The Effect of Protein Supplementation on Lactate Accumulation During Submaximal and Maximal Exercise

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Eleven subjects performed a graded exercise test after 1 week of protein supplementation (PRO) or glucose polymer placebo (CON), randomly assigned in a double blind fashion. The exercise consisted of 3-min graded exercise bouts separated by 10 min of active recovery at zero pedal resistance. Subjects then performed a 30-sec Wingate test (WIN) to assess performance during supramaximal exercise. Blood samples were obtained in the last 15 sec of each exercise and recovery period. PRO resulted in a decrease in blood lactate following 120% VO\textsubscript{2}max and WIN, an increase in blood alanine at all time points, and lower postexercise muscle lactate and glycogen. Resting muscle GPT activity was 47% higher during the PRO trial. Mean power output during the WIN did not differ between PRO and CON. The WIN fatigue index was not significantly different between PRO and CON. The increased alanine may reflect increased transamination of pyruvate, thereby reducing the accumulation of lactate, which in turn had a marginal effect on performance during supramaximal exercise.

In a recent study from our laboratory (in review), subjects fed a protein supplement during an 8-week training program experienced a more rapid training-induced decrease in the blood lactate response to submaximal exercise compared to a matched set of controls. This may indicate either an accelerated adaptation of metabolic factors involved in reduced lactate response (e.g., increased activity in the tricarboxylic acid [TCA] cycle enzymes, increased size and number of mitochondria, increased myoglobin content) or, alternatively, an acute effect of protein supplementation on the blood lactate response to exercise. Because the protein supplement was rich in branched chain amino acids (BCAA) and glutamate, the latter possibility is consistent with studies that have shown increased transamination of pyruvate to alanine in the presence of elevated BCAA availabil-

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ity (16). Increased transamination of pyruvate has been suggested as an alternative to lactate formation and may thus decrease lactate production (15).

The purpose of the present study was to determine whether there is an effect of protein supplementation independent of training adaptations on blood and muscle lactate concentrations after submaximal and supramaximal bicycle exercise in untrained subjects. The effect of the protein supplement on performance of supramaximal exercise was also evaluated.

Methods

Procedures were approved by the university’s Human Subjects in Research review committee prior to initiation of this project. The subjects were fully informed of the purpose and possible risks and benefits of their participation in the study, and all signed an informed consent form.

Nine males and two females volunteered for the study. Each subject was tested for maximum oxygen consumption (VO$_{2\text{max}}$) using a graded exercise test on a Monark cycle ergometer. This test consisted of cycling at a pedal rate of 60 rpm beginning at a power output of 60 watts and increasing by 30 watts every 4 minutes until the subject could no longer maintain the rpm above 50. Inspired volume was obtained using a Pneumoscan S-301 Spirometer. Expired gases were collected and sampled continuously from a mixing chamber. The fractional concentrations of oxygen (O$_2$) and carbon dioxide (CO$_2$) in the expired air were analyzed with an Applied Electrochemistry S-3A oxygen analyzer and a Beckman LB-2 carbon dioxide analyzer, interfaced with an Apple II+ computer. The gas analyzers were calibrated immediately before each test with gases of known concentration.

Experimental Design

Dosage Protocol. Following the VO$_{2\text{max}}$ test, subjects received either a protein supplement (Pro Mod, Ross Laboratories, Columbus, OH) in the amount of 0.8 grams per kilogram of body weight per day or a calorically equivalent daily amount of a glucose polymer (Polycose, Ross Laboratories). These treatments were administered in a double blind, randomized fashion. Supplements were mixed in a flavored shake to disguise the taste. Each dosage was divided into three feedings per day. The subject consumed the treatment in addition to his/her normal diet for 7 days. Subjects were instructed on how to maintain a diet record, and recorded all foods and beverages consumed during each 7-day trial. Diets were analyzed using commercially available computer software (Foodcomp ISU, Ames, IA). Each subject served as his/her own control.

Exercise Test Session. The same experimental test protocol was used following each of the 7-day treatment periods. The subject reported to the laboratory and performed an experimental ride consisting of riding a Monark cycle ergometer for 3 min at work rates of 40, 60, 80, and 100% VO$_{2\text{max}}$, followed by 2 min at 120% VO$_{2\text{max}}$. Each 3-min ride was separated by an active recovery period consisting of cycling for 10 min at 50 rpm with no pedal resistance. Following the final 10-min recovery period, after the ride at 120% VO$_{2\text{max}}$, the subject performed a 30-sec Wingate test (WIN). The resistance for the WIN was set at 0.09 kp-kg$^{-1}$ body weight for males and 0.075 kp-kg$^{-1}$ for females (1, 10). A
Monark cycle ergometer was interfaced with an Apple II+ microcomputer to determine power output during the WIN. This system allows for the determination of power output every 1 second. Mean power output, which was the average power output generated during the 30-sec period, and percent fatigue, which was the difference between peak power and the lowest 1-sec power output, were calculated by the computer based on the recordings of the pedal revolution rate.

**Tissue Sampling.** Blood samples were obtained from a Teflon catheter inserted into a superficial forearm vein. Samples were taken at rest, during the last 15 sec of each exercise period, and during the last 15 sec of each recovery period. Blood was placed into a test tube and 1 ml was deproteinized immediately (within 20 sec) in 2 ml of 8% perchloric acid for later analysis of blood lactate and alanine concentrations.

Muscle biopsies (2) were obtained from the m. vastus lateralis of 10 subjects before exercise and immediately after the WIN test. Each muscle sample was quickly frozen in liquid nitrogen following removal from the muscle. The muscle samples were dissected free of visible blood and connective tissue at −15°C. Each muscle sample was cut into 5- to 20-mg pieces, which were weighed to the nearest 0.1 mg, placed in plastic containers, and stored at −80°C. The muscle samples were later analyzed for glutamate-pyruvate transaminase (GPT) activity, citrate synthase (CS) activity, lactate concentration, and glycogen concentration. Muscle CS and GPT activity as well as blood lactate concentration were analyzed spectrophotometrically (11, 15, 18). Muscle lactate concentration, glycogen concentration, and blood alanine concentration were analyzed fluorometrically (11, 17).

**Statistics.** All results were analyzed using an analysis of variance for repeated-measures design. When a significant F ratio (p<0.05) was obtained, a Newman-Kuels multiple range test was used to locate significant mean differences. When p<0.05, mean differences were considered to be significant. Data are reported as means ±SE.

**Results**

There were no significant differences between the two trials in kilocalories, grams of protein, grams of fat, or grams of carbohydrate consumed per day (Table 1). During the PRO trial the subjects did consume a significantly greater amount of protein when the protein supplement was calculated into the diet.

There was no significant difference between PRO and CON trials in the amount of work performed during the graded exercise test. The power outputs, corresponding VO₂, and respiratory exchange ratio (RER) are presented in Table 2. Mean power output during the WIN (Table 3) did not differ significantly between treatments. However, differences in percent fatigue (Table 3) did approach significance (p<0.08).

Blood lactate concentrations were significantly lower in the PRO trial compared with the CON trial at intensities greater than 100% VO₂max (Figure 1). There were no significant differences in blood lactate concentrations at intensities below 100% VO₂max. The blood lactate concentrations during the rest interval following the ride at 100% VO₂max (CON, 5.70 ±0.52 mM; PRO, 4.48 ±0.55 mM) differed significantly. Significant differences in blood lactate concentrations were observed at 120% VO₂max (CON, 7.05 ±0.73 mM; PRO, 5.86 ±0.83 mM), the recovery period following the ride (CON, 9.40 ±0.98 mM; PRO,
### Table 1

**Dietary Analysis (mean ± SE) of the Subjects’ Diet During the 7 Days of Each Trial**

<table>
<thead>
<tr>
<th></th>
<th>Total KCAL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Grams PRO&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Grams FAT&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Grams CHO&lt;sup&gt;b&lt;/sup&gt;</th>
<th>g·kg&lt;sup&gt;−1&lt;/sup&gt; PRO&lt;sup&gt;b&lt;/sup&gt;</th>
<th>g·kg&lt;sup&gt;−1&lt;/sup&gt; PRO&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>CON</td>
<td>4368</td>
<td>141</td>
<td>161</td>
<td>470</td>
<td>1.58</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>±552</td>
<td>±14</td>
<td>±25</td>
<td>±78</td>
<td>±0.18</td>
<td>±0.18</td>
</tr>
<tr>
<td>PRO</td>
<td>4171</td>
<td>138</td>
<td>123</td>
<td>509</td>
<td>1.54</td>
<td>2.73&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>±368</td>
<td>±14</td>
<td>±11</td>
<td>±64</td>
<td>±0.21</td>
<td>±0.17</td>
</tr>
</tbody>
</table>

<sup>a</sup>Total kcals obtained from diet plus supplement; <sup>b</sup>Value obtained from diet only; <sup>c</sup>g·kg<sup>−1</sup> of body weight of protein obtained from diet plus supplement.

<sup>*</sup>Significant difference, *p* < 0.05, between PRO and CON.

### Table 2

**Power Outputs, Oxygen Consumption, and Respiratory Exchange Ratio With (PRO) and Without (CON) Protein Supplementation During Incremental Exercise**

<table>
<thead>
<tr>
<th>% VO&lt;sub&gt;2&lt;/sub&gt;max</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>120</th>
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<tr>
<td>Watts</td>
<td>102</td>
<td>164</td>
<td>223</td>
<td>292</td>
<td>360</td>
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<tr>
<td>±9</td>
<td>±12</td>
<td>±14</td>
<td>±16</td>
<td>±18</td>
<td></td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt; (l · min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>1.67</td>
<td>2.48</td>
<td>3.46</td>
<td>4.22</td>
<td>4.71</td>
</tr>
<tr>
<td>CON</td>
<td>±0.07</td>
<td>±0.12</td>
<td>±0.16</td>
<td>±0.20</td>
<td>±0.22</td>
</tr>
<tr>
<td>PRO</td>
<td>1.71</td>
<td>2.59</td>
<td>3.49</td>
<td>4.20</td>
<td>4.65</td>
</tr>
<tr>
<td>±0.04</td>
<td>±0.11</td>
<td>±0.13</td>
<td>±0.20</td>
<td>±0.15</td>
<td></td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>CON</td>
<td>0.84</td>
<td>0.89</td>
<td>0.97</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>±0.02</td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.01</td>
<td>±0.02</td>
</tr>
<tr>
<td></td>
<td>PRO</td>
<td>0.81</td>
<td>0.88</td>
<td>0.97</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
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<td>±0.01</td>
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8.18 ±0.88 mM), the period immediately following the WIN (CON, 12.19 ±0.87 mM; PRO, 10.64 ±1.11 mM), and the one following the WIN recovery period (CON, 13.49 ±0.87 mM; PRO, 12.10 ±0.87 mM). Blood alanine concentrations during the incremental exercise were significantly higher in PRO as compared with CON trials at all intensities and throughout recovery periods (Figure 2).

Muscle lactate concentrations did not differ significantly between pre-CON and pre-PRO (Figure 3). However, following the Wingate test they were
Table 3
Mean Power Output and % Fatigue During 30-sec Wingate Test
With (PRO) and Without (CON) Protein Supplementation

<table>
<thead>
<tr>
<th>Power output (watts)</th>
<th>% Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>556.66</td>
</tr>
<tr>
<td>PRO</td>
<td>583.82</td>
</tr>
<tr>
<td></td>
<td>±47.14</td>
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<td></td>
<td>±46.25</td>
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</table>

*p < 0.08 between PRO and CON.

Figure 1 — Blood lactate concentrations during incremental exercise with (PRO) and without (CON) protein supplementation (*p<0.05). Values are mean ±SE, n=11. W = Wingate Anaerobic Test. R = 10 min active recovery between bouts.

significantly lower in post-PRO (21.14 ±1.6 μmoles·gram⁻¹) as compared with post-CON (26.04 ±1.7 μmoles·gram⁻¹) (Figure 3). Muscle glycogen concentrations did not differ significantly between pre-CON and pre-PRO (Figure 4), but they were significantly lower following the Wingate test in post-PRO (58.29 ±7.5 μmoles·gram⁻¹) as compared with post-CON (69.65 ±5.6 μmoles·gram⁻¹). There was no significant difference in the preexercise activity of citrate synthase (CS) between the CON and PRO trials (Table 4). However, preexercise glutamate-pyruvate transaminase (GPT) activity was significantly higher in the PRO trial (10.87 ±1.5 μmoles·gram⁻¹·min⁻¹) as compared with the CON trial (7.40 ±0.84 μmoles·gram⁻¹·min⁻¹) (Table 4).

Discussion
The results of the present study demonstrate that protein supplementation was associated with an attenuated accumulation of lactate in forearm venous blood during exercise greater than 100% VO₂max, and in muscle immediately following
Figure 2 — Blood alanine concentrations during incremental exercise with (PRO) and without (CON) protein supplementation (*p<0.05). Values are mean ±SE, n=11. W = Wingate Anaerobic Test. R = 10 min active recovery between bouts.

Figure 3 — Muscle lactate concentrations with (PRO) and without (CON) protein supplementation before and after incremental exercise (*p<0.05). Values are mean ±SE, n=10.
Figure 4 — Muscle glycogen concentrations with (PRO) and without (CON) protein supplementation before and after incremental exercise (*p<0.05). Values are mean ±SE, n=10.

Table 4

<table>
<thead>
<tr>
<th>Activity</th>
<th>CON</th>
<th>PRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate-pyruvate transaminase</td>
<td>7.40 ± 0.84</td>
<td>10.87* ± 1.5</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>16.02 ± 1.37</td>
<td>15.36 ± 1.03</td>
</tr>
</tbody>
</table>

*Significant difference, p < 0.05, between PRO and CON.

high intensity exercise. This finding is in agreement with that of Maresh et al. (13), who reported a decrease in forearm blood lactate following a Wingate test when the subjects consumed an amino acid supplement. These researchers attributed the lower blood lactate response to (a) increased transamination of amino acids with pyruvate, (b) increased oxidation of amino acids, (c) reduced efflux of lactate from muscle to blood, or (d) reduced intracellular ammonia accumulation.

If transamination were increased when subjects ingested supplemental amino acids, muscle lactate production and its subsequent accumulation in both muscle and blood may be decreased (15). Unfortunately, neither lactate production nor transamination rates were measured in either the Maresh et al. study or the
present study. However, the reduced muscle lactate accumulation and increased activity of muscle GPT observed in the present study support the proposed increase in transamination. In addition to reduced lactate accumulation, increased transamination should result in greater formation of alanine equimolar to the decrease in lactate production.

In the present study, blood alanine concentration was elevated throughout the experimental period in the PRO trial while blood lactate concentration was decreased only during the more intense work rates and their recovery periods. This lack of equimolar change in alanine and lactate does not necessarily exclude increased transamination as the explanation for lower blood lactate response in PRO, as there may be different metabolic rates and anatomic sites of disposal for lactate and alanine. Further research should examine the effect of protein supplementation on specific production rates of lactate and alanine to determine whether the lower blood lactate concentration is reflective of increased flux through the GPT reaction.

Muscle GPT Activity

The increase in muscle GPT activity observed in the present study is probably due to an increase in the amount of enzyme brought about by the increased availability of amino acids used as a substrate for GPT and as a substrate for GPT production (15, 20). The activity of aminotransferases are reported to increase sixfold (6) and threefold (8) in the liver, and by 90% in muscle tissue (14) of rats adapted to a high-protein diet. The finding in the present study that GPT activity increased during the high-protein diet is consistent with the fact that the majority of BCAA catabolism and alanine formation occurs in skeletal muscle (4, 7, 8, 16). The increase in GPT activity would allow GPT to compete with lactate dehydrogenase for pyruvate, thereby increasing the production of alanine and perhaps decreasing the accumulation of lactate in blood and muscle (15).

Lactate Clearance

An alternative explanation for the reduced lactate accumulation after protein supplementation is that an increase in lactate clearance could have contributed to it. Alanine and other amino acids have been shown to increase the release of glucagon (3), which activates gluconeogenic enzymes (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, and glucose 6-phosphatase) and inactivates pyruvate kinase (6, 9). Therefore there may have been an increase in hepatic gluconeogenesis, which theoretically could reduce the accumulation of blood lactate independent of any effect of the protein supplement on lactate production. Although glucagon was not measured in the present study, enhanced hepatic gluconeogenesis by the protein supplement cannot be ruled out as a possible explanation for the reduced blood lactate accumulation.

Muscle Glycogen

Because glucagon promotes glycogenolysis, the greater glycogen used in the PRO trial may be related to the possible increase in glucagon caused by the supplement. Whether this extra glycogen lost was oxidized, used to provide pyruvate for
de novo alanine synthesis (4, 16), or met with another fate cannot be answered from the present data. The finding that RER was unaffected by the protein supplement, however, may indicate that carbohydrate oxidation was maintained despite the lower glycogen levels and supports the possibility that glycogen was used in greater amounts to provide pyruvate for alanine synthesis. On the other hand, it is possible that RER is not a sensitive enough indicator to detect differences in glycogen oxidation in the amount that may have occurred in this study. If the PRO supplement increased glycogen oxidation, this could have a detrimental effect on prolonged endurance performance. Therefore it is important that further research be done to (a) identify the mechanism of the protein-induced accelerated glycogen use, (b) identify the fate of the increased glycogen used, and (c) evaluate the possible effects this may have on endurance performance.

**Performance Test**

Although the concentrations of blood and muscle lactate during the PRO trial were lower, there were only slight differences in performance measures during the WIN test. There was no difference in mean power output (Table 3) between the two trials. These results are in agreement with those reported by Maresh et al. (13). However, the percent fatigue during the PRO trial was slightly lower and approached a significant difference at p<0.08. We realize that this value exceeds the level of significance previously set, but thought it merited some discussion. This small difference in percent fatigue may indicate that the individuals were able to maintain a relatively higher power output over the 30-sec period.

The decrease in muscle and blood lactate concentrations may account for the decrease in percent fatigue during the PRO trial because cellular acid-base disturbances has been closely linked with the fatigue process (12, 19). However, because muscle pH was not measured in this study, we cannot conclude that the acid-base disturbance was lessened in the PRO trial. In addition, lactic acid accumulation is not the sole determinant of muscular fatigue and exhaustion (19), which may occur due to (a) depletion of creatine phosphate stores, (b) local increases in inorganic phosphate, and/or (c) the inability to generate adenosine triphosphate at sufficient rates (5, 12, 19).

**Summary**

In summary, ingestion of a protein supplement for 7 days reduced the concentration of lactate in muscle and blood after submaximal and supramaximal exercise. In addition, blood alanine concentrations were elevated during all exercise intensities studied. Resting muscle GPT activity was increased 47% after protein supplementation, and glycogen use during exercise after supplementation was increased. Despite these apparent adaptations to the protein supplementation, performance of the Wingate test was unaffected except for a trend toward lower percent fatigue after protein supplementation. We conclude that protein supplementation affects exercise metabolism in a way that may be consistent with elevated transamination and, consequently, reduced production of lactate. Additional research is recommended to test the validity of this hypothesis, to identify the mechanisms for reduced lactate accumulation, and to evaluate the possible effect of these adaptations on the performance of both short-term intense exercise and endurance exercise.
References


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