Stimulatory effect of insulin on creatine accumulation in human skeletal muscle

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Steenge, G. R., J. Lambourne, A. Casey, I. A. Macdonald, and P. L. Greenhaff. Stimulatory effect of insulin on creatine accumulation in human skeletal muscle. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E974–E979, 1998.—This study investigated the effect of insulin on plasma and muscle creatine accumulation and limb blood flow in humans after creatine administration. Seven men underwent a 300-min euglycemic insulin clamp combined with creatine administration on four separate occasions. Insulin was infused at rates of 5, 30, 55, or 105 mU·m⁻²·min⁻¹, and on each occasion 12.4 g creatine was administered. During infusion of insulin at rates of 55 and 105 mU·m⁻²·min⁻¹, muscle total creatine concentration increased by 4.5 ± 1.4 (P < 0.05) and 8.3 ± 1.0 mmol/kg dry mass (P < 0.05), and plasma creatine concentrations were lower at specific time points compared with the 5 mU·m⁻²·min⁻¹ infusion rate. The magnitude of increase in calf blood flow (plethysmography) was the same irrespective of the rate of insulin infusion, and forearm blood flow increased to the same extent as the three highest infusion rates. These findings demonstrate that insulin can enhance muscle creatine accumulation in humans but only when present at physiologically high or supraphysiological concentrations. This response is likely to be the result of an insulin-mediated increase in muscle creatine transport rather than creatine delivery.

phosphocreatine; muscle fatigue; exercise

CREATINE, in its free and phosphorylated forms, plays an important role in the regulation of muscle energy metabolism and fatigue development (22, 35). Increasing dietary creatine intake from 1–2 g/day (in a normal healthy, meat-eating individual) to 20 g/day for a period of 4–6 days has been shown to increase muscle total creatine (TCr) concentration by ~20%, with large interindividual variation (5–30%; 15, 18, 20). It has also been reported that this regimen of creatine supplementation can sustain muscle ATP resynthesis and augment performance during repeated bouts of maximal exercise (1, 5, 6, 10, 16). However, it appears that the ergogenic and metabolic effects of creatine ingestion are dependent on the magnitude of the increase in muscle TCr during supplementation. Specifically, it has been suggested that an increase in muscle TCr content in excess of 20 mmol/kg dry muscle (dm) is required to exert an ergogenic effect on muscle power output (6) and postexercise phosphocreatine (PCr) resynthesis (15). Indeed, this may be one reason for the reported lack of effect of creatine supplementation on exercise performance (30, 32).

Recently, Green et al. (13) reported that ingestion of creatine in combination with a carbohydrate-containing solution increased muscle TCr by ~25%. This was 60% greater than the increase observed when creatine alone was ingested. Moreover, the authors demonstrated that the ingestion of creatine in conjunction with carbohydrate increased muscle TCr in all subjects by >20 mmol/kg dm. However, only half of the subjects who ingested creatine alone had an increase of this magnitude. In accordance with published animal experiments (19, 23, 24), the authors proposed that the increase in muscle creatine accumulation originated from carbohydrate-mediated insulin release, which would stimulate sodium-dependent muscle creatine transport. In the study of Green et al., subjects ingested 94 g of carbohydrate (in the form of glucose and simple sugars) with each 5-g dose of creatine to achieve physiologically high plasma insulin concentrations during the 1st h after ingestion. However, the quantity of ingested carbohydrate proved to be close to the limit of palatability. The concentration of insulin necessary to stimulate muscle creatine accumulation in humans is presently unknown. On the basis of previously published work involving a mouse muscle cell line (31), isolated rat skeletal muscle (19), and human volunteers (13), it is hypothesized that a concentration close to the upper physiological limit would be required to promote this response.

Insulin has also been reported to stimulate muscle blood flow (2), a response that cannot be observed in vitro. Therefore, it could be concluded that the increase in muscle creatine accumulation observed after creatine and carbohydrate consumption in humans may have at least partly resulted from an insulin-mediated increase in muscle blood flow and thereby muscle creatine availability. In this context, submaximal exercise performed before creatine ingestion has been shown to augment muscle creatine accumulation (18).

The aims of the present study were, first, to determine whether insulin per se could stimulate in vivo creatine accumulation in human skeletal muscle. The second aim was to determine at what plasma insulin concentration a response, if any, was evident, and the final aim was to determine whether an insulin-mediated increase in muscle blood flow was associated with any observed increase in muscle creatine accumulation.

MATERIALS AND METHODS

Subjects. Seven moderately active, healthy, nonvegetarian men (age 25.9 ± 3.0 yr, weight 72.6 ± 3.4 kg, height 1.81 ±...
5-min intervals for monitoring blood glucose concentration. Visit, 25 ml of arterialized venous (a-v) blood were obtained at each administration and to maintain a steady-state plasma concentration. These procedures had been found to increase plasma creatine concentration to "physiologically high" and supraphysiological serum insulin concentrations. As illustrated in Fig. 1, insulin infusion started at t = 0 min. After an equilibration period of 60 min, subjects ingested 5 g of creatine monohydrate (Experimental & Applied Sciences, Denver, CO) dissolved in 250 ml warm water. A nasogastric tube was then used for enteral infusion of an isotonic creatine solution (100 mmol/l, 2.5 ml/min) from t = 75 min until the end of the experiment. In a pilot study, these procedures had been found to increase plasma creatine concentration to ~1 mmol/l within 60 min of creatine administration and to maintain a steady-state plasma concentration at this level thereafter. The total quantity of creatine administered during each treatment was 12.4 g.

Blood sampling and analysis. During each experimental visit, 25 ml of arterialized venous (a-v) blood were obtained at 5-min intervals for monitoring blood glucose concentration (YSI 2300 STATplus, YSI, Yellow Springs, OH). At 15-min intervals, 5 ml of a-v blood were collected into lithium heparin containers and, after centrifugation (3,000 rpm for 10 min), the plasma was removed and stored frozen at −20°C. Creatine and creatinine concentrations were determined on these samples at a later date according to the method of Dunnott et al. (9) with high-performance liquid chromatography (System Gold 507, Beckman, Bucks, UK). At 30-min intervals, a further sample of 5 ml of a-v blood was collected and allowed to clot, and, after centrifugation, the serum was stored frozen at −20°C. Insulin was measured on these samples at a later date with a radioimmunoassay (Diagnostic Products, Los Angeles, CA).

Blood flow measurements. Forearm and calf blood flow were measured simultaneously at 20-min intervals by venous occlusion plethysmography with a mercury-in-rubber strain gauge (36). Changes in gauge resistance were recorded with commercial computer hardware and software (MacLab, Mega System, New Zealand and Macintosh LC, Cupertino, California). The average slope of five occlusions was used to calculate forearm and calf blood flow as milliliters per 100 ml tissue per minute.

Muscle sampling and analysis. Muscle biopsy samples were obtained from the vastus lateralis muscle immediately before and at the end of each insulin clamp with the percutaneous needle biopsy technique (4). To enable true resting muscle PCr and free creatine concentrations to be achieved, biopsy samples were frozen in liquid nitrogen 1 min after removal from the limb (33). Samples were then freeze-dried and stored at −80°C for subsequent metabolite analysis. After removal of visible blood and connective tissue, muscle samples were powdered, and ATP, PCr, and free creatine concentrations were determined spectrophotometrically with the method of Harris et al. (17). To reduce the variance in nonmuscle constituents, PCr and creatine concentrations were adjusted with the highest ATP concentration from each pair of samples (18). TCr was calculated by adding PCr and free creatine concentrations. In addition, 1.5–3.5 mg of muscle powder from each biopsy was solubilized in NaOH (0.1 mol/l) by heating at 80°C for 20 min, and the neutralized extract was then used for the spectrophotometric determination of muscle glycogen concentration (17).

Statistical analysis. A two-way ANOVA (time and treatment effects, Minitab, Clecom, Birmingham, UK) was performed to detect differences in plasma creatine, serum insulin, and blood flow responses between treatments. When comparisons of muscle metabolites across treatments were made, metabolite concentrations were initially analyzed with a balanced analysis for repeated measures (ANOVA, Minitab, Clecom). If significance was achieved, a Student's paired t-test was used to locate differences between treatments. Relationships between variables were examined by computing the Pearson product-moment correlation coefficient (r). The total area under the plasma creatine-time curve was calculated with the least squares method. Statistical significance was declared at P < 0.05, and all values are means ± SE.

RESULTS

Serum insulin. Infusion of insulin at rates of 5, 30, 55, and 105 mU·m^{-2}·min^{-1} resulted in steady-state serum insulin concentrations of 12 ± 0.2, 56 ± 2, 109 ± 5, and 199 ± 5 mU/l, respectively, within 60 min of the onset of infusion (Fig. 2). The total volumes of 20% glucose solution infused to maintain these circulating insulin concentrations were 75 ± 60, 800 ± 60, 1,075 ± 60, and 1,180 ± 80 ml, respectively.

![Fig. 1. Study protocol.](http://ajpendo.physiology.org.)
Plasma creatine. Basal plasma creatine concentrations were similar between treatments (75 ± 8 µmol/l). As illustrated in Fig. 3, plasma creatine concentration increased markedly during the first hour after creatine administration on each treatment (P < 0.001) and remained elevated at a steady state thereafter. However, plasma creatine concentrations were significantly lower at specific time points when insulin was infused at rates of 55 (P < 0.05) and 105 mU·m⁻²·min⁻¹ (P < 0.05), when compared with the infusion rate of 5 mU·m⁻²·min⁻¹. Furthermore, the total area under the plasma creatine-time curve (196 ± 20, 173 ± 12, 160 ± 16, and 156 ± 13 mmol·l⁻¹·min at the insulin infusion rates of 5, 30, 55, and 105 mU·m⁻²·min⁻¹, respectively) was negatively correlated with the infused dose of insulin (r = -0.885, P < 0.01).

Blood flow. The forearm and calf blood flow values immediately before and at the end of each insulin clamp are presented in Table 1. Before insulin infusion, neither forearm nor calf blood flow differed between experimental treatments. Infusion of insulin at rates of 30, 55, and 105 mU·m⁻²·min⁻¹ resulted in similar increases in forearm blood flow of ~65% within 100 min, but no response was observed at the 5 mU·m⁻²·min⁻¹ infusion rate. This insulin-mediated increase in forearm blood flow was maintained for the remainder of each infusion period, such that forearm blood flow was higher at the end of the 30, 55, and 105 mU·m⁻²·min⁻¹ treatments when compared with the 5 mU·m⁻²·min⁻¹ infusion rate (Table 1). The calf blood flow response was similar for all treatments; it gradually increased over the initial 120 min to ~40% above baseline (P < 0.001), which was then sustained until the end of the experiment (Table 1).

Muscle metabolites. Muscle ATP, PCr, and glycogen concentrations before and after insulin and creatine administration are presented in Table 2. Before administration, muscle metabolite concentrations were not different between treatments. After administration, not significant changes were observed in muscle PCr and ATP concentrations within and across treatments. Infusion of insulin at rates of 5, 30, and 55 mU·m⁻²·min⁻¹ had no effect on muscle free creatine concentration. However, free creatine increased by 6.9 ± 1.9 mmol/kg dm (P < 0.05) during infusion of insulin at a rate of 105 mU·m⁻²·min⁻¹, but this increase was not significantly different compared with the infusion rate of 5 mU·m⁻²·min⁻¹. No change in muscle TCr concentration was observed when insulin was infused at rates of 5 and 30 mU·m⁻²·min⁻¹. As Fig. 4 illustrates, significant increases were observed at the 55 and 105 mU·m⁻²·min⁻¹ infusion rates, and the magnitude of increase was greatest at the highest infusion rate (P < 0.05). There was a strong correlation between the dose of insulin infused and the change observed in muscle TCr concentration (r = 0.977, P < 0.001).

Similar to TCr, muscle glycogen concentration increased significantly when insulin was infused at rates of 55 and 105 mU·m⁻²·min⁻¹ (Table 2). However, the magnitude of the increase was not different between

Table 1. Mean forearm and calf blood flow immediately before and after 300 min of insulin infusion combined with oral and nasogastric administration of creatine

<table>
<thead>
<tr>
<th>Infusion Rate, mU·m⁻²·min⁻¹</th>
<th>Forearm</th>
<th>Calf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>300 min</td>
</tr>
<tr>
<td>5</td>
<td>1.6 ± 0.1</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>1.8 ± 0.3</td>
<td>2.7 ± 0.4*</td>
</tr>
<tr>
<td>55</td>
<td>1.3 ± 0.2</td>
<td>2.5 ± 0.4*</td>
</tr>
<tr>
<td>105</td>
<td>1.7 ± 0.2</td>
<td>2.6 ± 0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in ml·100 ml⁻¹·min⁻¹. *P < 0.001 significantly different from 0 min.
Table 2. Muscle metabolite concentrations of vastus lateralis before and after 300 min of insulin infusion combined with oral and nasogastric administration of creatine

<table>
<thead>
<tr>
<th>Insulin Infusion, mU·m⁻²·min⁻¹</th>
<th>Precreatine</th>
<th>Postcreatine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ATP</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>23.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>24.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>23.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>24.0 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry muscle. P, creatine; Pcr, phosphocreatine; Tcr, total creatine. *P < 0.05, significantly different from Precreatine treatment. †P < 0.05, ‡P < 0.01, §P < 0.001, significantly different from 105 mU·m⁻²·min⁻¹ insulin infusion rate.

Discussion

We have recently demonstrated that creatine accumulation can be substantially augmented in human skeletal muscle when creatine is ingested in conjunction with large quantities of simple carbohydrates (13). It was speculated that insulin, released as a consequence of carbohydrate ingestion, was responsible for this enhancement of muscle creatine transport. This hypothesis was supported by findings from animal studies showing that insulin could increase whole body creatine retention in vivo (24) and could stimulate cellular creatine uptake in vitro (19). Over 90% of creatine enters skeletal muscle by binding to a specific transporter protein (35). This process is saturable, is sodium dependent, and allows creatine to enter the muscle against a concentration gradient (25, 31, 34). It has been proposed that insulin facilitates sodium-dependent creatine transport by increasing muscle sodium-potassium pump activity (7, 21, 28, 31). In support of this hypothesis, Odom et al. (31) demonstrated that insulin, insulin-like growth factor I, triiodothyronine, and amylin, all of which are known to stimulate sodium-potassium activity, increased creatine accumulation in a muscle cell line. Furthermore, ouabain, an inhibitor of sodium-potassium ATPase activity, was shown to reduce cellular creatine uptake in vitro (11, 31). All of these studies, however, employed animal or muscle cell line models, and all used supraphysiological concentrations of insulin to augment cellular creatine transport. As a result of this, there is currently little, if any, published information concerning the effects of insulin on muscle creatine accumulation in humans. The principal aim of the present study therefore was to examine the relationship between insulin availability and muscle creatine accumulation in humans. We were able to demonstrate that insulin augmented muscle creatine accumulation but only when present at high (~100 mU/l) or supraphysiological (~200 mU/l) concentrations. In line with this finding, we were able to show that the plasma creatine concentration was lower at several time points after administration when insulin was clamped at 100 and 200 mU/l (55 and 105 mU·m⁻²·min⁻¹), indicating that muscle creatine transport was increased under these conditions.

Previous studies have demonstrated that it takes 4–6 days of creatine supplementation at a rate of 20 g/day to increase muscle TCr concentration by ~20 mmol/kg dm (15, 18, 20). It is therefore not surprising that the changes in muscle TCr concentrations in the present study, where a total dose of 12.4 g was administered, were considerably lower than those previously reported. However, extrapolating from the present data what the increase in muscle TCr might have been if 20 g of creatine had been administered for a period of 5 days, we can estimate that at the 5 mU·m⁻²·min⁻¹ insulin infusion rate (control) an increase of ~20 mmol/kg dm would have occurred. This fits well with previous studies involving 5 days of creatine supplementation at a rate of 20 g/day (13, 15). Similarly, the estimated 33 mmol/kg dm increase in muscle TCr content at the 55 mU·m⁻²·min⁻¹ insulin infusion rate is in good agreement with our previous work showing an increase of 33.4 ± 3.4 mmol/kg dm after the ingestion of 5 g creatine with 94 g carbohydrate four times daily for 5 days (13). This also confirms that the effect of carbohydrate on muscle creatine transport that we previously observed was insulin mediated. Indeed, the area under the plasma creatine-time curve was nega-
The same is true of more conventional regimens of muscle creatine loading. It would seem, therefore, that the increase in muscle creatine accumulation after submaximal exercise observed by Harris et al. may have been attributable to an exercise-induced increase in muscle insulin sensitivity. In support of this suggestion, Green et al. (14) demonstrated that the ingestion of 94 g of simple carbohydrates in combination with 5 g of creatine appeared to eliminate the stimulatory effect of exercise on muscle creatine transport. This is presumably a result of the marked increase in serum insulin concentration overshadowing any effect of exercise on insulin sensitivity.

In conclusion, the present study demonstrates that insulin can enhance muscle creatine accumulation in human skeletal muscle but only when present at high or supraphysiological concentrations. Furthermore, it would appear that this effect is achieved by an insulin-mediated augmentation of sodium-dependent creatine transport, rather than an insulin-mediated increase in muscle creatine delivery. These findings will be of interest to individuals wishing to maximize muscle creatine accumulation in an attempt to improve exercise performance, particularly because the magnitude of the increase in exercise performance and postexercise PCr resynthesis appears to be related to the extent of muscle creatine retention during supplementation (6, 15). The present findings also suggest that ingesting creatine with anything other than large quantities (~100 g) of simple carbohydrates will be no more effective at increasing muscle creatine retention than ingesting creatine alone.

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