocrit and hemoglobin.

The decrease in the concentration in serum of both alanine and proline was found to be highly correlated with the increase in serum FFA (for alanine versus FFA, \(r=-0.986, p<0.05\); and for proline versus FFA, \(r=-0.961, p<0.05\)). The total 24-h urine volume was significantly less on the day of exercise than on the control day \((978\pm187 \text{ ml} \text{versus} 1,480\pm245 \text{ ml}; p<0.05)\).

DISCUSSION

This study measured plasma urea, glucose, free fatty acids and amino acid concentrations during a prolonged endurance run \((60\% \bar{V}_{O_{max}})\) on a treadmill involving eight highly trained distance runners. On the basis of the present results it appears that in prolonged endurance running involving highly trained subjects, there is a progressive decrease in the plasma concentration of alanine and proline and a progressive increase in the plasma concentration of free fatty acids. Previous studies reported that during intense exercise \((70\% \bar{V}_{O_{max}})\) lasting less than 1 h, there was no significant increase in the concentration of total plasma amino nitrogen \([11,15,17]\) but there was a marked increase in the concentration of plasma alanine \([12,20,25]\). In moderate to heavy exercise of longer duration \( (>2 \text{ h})\), it was almost always found that there was a decrease in the total amino nitrogen content in blood serum \([12,20]\). Ahlborg et al. \([26]\) also found that the arterial proline concentration was significantly lower after 4 h of exercise at 30\% \(V_{O_{max}}\), and that the alanine concentration was significantly lower at the end of exercise than at 40 min of exercise. In that study, data were obtained only at rest, at 40 min of exercise and at the end of exercise, and it is particularly interesting in the present study to observe a continuing decrease in proline and alanine concentrations at 30 min intervals over a 3-h period. Ferrannini et al. \([27]\) observed a

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significant negative correlation between blood FFA/glucose and alanine concentrations at rest, when subjects were infused with lipid. When our data is analyzed in the same way, namely FFA/glucose vs. % change from rest, the correlation is $r = -0.94$ ($p < 0.01$). A decrease in the concentration of a plasma amino acid could be due to a decrease in the rate of release, or an increase in the rate of disappearance of the amino acid. Ferrannini et al. suggest that elevated FFA levels impair insulin-mediated glucose uptake by the muscle, such that the production of alanine from glucose via pyruvate is restricted.

The control experiment (in which the serum FFA concentration did not change in the first 2 h and increased only marginally after 3 h) indicated that the observed increase in the serum FFA concentration in the prolonged exercise could not be due to heparin induced lipoprotein lipase activity. The increase in FFA concentration indicates the increase in FFA mobilization in the prolonged exercise. This is consistent with the fact that in prolonged moderately heavy exercise the lactate concentration does not increase significantly but there is a significant increase in catecholamine concentration. An increase in lactate concentration suppresses FFA mobilization whereas an increase in catecholamine concentration increases FFA mobilization.

The correlation between a decrease in serum proline and alanine concentrations, and an increase in serum FFA concentration suggests that FFA plays a central role in the metabolism of these two amino acids. FFA could mediate this effect on alanine by reducing the availability of glucose derived pyruvate. However, this cannot be the case with proline, and here the mechanism is more likely to be an enhanced uptake by the liver. In fact Ahlborg et al. [26] have demonstrated augmented uptake of alanine, proline, theonine, serine and glycin during prolonged exercise. The decrease in the serum lysine concentration is also interesting. The overall decrease in serum free amino acids measured did not change the 24-h total urinary excretion of urea on the exercise day as compared with that on the control day. There was in fact a significant decrease in the total 24-h urine volume on the day of exercise as compared to that on the control day such that there was a corresponding decrease (although not significant) in the total urinary excretion of urea. Whether nitrogen was lost by other means, e.g. in the form of sweat, or urea production was depressed in the recovery period or this was simply the result of the exercise period (3 h) being only a small part of 24-h period of urine collection is difficult to quantify. Extending the period of urine collection after the exercise day may have shown an increase in urinary urea excretion. No measurement was made on sweat nitrogen loss. It is possible that nitrogen is excreted as fecal ammonia. Although not statistically significant, the serum urea concentration increases with the exercise duration and this increase more than accounts for the total decrease in serum amino acids levels.

Ji et al. [28] found reduced levels of a number of amino acids after exercise in trained rats, including alanine, lysine and proline. They found that the decrease in alanine particularly surprising, because mitochondrial alanine aminotransferase
(ALT) is increased after training. However, they found that although ALT is increased by training in the rested state, the activity is suppressed following an exercise bout, and they postulate that the mechanism of the enzyme inhibition could be linked to generation of free radicals by exercising muscle.

In summary it is observed that in endurance running on a treadmill involving highly trained distance runners, serum alanine and proline concentrations progressively decrease and serum FFA concentration progressively increases.

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REFERENCES


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