Amino Acids (2005) 28: 71–76
DOI 10.1007/s00726-004-0143-z

Amino Acids
Printed in Austria

The addition of fenugreek extract (*Trigonella foenum-graecum*) to glucose feeding increases muscle glycogen resynthesis after exercise

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Received May 20, 2004
Accepted September 4, 2004
Published online December 2, 2004;

Summary. The purpose of this study was to determine the effects of ingesting an oral supplement containing 4-Hydroxyisoleucine (4-OH-Ile, isolated from fenugreek seeds [*Trigonella foenum-graecum*]) with a glucose beverage on rates of post-exercise muscle glycogen resynthesis in trained male cyclists. Following an overnight fast (12 hr), subjects completed a 90-minute glycogen depletion ride after which a muscle biopsy was obtained from the vastus lateralis. Immediately and 2 hours after the muscle biopsy, subjects ingested either an oral dose of dextrose (Glu) ($1.8\,g \cdot kg\ BW^{-1}$) or 4-OH-Ile supplement (Glu + 4-OH-Ile, including $2.0\,mg \cdot kg^{-1}$ 4-OH-Ile with the same oral dose of dextrose) with a second muscle biopsy 4 hours after exercise. Post exercise muscle glycogen concentration was similar for both trials. Overall, there was a significant increase in glucose and insulin concentrations from time 0 throughout the majority of the 4-hour recovery period, with no significant differences between the two trials at any time point. Although muscle glycogen concentration significantly increased from immediately post exercise to 4 hr of recovery for both trials, the net rate of muscle glycogen resynthesis was 63% greater during Glu + 4-OH-Ile (10.6 ± 3.3 vs. $6.5 \pm 2.6\,g \cdot kg$ wet $wt.^{-1} \cdot hr.^{-1}$ for the Glu + 4-OH-Ile and Glu trials, respectively). These data demonstrate that when the fenugreek extract supplement (4-OH-Ile) is added to a high oral dose of dextrose, rates of post-exercise glycogen resynthesis are enhanced above dextrose alone.

Keywords: Glucose uptake – 4-OH-Isoleucine – Insulin – Muscle recovery – Post-exercise nutrition

Introduction

It has been clearly established that regular carbohydrate feedings are required soon after glycogen depleting muscle work to maximize the rates of muscle glycogen resynthesis (Blom et al., 1987; Ivy et al., 1988a; Reed et al., 1989; Rotman et al., 2000; Tarnopolsky et al., 1997; Zawadzki et al., 1992). It is also clear that post exercise glucose uptake by the skeletal muscle is due to a combination of insulin dependent (nutrient stimulated) and insulin independent (contractile mediated) mechanisms. Prior research has attempted to promote an increase in the post-exercise rate of glycogen synthesis by varying the source of carbohydrate (CHO) (Blom et al., 1987; Reed et al., 1989), the CHO dose (Ivy et al., 1988b), the timing of CHO ingestion (Ivy et al., 1988a), the inclusion of protein (Ivy et al., 2002; Jentjens et al., 2001; Tarnopolsky et al., 1997; van Hall et al., 2000; Zawadzki et al., 1992), and the inclusion of specific essential and non essential amino acids in combination with the CHO (Bowtell et al., 1999; Robinson et al., 2003; Yaspelkis and Ivy, 1999).

It has been previously suggested that the inclusion of protein with the CHO in solution has been shown to increase insulin release, which may further promote glucose uptake (Zawadzki et al., 1992). Insulin has also been shown to promote glycogen synthase activity (Ivy et al., 1988b). While some research has demonstrated an increase in the rate of muscle glycogen resynthesis using a CHO + protein feeding protocol vs. CHO only (Ivy et al., 2002; Zawadzki et al., 1992) others have demonstrated limited effects (Carrithers et al., 2000; Jentjens et al., 2001; van Hall et al., 2000). However, since both isocaloric and hypercaloric feeding protocols have been used it becomes more difficult to distinguish the unique effects of protein and amino acid preparations when added to CHO.

Additional research has described the use of essential and non-essential amino acids (AA) in addition to CHO in solution. While the use of arginine has been suggested to increase the availability of glucose for muscle glycogen storage (Yaspelkis et al., 1999), it has been suggested that L-arginine would not contribute to post-exercise muscle glycogen storage efforts (Robinson et al., 2003). Similarly, the use of glutamine has been suggested to promote liver CHO storage without altering rates of muscle glucose

uptake (Bowtell et al., 1999). In contrast, leucine has been shown to stimulate glucose uptake in the rat soleus muscle by way of an insulin-independent mechanism (Nishitani et al., 2002). However, these amino acids represent commonplace amino acids widely available in a variety of dietary sources.

Fenugreek seeds (Trigonella foenum graecum L.) contain a relatively high concentration of 4-Hydroxyisoleucine (4-OH-Ile), which is a unique amino acid that is not found in mammalian muscle tissue but is a common ingredient in some Indian cooking. This amino acid has demonstrated unique insulinotropic properties under resting conditions in the presence of moderate hyperglycemia by way of direct stimulation of the pancreatic β-cells (Broca et al., 1999, 2000) and has been suggested as an alternative approach to the treatment of non-insulin dependent diabetes mellitus (Sauvaire et al., 1998). However, the effects of fenugreek and/or 4-OH-Ile on glucose uptake and the resultant rate of post-exercise muscle glycogen resynthesis have not been investigated in human subjects.

The purpose of this study was to determine the effects of CHO alone vs. CHO + 4-OH-Ile on the rates of post-exercise muscle glycogen resynthesis in recreationally trained male cyclists. Given the previous research, which has demonstrated a potential of this novel amino acid to stimulate pancreatic β-cells and subsequent insulin release under resting conditions, we hypothesized that circulating insulin concentrations would be elevated and lead to an increased rate of post-exercise muscle glycogen resynthesis when 4-OH-Ile was added to the post-exercise carbohydrate bolus.

Methodology

Subjects

A total of six trained male cyclists completed the study (see Table 1 for descriptive data). The University Internal Review Board approved the study, and subjects provided written consent prior to participation.

Preliminary testing

Peak VO_2 was measured for each subject using a ramp protocol ($40\,W \cdot min^{-1}$) and an electronically braked cycle ergometer (Cardgirus Medical Pro, Spain). Expired gases were collected during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT) and analyzed at 15-second intervals. Ventilatory threshold (VT) was determined using a combination of the V-slope method, the ventilatory equivalent method and the excess CO_2 method (Gaskill et al., 2001). Body composition was determined at residual lung volume using hydrodensitometry. The average of three measurements of residual lung volume was calculated using the same Parvomedics calibrated metabolic cart (oxygen dilution). Net underwater weights were recorded using a digital scale (Exertech, Dresbach, MN). Body density was converted to body composition using the Siri equation (Siri, 1993).

Table 1. Subject descriptive data (n = 6). Data are expressed as mean ± SD

Age (yr)	26.0 ± 5.2
Height (cm)	178.0 ± 8.6
Weight (kg)	73.8 ± 8.5
Body fat (%)	12.0 ± 5.0
Peak VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	58.3 ± 3.8
VO_2 at VT ($ml \cdot kg^{-1} \cdot min^{-1}$)	36.4 ± 4.2

Design

Subjects completed a placebo controlled, double-blind crossover design. The *Placebo Trial* (*Glu*) included dextrose ($1.8\,g \cdot kg^{-1}$ BW) plus placebo capsules (micro crystallized cellulose) provided approximately 15 minutes post exercise and again at 120 minutes after the first feeding. The *Experimental Trial* (*Glu + 4-OH-Ile*) included dextrose ($1.8\,g \cdot kg^{-1}$ BW) plus the experimental supplement extracted from Fenugreek seeds (patent pending, Technical Sourcing International, Ltd). In addition to the 4-OH-Ile ($2.0\,mg \cdot kg^{-1}$ BW), there were also trace amounts of other amino acids (see Table 2). Since the inclusion of the AA supplement to the Glu did not substantially change the overall caloric content of the post-exercise drinks, both trials were essentially eucaloric. The feeding schedule was identical for the Glu and Glu + 4-OH-Ile trials. Trial order was randomly assigned and counter-balanced with each subject completing both trials within a 2-week period with at least seven days between trials.

Experimental protocol

Following an overnight fast (12 hr), subjects reported to the lab at 0600. Subjects completed a 10-minute warm up at approximately 55% peak VO_2. Thereafter, subjects completed a series of ten intervals, which included two minutes at approximately 80% peak VO_2 followed by four minutes at approximately 50% peak VO_2. After the series of 10 intervals, subjects completed 8 minutes at 60% peak VO_2 followed by 12 minutes at 50%

Table 2. Amino acid profile of the 4-OH-Ile supplement

Amino acid	mg/kg BW dose
Arginine	0.07
Aspartate	0.11
Threonine	0.02
Serine	0.07
Glutamate	0.16
Glycine	0.06
Alanine	0.10
Cyseine	0.06
Valine	0.03
Methionine	0.01
Isoleucine	0.02
Leucine	0.02
Phenylalanine	0.05
Ornithine	0.01
Lysine	0.01
Histidine	0.01
Tyrosine	0.04
4-OH-Ile	2.00

Total amino acids (40%, 4-hydroxyisoleucine is approximately 25% of the 40%), alkaloids (35%, primarily Trigonelline), protein/peptides (5%), fiber (1%), Ash (2%), moisture (12%), lipids, etc (5%)

peak VO_2. During the exercise trials, subjects were allowed to consume water ad libitum. Immediately upon completion of the 90-minute depletion ride, subjects were instructed to towel off and change into dry clothes.

With the subject in the supine position, an indwelling venous catheter (20 ga.) was inserted into an arm vein and kept patent using a continuous saline drip (0.45 normal saline, drip rate approximately $0.75\,ml \cdot min^{-1}$). Blood samples were collected in untreated tubes and allowed to clot on ice for 15 minutes. Samples were then spun for 15 minutes at 4000 rpm in a refrigerated centrifuge (4°C). The subsequent serum was removed and separated between two different tubes and stored at −30°C for later analyses of glucose and insulin. The leg was prepared and a muscle biopsy was obtained from the midsection of the vastus lateralis muscle. The initial post exercise biopsy and blood sample were collected approximately 10-minutes post-exercise.

Following the biopsy and the collection of a baseline blood sample, each subject received an oral dose of dextrose ($1.8\,g \cdot kg^{-1}$ BW, Fisher Scientific) and either placebo or experimental ($2.0\,mg \cdot kg^{-1}$ BW 4-OH-Ile) capsules. After the initial ingestion of the dextrose beverage and supplement, subsequent blood samples were obtained every 10 minutes until 60 minutes and every 30 minutes thereafter until 120 minutes. At 120 minutes post feeding, a second oral dextrose solution (1.8 g/kg BW) with either placebo or experimental ($2.0\,mg \cdot kg^{-1}$ BW 4-OH-Ile) capsules was ingested. Subsequent blood samples were obtained at 10-minute intervals until 180 minutes and every 30 minutes thereafter until 240 minutes. During the 4-hour post-exercise session, subjects remained in a supine and/or partially seated position in a hospital bed and were allowed to study, read, and/or watch television.

At 4-hours following the initial feeding, a second biopsy sample was obtained from a second incision in the vastus lateralis made approximately 4 cm proximal to the initial biopsy site on the same leg. After excess blood and any connective tissue or fat were removed, tissue samples were immersed in liquid nitrogen within approximately 1-minute post biopsy.

Tissue and blood analyses

Blood samples were analyzed for glucose in duplicate using an enzymatic spectrophotometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.). Insulin was also analyzed in duplicate using an enzymatic spectrophotometric ELISA method (EIA2935, DRG International). Average intra-assay coefficient of variation for glucose and insulin was <5%.

Muscle glycogen was analyzed using a similar enzymatic spectrophotometric method (Infinity glucose (HK) liquid stable reagent, ThermoTrace Ltd.) after tissue preparation. Samples (22.6 ± 1.7 mg wet weight) were weighed upon removal from a −80°C freezer. Samples were placed in 1 ml, 1 N HCL solution and homogenized using a manual mortar and pestle tissue grinder. Once homogenized, samples were incubated at 95.6°C for three hours. After the incubation, 0.5 ml, 1 N NaOH was added to 0.5 ml of boiled tissue sample to normalize pH. Samples were analyzed in triplicate against known glycogen and glucose controls run at the same time. Muscle glycogen concentrations were expressed in $mmol \cdot kg^{-1}$ wet weight of muscle. Glycogen resynthesis rate was expressed as $mmol \cdot kg^{-1} \cdot hr^{-1}$ wet weight of muscle and calculated from the following equation: $Rate = (G_{post} - G_{pre})/t$, where G_{post} is the glycogen concentration at 4 hours post feeding, G_{pre} is the glycogen concentration immediately post exercise, prior to feeding, and t is the time between biopsies.

Statistics

Glucose, insulin and muscle glycogen were analyzed using a repeated measures ANOVA (trial × time). The difference in the overall rate of muscle glycogen resynthesis was analyzed with a 2-tailed dependent t-test. Statistical significance was established using an alpha level of $p < 0.05$. Data were analyzed using SuperAnova for the Macintosh.

Results

Blood glucose

There were no significant differences in the blood glucose concentration between the Glu and Glu + 4-OH-Ile trials at any time point. However, the main effect for time indicated a statistically significant increase in blood glucose from time 0 (pre-feeding, immediately post exercise) at minutes 20 and every time point thereafter until 210 minutes. By 240 minutes post initial feeding, blood glucose had returned to pre-feeding values (see Fig. 1). The overall mean glucose for the entire 240 minute period was also

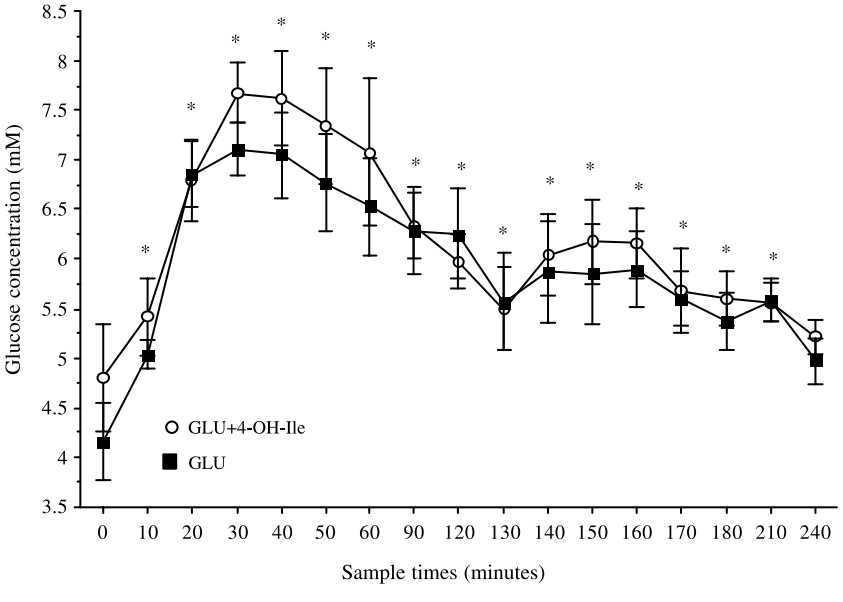

Fig. 1. Changes in blood glucose in response to the oral glucose and experimental feedings (Glu vs. Glu + 4-OH-Ile). Feedings occurred immediately after exercise post exercise biopsy post (time 0) and again at 120 minutes of recovery. * – glucose > time 0, $p < 0.05$ (main effect of time). There were no differences between trials at any time point

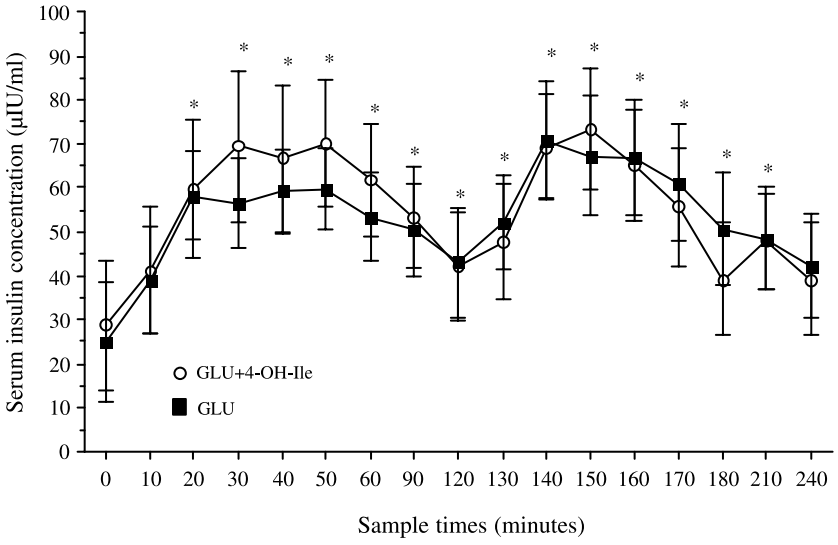

Fig. 2. Changes in serum insulin in response to the oral glucose and experimental feedings (Glu vs. Glu + 4-OH-Ile). Feedings occurred immediately post time 0 and again at 120 minutes. * – Insulin > time 0, $p < 0.05$ (main effect time). There were no differences between trials at any time point

not significantly different between the trials (5.9 ± 0.8 and 6.2 ± 0.7 mmol for the Glu and Glu + 4-OH-Ile trials, $p = 0.25$).

Serum insulin

There were no significant differences in the serum insulin concentration between the Glu and Glu + 4-OH-Ile trials at any time point. However, the main effect for time indicated a statistically significant increase in insulin from time 0 (pre-feeding, immediately post exercise) at minutes 20 and every time point thereafter until 210 minutes. By 240 minutes post initial feeding, serum insulin had returned to pre-feeding values (see Fig. 2). The overall mean insulin for the entire 240 minute period was also not significantly different between the trials (44.6 ± 18.5 and 43.94 ± 15.7 μIU/ml for the Glu and Glu + 4-OH-Ile trials, $p = 0.94$).

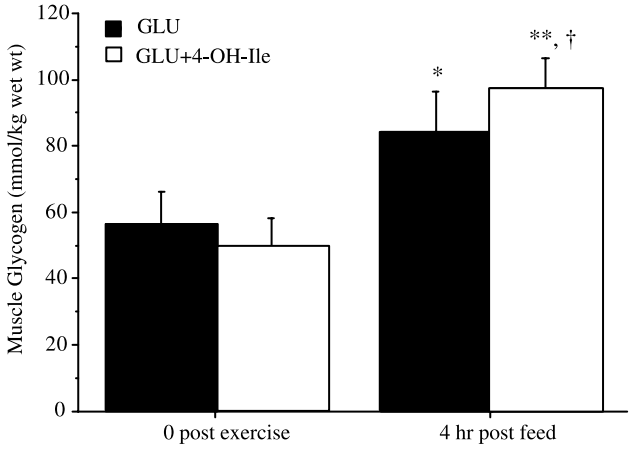

Fig. 3. Changes in muscle glycogen (vastus lateralis) in response to the oral glucose and experimental feedings (GLU vs. GLU + 4-OH-Ile). Feedings occurred immediately after post exercise biopsy (0 post exercise) and again at 120 minutes (4 hr post feed). * – significant increase from 0 post exercise (GLU), $p < 0.05$, ** – significant increase from 0 post exercise (GLU + 4-OH-Ile), $p < 0.05$, † – 4 hr post feed (GLU + 4-OH-Ile) vs. 4 hr post feed (GLU), $p < 0.05$

Muscle glycogen

The trial × time interaction for the measure of muscle glycogen was significant ($p < 0.05$) indicating a difference in the rate of muscle glycogen resynthesis between the Glu and Glu + 4-OH-Ile trials. There was no significant difference immediately post exercise between the treatments showing equal glycogen depletion for both trials. Both the Glu and Glu + 4-OH-Ile trials demonstrated a significant increase in muscle glycogen following the 4-hour recovery/feeding period. However, at 4-hours, muscle glycogen was significantly higher for the Glu + 4-OH-Ile trial compared to the placebo trial (see Fig. 3). Similarly, calculated rates of muscle glycogen resynthesis were significantly higher for the Glu + 4-OH-Ile trial (10.6 ± 3.3 vs. 6.5 ± 2.6 g · kg wet wt.$^{-1}$ · hr.$^{-1}$ for the Glu + 4-OH-Ile and Glu trials, respectively). This pattern for glycogen resynthesis was noted for each subject (see Table 3).

Discussion

The purpose of this study was to determine the effects of a fenugreek seed extract providing 2.0 mg · kg^{-1} BW 4-OH-Ile in addition to trace amounts of other amino acids on post-exercise muscle glycogen resynthesis. The main findings indicate that in combination with the large oral car-

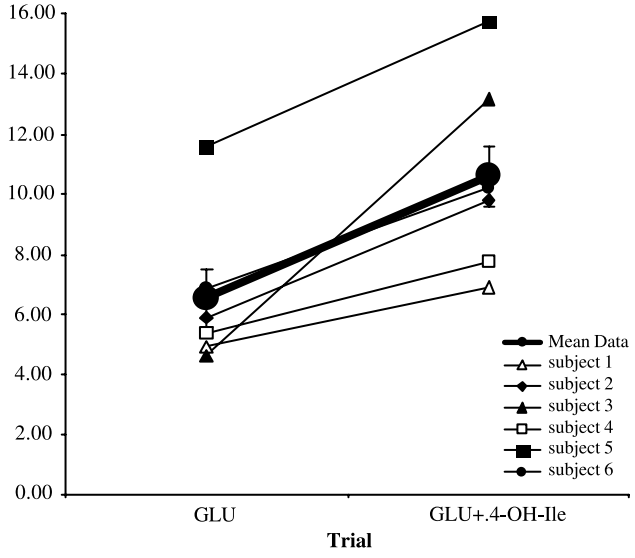

Fig. 4. Individual responses in the rates of muscle glycogen resynthesis for both the Glu + 4-OH-Ile and the Glu trials. The mean rate of muscle glycogen resynthesis was significantly higher for the Glu + 4-OH-Ile (10.6 ± 3.3 vs. $6.5 \pm 2.6\,g \cdot kg$ wet $wt.^{-1} \cdot hr.^{-1}$ for the Glu + 4-OH-Ile and Glu trials, respectively, $p < 0.05$)

Table 3. Changes in muscle glycogen (vastus lateralis) in response to the oral glucose and experimental feedings (Glu vs. Glu + 4-OH-Ile). Feedings occurred immediately after post exercise biopsy (0 post exercise) and again at 120 minutes (4 hr post feed)

	Pre	Post
Glu	50.3 ± 27.7	77.0 ± 33.1 *
Glu + 4-OH-Ile	52.5 ± 20.0	97.0 ± 21.2 **,†

* – $p < 0.05$ vs. pre exercise (Glu), ** – $p < 0.05$ vs. pre exercise (Glu + 4-OH-Ile), † – $p < 0.05$ Glu + 4-OH-Ile Glu (4 hr post feed)

bohydrate bolus feedings ($1.8\,g \cdot kg^{-1}$ BW), the added 4-OH-Ile promoted a 63% higher rate of post-exercise muscle glycogen resynthesis compared to carbohydrate alone.

Past research has indicated that the rate of muscle glycogen resynthesis can proceed rapidly if a regular feeding schedule is established during the immediate hours post glycogen depleting exercise (Bergstrom and Hultman, 1967; Blom et al., 1987; Ivy and Holloszy, 1981). However, when carbohydrate is provided at a rate above $1.5\,g \cdot kg^{-1}$ BW it does not further accelerate glycogen resynthesis (Blom et al., 1987). The rate limiting mechanisms associated with post-exercise muscle glycogen resynthesis include glucose transport into and glycogen synthesis within the muscle and/or the subsequent rate of total CHO oxidation during the post-exercise recovery period.

Because insulin is a strong potentiator of glycogen synthesis in the muscle and glucose transport by activating GLUT4 translocation, previous research has attempted to alter or amplify insulin secretion to hasten glycogen recovery in the skeletal muscle. Although past research has demonstrated that protein as well as specific dietary amino acids may enhance insulin release (Floyd et al., 1966; Nuttall et al., 1984; Pallotta and Kennedy, 1968; Rabinowitz et al., 1966), the link between amplified insulin release and elevated glucose uptake and the subsequent rate of muscle glycogen resynthesis has demonstrated mixed results. Where some research has indicated an increase in the rate of muscle glycogen recovery when protein is added to the carbohydrate source (Ivy et al., 2002; Tarnopolsky et al., 1997; Zawadzki et al., 1992), others have noted no significant increase when protein or dietary amino acids are consumed with carbohydrate (Carrithers et al., 2000; Jentjens et al., 2001; Rotman et al., 2000; van Hall et al., 2000).

Past results indicate that although the use of supplemental protein and/or dietary amino acids in combination with oral glucose solutions in upwards of $1.8\,g \cdot kg^{-1}$ may result in an acute increase in insulin release, it does not guarantee an acceleration of glucose uptake by the depleted skeletal muscle. The increase in insulin release may also promote an increase in whole body glucose disposal and storage, stimulating glucose uptake by the liver, adipose tissue, and non-depleted skeletal muscle. The discrepancies in the literature regarding the effects of amplified insulin release on accelerating muscle glycogen resynthesis post-exercise suggests an upper physiological limit of glucose uptake by the depleted skeletal muscle that is dependent on an insulin-mediated mechanism. It is probable that the contractile mediated mobilization of GLUT4 is more influential on the overall rates of skeletal muscle glucose uptake as compared to the post exercise concentrations of circulating insulin.

Past research further suggests that the rate of glucose uptake and the concomitant rate of muscle glycogen resynthesis is likely a function of, but may not be limited to the initial muscle glycogen concentration, total skeletal GLUT4 protein content, degree of GLUT4 translocation, glycogen synthase activity, and rates of whole body CHO oxidation during the recovery period. Other potential mechanisms that may further increase the rate of muscle glycogen resynthesis may include enhanced insulin receptor activity or amplified GLUT4 translocation independent of the traditionally proposed contractile and insulin regulated processes. Regardless of the concept of multiple GLUT4 pools (insulin and contraction dependent), there

are limited data to support the concept of GLUT4 translocation independent of the primary mechanisms mediated by muscle contraction and insulin. However, a recent study by Nishitani et al. (2002) has demonstrated that leucine promotes glucose uptake in insolated rat soleus muscle by way of what was described as an insulin-independent mechanism.

In summary, we have demonstrated that compared to CHO alone, CHO + 4-OH-Ile can promote a more rapid rate of glycogen resynthesis (63% higher) following glycogen depleting cycle exercise in trained male cyclists. Based on the similarity in blood glucose and insulin between trials, the combination of CHO + 4-OH-Ile appears to enhance muscle glycogen resynthesis without altering circulating insulin. Further research should evaluate the underlying mechanisms of this unique amino acid and its effect on muscle glycogen recovery and subsequent exercise performance during multiple days of arduous exercise when glycogen concentrations are consistently challenged.

Acknowledgements

The authors would like to thank Dr. Theodore Zderic for his assistance in preparing our lab group for the glycogen assays.

Disclosure

This study was funded by a grant from Technical Sourcing International, Inc. Missoula, MT.

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