J Appl Physiol
89: 1165–1171, 2000.

Protein- and carbohydrate-induced augmentation of whole body creatine retention in humans

G. R. STEENGE, E. J. SIMPSON, AND P. L. GREENHAFF
School of Biomedical Sciences, University Medical School, Queen's Medical Centre, Nottingham NG7 2UH, United Kingdom

Received 22 October 1999; accepted in final form 21 April 2000

Steenge, G. R., E. J. Simpson, and P. L. Greenhaff. Protein- and carbohydrate-induced augmentation of whole body creatine retention in humans. *J Appl Physiol* 89: 1165–1171, 2000.—This study investigated the effect of creatine supplementation in conjunction with protein and/or carbohydrate (CHO) ingestion on plasma creatine and serum insulin concentrations and whole body creatine retention. Twelve men consumed 4 × 5 g of creatine on four occasions in combination with *1*) 5 g of CHO, *2*) 50 g of protein and 47 g of CHO, *3*) 96 g of CHO, or *4*) 50 g of CHO. The increase in serum insulin was no different when the protein-CHO and high-CHO treatments were compared, but both were greater than the response recorded for the low-CHO treatment (both $P < 0.05$). As a consequence, body creatine retention was augmented by ~25% for protein-CHO and high-CHO treatments compared with placebo treatment. The areas under creatine- and insulin-time curves were related during the first oral challenge ($r = -0.920$, $P < 0.05$) but not after the fourth ($r = -0.342$). It is concluded, first, that the ingestion of creatine in conjunction with ~50 g of protein and CHO is as effective at potentiating insulin release and creatine retention as ingesting creatine in combination with almost 100 g of CHO. Second, the stimulatory effect of insulin on creatine disposal was diminished within the initial 24 h of supplementation.

insulin; muscle metabolism; diet; exercise

MOST OF THE BODY CREATINE pool is restricted to skeletal muscle, where it plays a pivotal role in maintaining energy homeostasis (for extensive reviews, see Refs. 28, 29). The muscle total creatine store (phosphocreatine and free creatine) in healthy, nonvegetarian subjects is, on average, 124 mmol/kg dry mass (dm), but it can vary widely among individuals (100–150 mmol/kg dm; Refs. 10, 11). Dietary creatine supplementation at a rate of 20 g/day for 5 days has been shown to increase muscle total creatine content by 20% on average (11). A similar, but more gradual, increase can be obtained when creatine is ingested at a rate of 2 g/day for 28 days (13). It has been widely reported that elevating the muscle total creatine store can enhance performance during high-intensity exercise (1, 2, 8, 21, 26, 27). As a result of these publications, creatine supplementation has become popular among athletes wishing to improve athletic performance. It is also possible that creatine supplementation may be of therapeutic benefit for patients with muscular and neurological disorders (14, 20, 22, 25).

It has become apparent that the metabolic and physiological effects of creatine supplementation are positively related to the extent of muscle creatine accumulation during supplementation. Specifically, we have suggested that, to exert an optimal effect on performance and metabolism, it would be desirable to increase the muscle total creatine content by at least 20 mmol/kg dm (2, 7). As already stated, creatine supplementation at a rate of 20 g/day for 5 days can increase the muscle total creatine content by 20% on average (20 mmol/kg dm). However, it is important to note that the variation among individuals is large (0–40 mmol/kg dm; Refs. 5, 8, 11). This variation in creatine accumulation during supplementation can be partly accounted for by differences in presupplementation muscle creatine concentrations and possibly in muscle fiber-type distribution, but it remains unclear why muscle creatine accumulation can be different by up to sixfold among individuals with similar presupplementation creatine concentrations (2).

Our laboratory has previously reported that creatine ingested in combination with simple carbohydrates (CHO) substantially increased muscle creatine accumulation compared with the ingestion of creatine alone (5). Furthermore, ingestion of creatine in conjunction with CHO reduced the interindividual variability in the magnitude of muscle creatine accumulation, such that all subjects demonstrated an increase in muscle total creatine content ≥20 mmol/kg dm. In agreement with animal-based research, it was proposed that the stimulatory effect of CHO on muscle creatine accumulation was due to insulin-enhancing muscle creatine uptake, probably by stimulating sodium-potassium pump activity (12, 19). Recently, our laboratory has confirmed that insulin can increase creatine accumulation in human skeletal muscle but only when present at a concentration close to, or in excess of, 100 mU/l (24). On the basis of these findings, it is clear that creatine supplements would need to be ingested with

Address for reprint requests and other correspondence: P. L. Greenhaff, School of Biomedical Sciences, Univ. Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

very large quantities of simple CHO to achieve an insulin-mediated stimulation of muscle creatine transport. Indeed, in the study by Green et al. (5), the ingestion of ~100 g of simple CHO with each 5-g dose of creatine proved to be close to the limit of palatability over the 5 days of supplementation.

Glucose is the principal regulator of pancreatic insulin release, but several proteins are also known to stimulate insulin secretion (23). Moreover, it has been reported that the ingestion of proteins in combination with CHO can result in a greater increase in serum insulin concentrations than would be expected from the sum of their individual responses (31). The aim of the present study, therefore, was to examine whether the ingestion of creatine in combination with a solution containing ~50 g of protein and ~50 g of simple CHO could increase serum insulin concentration to a level similar to that observed after the ingestion of ~100 g of simple CHO. Our second aim was to determine whether this would facilitate creatine retention toward that reported with larger quantities of simple CHO. In practical terms, this would make the insulin-mediated augmentation of muscle creatine accumulation a more feasible option than the present procedure of ingesting creatine with very large quantities of CHO.

METHODS

Subjects. Twelve healthy, nonvegetarian men (age 26.9 ± 2.2 yr, body mass 79.7 ± 2.9 kg, height 1.80 ± 0.02 m, and body mass index 24.7 ± 0.8 kg/m^2) volunteered for the present study, which was approved by the University of Nottingham Medical School Ethics Committee. Before participation, subjects underwent routine medical screening, completed a general health questionnaire, and verified that they had not consumed creatine supplements in the past 3 mo. Each subject gave his informed consent to take part in the study and was aware that withdrawal from the study was possible at any time.

Study protocol. All volunteers visited the laboratory on an afternoon and on the following morning on four different occasions. Each occasion was separated by at least 2 wk to ensure similar basal plasma and muscle creatine concentrations among experimental treatments. For the first experimental visit, subjects arrived in the laboratory at noon, after having fasted for 4 h and having voided their bladder immediately before arrival. Subjects then rested on a bed in a supine position for ~4 h. During this time, one hand was placed in a hand-warming unit (~55°C) to arterialize the venous drainage of the hand (4). After 15 min, a vein on the dorsal surface of the hand was cannulated to obtain arterialized venous blood samples. The cannula was kept patent throughout each visit by using an isotonic saline drip.

After the collection of a basal blood sample, subjects ingested 5 g of creatine monohydrate (Experimental and Applied Sciences, Golden, CO) dissolved in 200 ml of warm, sugar-free, diluted orange juice. Thirty minutes after consumption of the creatine solution, one of the following drinks was consumed over a 5-min period in a randomized order: 500 ml of low-calorie Lucozade (Smithkline Beecham, Coleford, UK) containing 5 g of simple CHO (placebo treatment); 500 ml of Protein Forte (Fresenius, Bad Homburg, Germany) containing 47 g of simple CHO and 50 g of ultrafiltrated milk protein (protein-CHO treatment); 250 ml of Lucozade and 250 ml of low-calorie Lucozade containing 50 g of simple CHO (low-CHO treatment); or 500 ml of Lucozade containing 94 g of simple CHO (high-CHO treatment).

The simple CHO in all formulations was almost exclusively in the form of glucose. Lucozade contains no nutrients other than CHO. The Protein Forte also contained small amounts of fat, electrolytes, and minerals (12.5 g fat, 450 mg sodium, 750 mg potassium, <675 mg chloride, 750 mg calcium, 300 mg magnesium, 500 mg phosphorous, and 15 mg iron).

The 30-min delay between the creatine and subsequent treatment-fluid ingestion enabled plasma creatine concentrations to rise above the K_m for muscle creatine transport (15, 16). Furthermore, we have previously shown that this procedure produces peak creatine and insulin concentrations at similar time points and stimulates muscle creatine accumulation in humans (5).

Arterialized venous blood samples were obtained at regular intervals for 220 min after ingestion of the creatine-containing solution. After 225 min, subjects consumed a second creatine solution, and 30 min later they ingested a second treatment solution of the same composition as that previously ingested. Subjects then left the laboratory and were instructed to ingest their third creatine and treatment solutions, which were provided, at 9 and 9:30 PM, respectively. The next morning, subjects returned to the laboratory at 8 AM, after having fasted overnight, and they consumed their final creatine and treatment solutions as described above. As on the initial visit, arterialized venous blood samples were collected immediately before and for 220 min after the ingestion of the fourth creatine solution. The total dose of creatine ingested over the 24-h period of supplementation was 20 g (4 × 5 g). Subjects collected their urine for a period beginning immediately after the ingestion of the first creatine solution to 24 h after the ingestion of the final creatine solution.

The volunteers refrained from strenuous exercise and had no alcohol and meat intake for the 24 h before and during the 40-h period of urine collection. Furthermore, dietary intake was controlled during supplementation to ensure consistent energy intake across treatments (inclusive of energy contained in treatment solutions). On the first day of each experimental treatment, subjects consumed a standardized breakfast at 8 AM and then remained fasted until their arrival in the laboratory at noon. Before leaving the laboratory, subjects were provided with a ready-to-make meal and several snacks, which they were instructed to consume between 6 and 8 PM that evening.

Blood sampling and analysis. Arterialized venous blood samples were obtained before and 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 220 min after ingestion of the first and fourth creatine solutions during each 24-h period of supplementation. Whole blood glucose concentration was measured immediately by using a β-glucose photometer (Hemocue, Ängelholm, Sweden). Then, 5 ml of blood were placed into lithium heparin tubes, and, after centrifugation (3,000 rpm for 10 min), the plasma was removed and stored frozen at −20°C. Plasma creatine concentrations were determined at a later date by using HPLC (System Gold, Beckman Instruments, Bucks, UK) according to the method of Dunnett et al. (3). A second 5-ml blood sample was allowed to clot for 20 min, and, after centrifugation (3,000 rpm for 10 min), the serum was stored frozen at −20°C. Serum insulin concentration was measured at a later date with a radioimmunoassay (Diagnostic Products, Los Angeles, CA).

Urine collection and analysis. All urine was collected in purpose-built bottles, after which the total mixed volume was recorded and a 5-ml aliquot was removed and stored frozen at −20°C. Urinary creatine and creatinine concentrations were

measured at a later date by using HPLC (3). Total creatine excretion was calculated by summing creatine and creatinine excretion. Percent whole body creatine retention was calculated as creatine ingested (g)/urinary creatine excretion (g) * 100.

Statistical analysis. Differences in blood glucose, plasma creatine, and serum insulin concentrations within and between treatments and between the initial and the fourth oral challenges were calculated by using two-way ANOVA. Differences in the area under the plasma creatine-time curve and the serum insulin-time curve between treatments and between the first and the fourth oral challenges were also analyzed by using two-way ANOVA. One-way ANOVA was used to detect differences in urinary creatine output between treatments. When appropriate, Fisher's protected least significant difference post hoc test was performed to locate differences between treatments. The areas under the plasma creatine- and serum insulin-time curves were calculated by using the least squares method (accounting for baseline values), and the relationship between these variables was examined by computing the Pearson product-moment correlation coefficient (r). Statistical differences between variables were accepted at $P < 0.05$, and all values are presented as means ± SE.

RESULTS

Plasma creatine. Figure 1 shows plasma creatine concentrations immediately before and 220 min after the ingestion of the first and fourth creatine solutions in combination with placebo, protein-CHO, high-CHO, and low-CHO solutions. For all treatments, the peak plasma creatine concentration was reached within 60 min of creatine ingestion. During the first oral challenge, there was a treatment effect such that the plasma creatine response was blunted after the ingestion of the high-CHO solution compared with the placebo ($P < 0.01$) and low-CHO solutions ($P < 0.01$). The response after the ingestion of the protein-CHO solution was not different from that after the high-CHO solution. In addition, the area under the plasma creatine-time curve tended to be lower after the ingestion of the protein-CHO and high-CHO solutions compared with the placebo and low-CHO solutions, but these differences were not statistically significant (100 ± 5 and 101 ± 6 vs. 111 ± 7 and 109 ± 4 $mmol \cdot l^{-1} \cdot min^{-1}$, respectively). For all treatments, the plasma creatine concentration was significantly higher before and for 220 min after the ingestion of the fourth oral challenge compared with the first oral challenge ($P < 0.001$, Fig. 1*B*). However, these higher concentrations after the fourth oral challenge were at least partly attributable to an elevation in the basal plasma creatine concentration before the fourth oral challenge. For the fourth oral challenge, the plasma creatine response was blunted when creatine ingestion was followed by ingestion of the high-CHO solution compared with the placebo ($P < 0.001$) and also compared with the protein-CHO solution ($P < 0.001$).

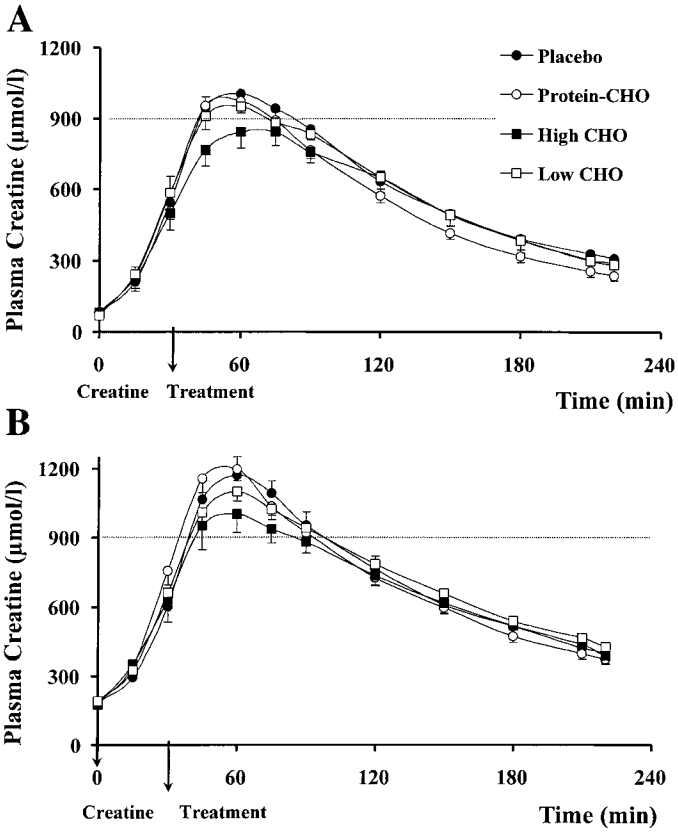

Fig. 1. Plasma creatine concentrations before and after the ingestion of 5 g creatine in a 200-ml solution followed 30 min later by the ingestion of 500 ml of solution containing 5 g of carbohydrates (CHO) (placebo), 50 g of protein and 47 g of CHO (protein-CHO), 94 g of CHO (high CHO), or 50 g of CHO (low CHO). *A*: 1st oral challenge; *B*: 4th oral challenge. Values are means ± SE. Dotted lines are included to facilitate comparison of curves across treatments and visits.

Blood glucose. Figure 2 shows blood glucose concentrations for each experimental treatment after the first and fourth oral challenges. On both occasions, the blood glucose concentration peaked within 30 min of the ingestion of the protein-CHO, high-CHO, and low-CHO solutions. As expected, blood glucose did not increase significantly from the presupplementation concentration (4.5 ± 0.1 mmol/l) for the placebo treatment. For the first and fourth oral challenges, the blood glucose response was significantly different among all treatments (high vs. low CHO, $P < 0.001$; low CHO vs. protein CHO, $P < 0.001$; protein CHO vs. placebo, $P < 0.05$; Fig. 2). When responses are compared between the first and fourth oral challenges within each treatment, blood glucose after the fourth oral challenge was no different from that observed after the first.

Serum insulin. Figure 3 shows serum insulin concentrations for each experimental treatment before and after the first and fourth oral challenges. On both occasions, the serum insulin concentration peaked within 30 min of consumption of the protein-CHO, high-CHO, and low-CHO solutions. During the placebo treatment, serum insulin did not rise significantly from the baseline concentration (11 mU/l). Insulin concentrations after ingestion of the protein-CHO and high-CHO solutions were higher compared with the low-CHO solution for both the first and fourth oral challenges (protein CHO vs. low CHO: first challenge $P < 0.001$, fourth challenge $P < 0.001$; high CHO vs. low CHO: first challenge $P < 0.001$, fourth challenge

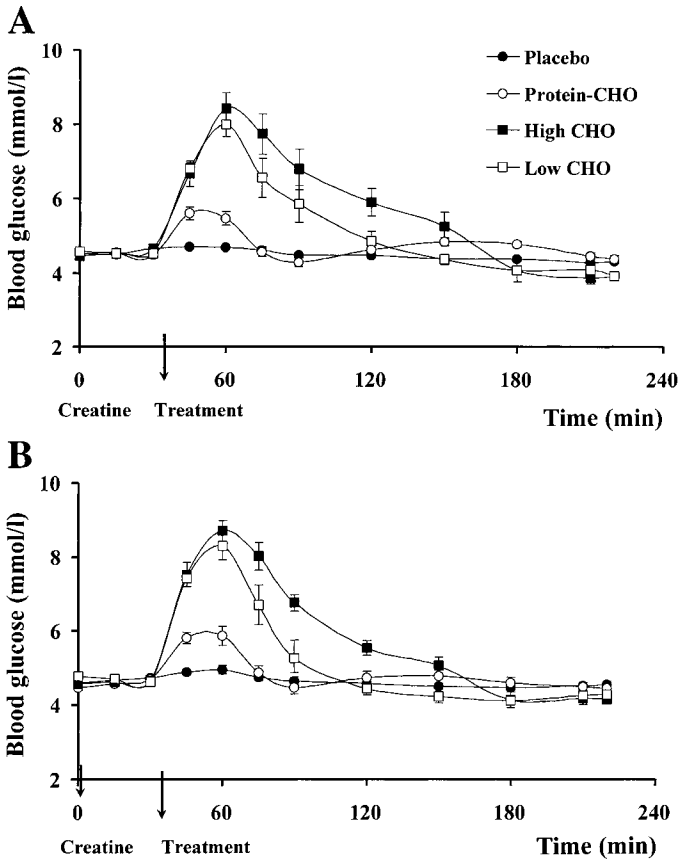

Fig. 2. Blood glucose concentrations before and after ingestion of 1st (*A*) and 4th (*B*) oral challenges. Values are means ± SE.

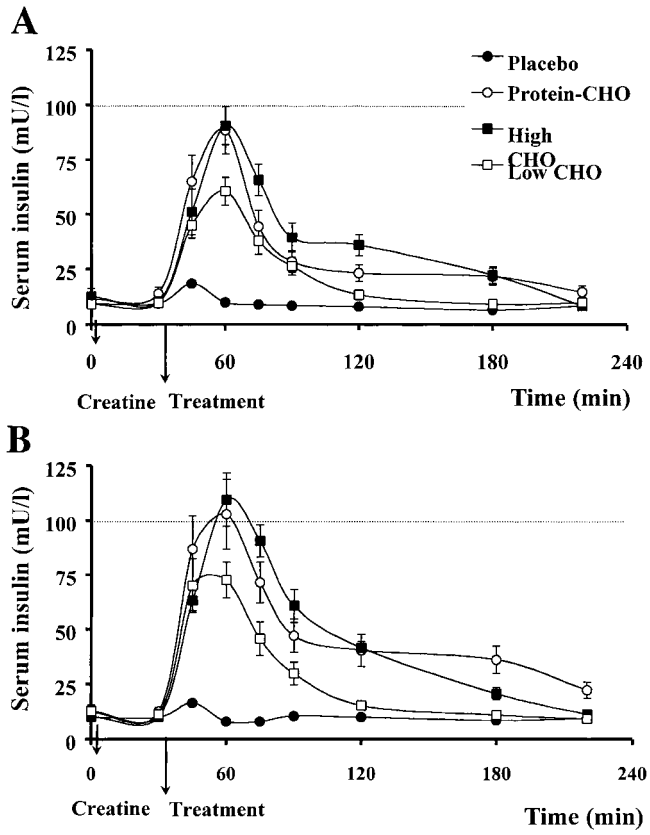

Fig. 3. Serum insulin concentrations before and after ingestion of 1st (*A*) and 4th (*B*) oral challenges. Values are means ± SE. Dotted lines are included to facilitate comparison of curves across treatments and visits.

$P < 0.001$). As a result, the area under the insulin-time curve was significantly greater for the protein-CHO and high-CHO treatments compared with the low-CHO and placebo treatments (Fig. 4). However, the area under the insulin-time curve for the protein-CHO treatment was no different from that observed for the high-CHO treatment for both the first and fourth oral challenges (Fig. 4). The serum insulin response and the area under the insulin-time curve were significantly greater after the fourth oral challenge compared with the first challenge for the protein-CHO (both $P < 0.001$), high-CHO (both $P < 0.001$), and low-CHO ($P < 0.01$ vs. $P < 0.001$) treatments (Figs. 3 and 4). The relationship between the area under the serum insulin-time curve and the plasma creatine-time curve after the first and fourth oral challenges is presented in Fig. 5. The two variables were negatively related after the first oral load ($r = -0.920$, $P < 0.05$) but not after the fourth ($r = -0.342$, $P > 0.05$).

Urinary creatine. Urinary creatine excretion was lower for the high-CHO ($P < 0.05$) and protein-CHO ($P < 0.05$) treatments compared with the placebo treatment (Table 1). As a consequence, whole body creatine retention was greater after the ingestion of creatine in combination with the protein-CHO and high-CHO solutions compared with the placebo solution (Fig. 6). The urinary creatine output for the low-CHO treatment was not significantly different from that for placebo, protein-CHO, and high-CHO treatments. The volume of urine collected over the 40-h period did not differ among treatments (Table 1).

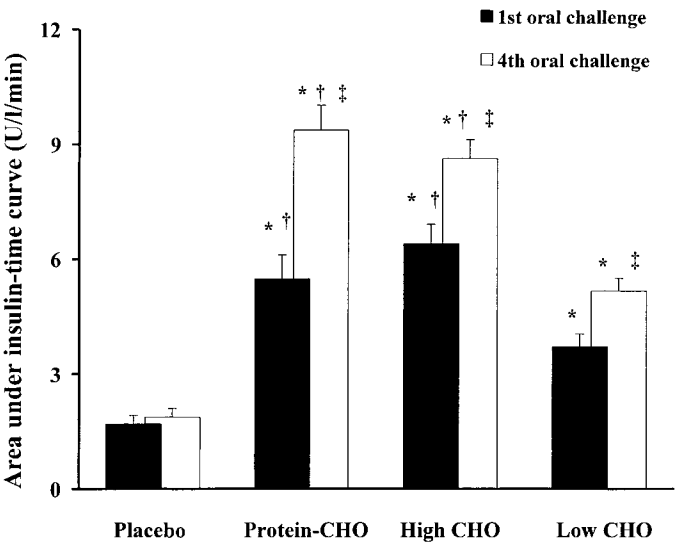

Fig. 4. Area under serum insulin-time curve after the 1st and 4th oral challenges. Values are means ± SE. *Significantly different from placebo, †low CHO, and ‡1st oral challenge, $P < 0.05$.

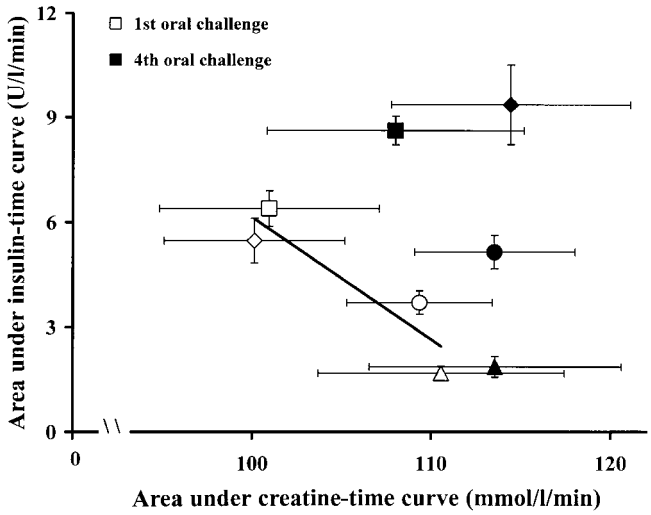

Fig. 5. Mean area under the plasma creatine-time curve plotted vs. mean area under the serum insulin-time curve for each experimental treatment for the 1st (open symbols; $r = -0.920$, $P < 0.05$) and 4th (solid symbols; $r = -0.342$, not significant) oral challenges for placebo (triangles), low-CHO (circles), protein-CHO (diamonds), and high-CHO (squares) treatment. Values are means ± SE. Thick line, line of best fit for 1st oral challenge.

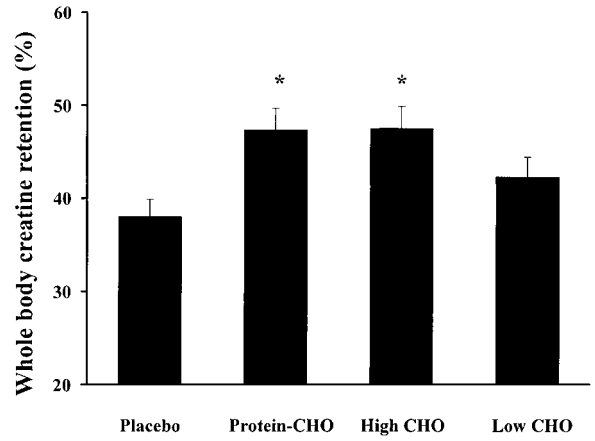

Fig. 6. Percent whole body creatine retention after the ingestion of 5 g creatine on 4 occasions over 24 h in conjunction with 500 ml of solution containing placebo, protein-CHO, high CHO, or low CHO. Values are means ± SE. *Significantly different from placebo, $P < 0.05$.

DISCUSSION

Our laboratory has previously reported that the ingestion of creatine in combination with 94 g of CHO on four occasions each day for 5 days resulted in a 60% greater increase in muscle creatine accumulation compared with the ingestion of creatine alone (5). It was suggested that the augmentation of muscle creatine accumulation occurred as a result of insulin-stimulating sodium-dependent muscle creatine transport (5). In vitro work has shown that insulin, insulin-like growth factor I, triiodothyronine, and amylin, all of which are known to stimulate sodium-potassium ATPase pump activity, enhances creatine transport in a muscle cell line (19). Recently, our laboratory demonstrated that insulin augments muscle creatine accumulation in vivo in humans when present at a concentration ≥100 mU/l (5, 24). In the study by Green et al. (5), subjects consumed 370 g of simple CHO per day for 5 days to achieve physiologically high serum insulin concentrations during the first hour after creatine administration. However, this quantity of CHO proved to be close to the limit of palatability. The important finding of the present study was that the ingestion of creatine, in conjunction with 47 g of simple CHO and 50 g of protein, resulted in a similar increase in serum insulin concentration to that achieved after the ingestion of 94 g of CHO. Furthermore, the insulin response was significantly greater than that observed after the ingestion of 50 g of CHO alone. The net effect of this was an increase in whole body creatine retention to the same extent as that seen after the high-CHO load. We would propose, therefore, that ingestion of creatine, in conjunction with a more moderate amount of CHO but in combination with protein, could be used as an alternative and as a more manageable method to maximize muscle creatine accumulation. This is important because, as already stated, the magnitude of the metabolic and physiological effects of creatine during exercise and recovery has been shown to be positively associated with the extent of muscle creatine accumulation during supplementation (2).

Table 1. *Total urinary creatine and creatinine excreted over a 40-h period*

	Placebo	Protein-CHO	High CHO	Low CHO
Urine volume, ml	5,320 ± 409	4,610 ± 433	4,823 ± 341	5,074 ± 415
Creatine excreted, g	8.7 ± 0.7	7.4 ± 0.5	7.6 ± 0.4	7.9 ± 0.7
Creatinine excreted, g	3.3 ± 0.2	3.2 ± 0.3	2.9 ± 0.2	3.3 ± 0.2
Total excreted, g	12.4 ± 0.8	10.5 ± 0.6*	10.5 ± 0.4*	11.2 ± 0.8
Creatine retention, g	7.6 ± 0.8	9.5 ± 0.6*	9.5 ± 0.4*	8.8 ± 0.8

Values are means ± SE; $n = 12$ subjects. CHO, carbohydrates. See METHODS for description of treatment groups. Urine collection began immediately after the ingestion of the first creatine or placebo solution. The amount of creatine ingested during supplementation was 20 g (4×5 g). *Significantly different from placebo, $P < 0.05$.

With the use of the urinary creatine excretion measurements and the assumption of a muscle mass equivalent to 40% of body mass, it was calculated that muscle total creatine concentration increased by ~7 mmol/kg dm during the 24 h of creatine supplementation combined with the placebo solution. This is in good agreement with the data of Harris et al. (11), who estimated an increase in muscle total creatine concentration of ~18 mmol/kg dm after the ingestion of 20–30 g creatine per day for 2 days. On the occasions in which creatine was consumed in combination with the protein-CHO and high-CHO solutions, the estimated muscle total creatine concentration increased by ~9 mmol/kg dm for both treatments. This finding confirms previous work (24) that insulin augments muscle creatine accumulation. However, the magnitude of the

increase (~25%) was less than that previously observed by Green et al. (60%; Refs. 5, 6) and can perhaps be partly accounted for by differences in the duration of creatine ingestion and periods of urine collection (40 vs. 24 h between studies). Based on the peak plasma creatine concentration, however, it appears more likely that, for reasons unknown, muscle total creatine accumulation was greater during the placebo treatment in the present study compared with the study by Green et al. (6) (1,091 ± 54 vs. 1,290 ± 98 μmol/l).

As previously reported (6, 11), the plasma creatine response after the ingestion of 5 g creatine varies markedly among individuals, and this is likely to be the reason why the areas under the plasma creatine-time curves were not statistically different across treatments in the present study. For all treatments, the plasma creatine concentrations were higher after the fourth oral challenge compared with the first. During the placebo treatment, this was almost entirely due to the elevation in the basal plasma creatine concentration before the fourth oral challenge (67 ± 7 vs. 190 ± 18 μmol/l). However, it can be clearly seen from Fig. 5 that the stimulatory effect of insulin on muscle creatine transport was markedly diminished after the fourth oral challenge for the high-CHO and protein-CHO treatments. This response, together with the elevation in basal plasma creatine concentrations, would account for the higher plasma creatine concentrations during the fourth oral challenge for the high-CHO and protein-CHO treatments. This aside, it is important to note from Fig. 5 that the augmentation of muscle creatine stores as a result of CHO and protein ingestion may have only occurred on the first day of supplementation.

At present, the mechanism by which skeletal muscle total creatine content is regulated is poorly understood. Creatine principally enters the muscle via binding to a specific transporter protein. This process is saturable and sodium dependent and moves creatine into the muscle against a high-concentration gradient (9, 17). Recently, it has been suggested that long-term exposure to high-plasma creatine concentrations may reduce the amount of the creatine transporter protein, thereby regulating muscle creatine content (9). Whereas this might be the case in the long term, it seems, from the present study, that acute regulation of flux through the creatine transporter would be more important in the control of muscle creatine homeostasis. Exactly what regulates flux via the creatine transporter is unresolved, but clearly the intracellular accumulation of creatine will be important, as will sodium-potassium pump activity. By way of example, it has been shown that a slowing of muscle creatine accumulation and/or transport occurs in parallel with an increase in muscle creatine stores (11, 15). Similarly, pharmacological inhibition of muscle sodium-potassium pump activity has been shown to inhibit cellular creatine transport (19), which would be expected given that creatine transport is sodium dependent.

There appears to be discrepancy in the literature concerning the effect of protein ingestion on insulin release. Some have found no effect of protein ingestion on insulin release (30), whereas others have found a marked increase in the insulin response after protein ingestion (18, 23, 31). The results of the present study confirm that ingestion of CHO, together with protein, has an insulin-potentiating and a blood glucose-moderating effect. Interestingly, the serum insulin concentrations were higher for the fourth oral challenge, compared with the initial oral challenge, for the protein-CHO, high-CHO, and low-CHO treatments. This may have been related to the subjects having fasted for only 4 h before the first oral load and for 12 h before the fourth.

In conclusion, the results of the present study indicate that the ingestion of creatine, in conjunction with ~50 g of protein and ~50 g of CHO, is as effective in stimulating pancreatic insulin release and whole body creatine retention as ingesting creatine in combination with almost 100 g of CHO. This information will be useful to individuals aiming to elevate their muscle total creatine store by supplementing with creatine, particularly those that regularly ingest CHO-protein supplements after exercise or several meal-replacement-type supplements per day (e.g., resistance-trained athletes). Finally, the potentiating effect of insulin on creatine disposal was less marked after the fourth oral challenge compared with the first. We would, therefore, propose that ingestion of CHO alone, or in combination with protein, in an effort to augment muscle creatine accumulation will probably only be highly effective on the first day of supplementation.

We thank Sujata Dissanayake for technical assistance and Fresenius (Bad Homburg, Germany) for kindly providing the Protein Forte drinks.

This work was funded by grant support from Experimental and Applied Sciences (Golden, CO).

REFERENCES

1. **Birch R, Noble D, and Greenhaff PL.** The influence of dietary creatine supplementation on performance during repeated bouts of maximal isokinetic cycling in men. *Eur J Appl Physiol* 69: 268–270, 1994.
2. **Casey A, Constantin-Teodosiu D, Howell S, Hultman E, and Greenhaff PL.** Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. *Am J Physiol Endocrinol Metab* 271: E31–E37, 1996.
3. **Dunnett M, Harris RC, and Orme CE.** Reverse-phase-ion-pairing high-performance liquid chromatography of phosphocreatine, creatine and creatinine in equine muscle. *Scand J Clin Lab Invest* 51: 137–141, 1991.
4. **Gallen IW and Macdonald IA.** Effect of two methods of heating on body temperature, forearm blood flow, and deep venous oxygen saturation. *Am J Physiol Endocrinol Metab* 259: E639–E643, 1990.
5. **Green AL, Hultman E, Macdonald IA, Sewell D, and Greenhaff PL.** Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. *Am J Physiol Endocrinol Metab* 271: E821–E826, 1996.
6. **Green AL, Simpson EJ, Littlewood JJ, Macdonald IA, and Greenhaff PL.** Carbohydrate ingestion augments creatine retention during creatine feeding in humans. *Acta Physiol Scand* 158: 195–202, 1996.
7. **Greenhaff PL, Bodin K, Söderlund K, and Hultman E.** The effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. *Am J Physiol Endocrinol Metab* 266: E725–E730, 1994.

8. **Greenhaff PL, Casey A, Short AH, Harris R, Söderlund K, and Hultman E.** Influence of oral creatine supplementation of muscle torque during repeated bouts of maximal voluntary exercise in man. *Clin Sci (Colch)* 84: 565–571, 1993.
9. **Guerrero-Ontiveros ML and Wallimann T.** Creatine supplementation in health and disease. Effects of chronic creatine ingestion in vivo: down-regulation of the expression of creatine transporter isoforms in skeletal muscle. *Mol Cell Biochem* 184: 427–437, 1998.
10. **Harris RC, Hultman E, and Norjesö L-O.** Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of the musculus quadriceps of man at rest. Methods and variance of values. *Scand J Clin Lab Invest* 33: 109–120, 1974.
11. **Harris RC, Söderlund K, and Hultman E.** Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clin Sci (Colch)* 83: 367–374, 1992.
12. **Haughland RB and Chang DT.** Insulin effects on creatine transport in skeletal muscle. *Proc Soc Exp Biol Med* 148: 1–4, 1975.
13. **Hultman E, Söderlund K, Timmons JA, Cederblad G, and Greenhaff PL.** Muscle creatine loading in men. *J Appl Physiol* 81: 232–237, 1996.
14. **Klivenyi P, Ferrante J, Matthews RT, Bogdanov MB, Klein AM, Andreassen OA, Mueller G, Wermer M, Kaddurah-Daouk R, and Beal MF.** Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat Med* 5: 347–350, 1999.
15. **Loike JD, Zalutsky DL, Kaback E, Miranda AF, and Silverstein SC.** Extracellular creatine regulates creatine transport in rat and human muscle cells. *Proc Natl Acad Sci USA* 85: 807–811, 1988.
16. **Möller A and Hamprecht B.** Creatine transport in cultured cells of rat and mouse brain. *J Neurochem* 52: 545–550, 1989.
17. **Nash SR, Giros B, Kingsmore SF, Rochelle JM, Suter ST, Gregor P, Seldin MF, and Caron MG.** Cloning, pharmacological characterisation and genomic localisation of the human creatine transporter. *Receptors Channels* 2: 165–174, 1994.
18. **Nuttall FQ, Gannon MC, Wald JL, and Ahmed M.** Plasma glucose and insulin profiles in normal subjects ingesting diets of varying carbohydrate, fat and protein content. *J Am Coll Nutr* 4: 437–450, 1985.
19. **Odoom JE, Kemp GJ, and Radda GK.** The regulation of total creatine content in a myoblast cell line. *Mol Cell Biochem* 158: 179–188, 1996.
20. **Pulido SM, Passaquin AC, Leijendekker WJ, Challet C, Wallimann T, and Rüegg UT.** Creatine supplementation improves intracellular Ca^{2+} handling and survival in mdx skeletal muscle cells. *FEBS Lett* 439: 357–362, 1998.
21. **Rossiter HB, Cannell ER, and Jakeman PM.** The effect of oral creatine supplementation on the 1000-m performance of competitive rowers. *J Sports Sci* 14: 175–179, 1996.
22. **Sipilia I, Rapola J, Simell O, and Vannas A.** Supplementary creatine as a treatment for gyrate atrophy of the coroid and retina. *N Engl J Med* 304: 867–870, 1981.
23. **Spiller GA, Jensen D, Pattison TS, Chuck CS, Whittam JH, and Scala J.** Effect of protein dose on serum glucose and insulin response to sugars. *Am J Clin Nutr* 46: 474–480, 1987.
24. **Steenge GR, Lambourne J, Casey A, Macdonald IA, and Greenhaff PL.** Stimulatory effect of insulin on creatine accumulation in human skeletal muscle. *Am J Physiol Endocrinol Metab* 275: E974–E979, 1998.
25. **Tarnopolsky M and Martin J.** Creatine monohydrate increases strength in patients with neuromuscular disease. *Neurology* 52: 854–857, 1999.
26. **Vandenberghe K, Goris M, Van Hecke P, Van Leemputte M, Vangerven L, and Hespel P.** Long-term creatine intake is beneficial to muscle performance during resistance training. *J Appl Physiol* 83: 2055–2063, 1997.
27. **Van Leemputte M, Vandenberghe K, and Hespel P.** Shortening of muscle relaxation time after creatine loading. *J Appl Physiol* 86: 840–844, 1999.
28. **Walker JB.** Creatine: biosynthesis, regulation and function. *Adv Enzymol Relat Areas Mol Biol* 50: 177–242, 1979.
29. **Wallimann T, Wyss M, Brdiczka D, Nicolay K, and Eppenberger HM.** Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the phosphocreatine circuit for cellular energy homeostasis. *Biochem J* 281: 21–40, 1992.
30. **Wolever TM and Bolognesi C.** Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, CHO and glycemic index. *J Nutr* 126: 2807–2812, 1996.
31. **Zawadzki KM, Yaspelkis BB III, and Ivy JL.** Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. *J Appl Physiol* 72: 1854–1859, 1992.