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Glycogen resynthesis and exercise performance with the addition of fenugreek extract (4-hydroxyisoleucine) to post-exercise carbohydrate feeding

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Summary. The purpose of this study was to determine the effect of adding fenugreek extract (FG) to post-exercise carbohydrate feeding on glycogen resynthesis and subsequent exercise performance in normoglycemic male endurance athletes. A muscle biopsy sample was obtained from the *vastus lateralis* from subjects prior to exercise for 5 h at 50% of peak cycling power ($52.1 \pm 3.3\%$ of VO_2 peak). A second muscle biopsy sample was obtained immediately after exercise. Immediately after and 2 h after the second biopsy subjects ingested either an oral dose of dextrose (GLU) ($1.8\,g \cdot kg\,BW^{-1}$) or GLU with FG containing $1.99 \pm 0.20\,mg \cdot kg^{-1}$ 4-hydroxyisoleucine (GLU + FG) in a randomized, cross-over, double blind design. At 4 h post-exercise a third biopsy was taken and subjects received a standardised meal along with FG or a placebo capsule. At 15 h post-exercise subjects underwent their final muscle biopsy before completing a simulated 40 km cycling time trial. There was no difference in muscle glycogen at any time between GLU and GLU + FG. Additionally, 40 km time trial performance was similar for average power output (221 ± 28 vs. 213 ± 16 watts) and for time to completion (69.7 ± 3.7 vs. 70.5 ± 2.2 min) for the GLU and GLU + FG, respectively. Despite earlier data to the contrary, the present results do not support an effect of fenugreek supplementation on glycogen resynthesis, even though this may have been the result of differences in experimental protocol.

Keywords: Glycogen – Fenugreek – Trigonella foenum-graecum – 4-Hydroxyisoleucine – Exercise

Introduction

Trigonella foenum graecum, commonly called fenugreek, is an annual plant originating in India and Northern Africa. The leaves and seeds are prepared into extracts or powders and used in traditional alternative medicine practice to treat several conditions including hyperglycemia and hyperlipidemia. The main component of this extract is the amino acid 4-hydroxyisoleucine (4-OH-Ile). Chronic 4-OH-Ile supplementation in the form of fenugreek has shown promising results in alleviating symptoms of diabetes in rodents (Raju et al., 2001; Vats et al., 2003; Mohamad et al., 2004; Thakran et al., 2004; Gad et al., 2006; Mohammad et al., 2006; Narender et al., 2006) and in humans (Madar et al., 1988; Sharma et al., 1990; Sowmya and Rajyalakshmi, 1999; Abdel-Barry et al., 2000; Gupta et al., 2001). These studies have demonstrated a decrease in blood glucose (Madar et al., 1988; Sharma et al., 1990; Abdel-Barry et al., 2000; Gupta et al., 2001; Raju et al., 2001; Vats et al., 2003; Mohamad et al., 2004; Gad et al., 2006; Mohammad et al., 2006), total cholesterol (Sharma et al., 1990; Sowmya and Rajyalakshmi, 1999; Narender et al., 2006), triglycerides (Sharma et al., 1990; Gupta et al., 2001; Narender et al., 2006), free fatty acids (Narender et al., 2006), and an increase in HDL cholesterol (Gupta et al., 2001) and pancreatic β-cell insulin release (Sauvaire et al., 1998). In addition, fenugreek has been shown to influence the insulin cell-signaling pathway (Broca et al., 2004; Vijayakumar et al., 2005), increasing glucose uptake in insulin sensitive tissues. These effects, along with the up-regulation of metabolic enzymes (Raju et al., 2001; Vats et al., 2003; Mohamad et al., 2004; Gad et al., 2006; Mohammad et al., 2006) and increased muscle and liver glycogen storage (Vats et al., 2003; Ruby et al., 2005; Gad et al., 2006), are consistent with the adaptations that occur with regular exercise training, suggesting that fenugreek supplementation may increase exercise capacity.

Muscle glycogen is a major energy substrate during exercise and thus is critical to performance (Hermansen et al., 1967; Burke and Hawley, 1999). When the muscle glycogen concentration is reduced by a bout of exercise it

must be resynthesized during recovery for individuals to perform at a high level during subsequent exercise sessions. Post-exercise intake of carbohydrate has been repeatedly shown to increase the rate of glycogen synthesis (Ivy, 2001). Essential to glycogen synthesis is glucose flux into the muscle, which can occur via insulin dependent and insulin independent (muscle contraction) pathways (Hayashi et al., 1997; Goodyear and Kahn, 1998). The inclusion of protein or amino acids in post exercise carbohydrate feedings has been suggested to increase insulin (Zawadzki et al., 1992) and thus promote glycogen synthase activity (Ivy et al., 1988). However, other research has demonstrated no benefit with the addition of protein or amino acids to a high carbohydrate, post-exercise feedings (Carrithers et al., 2000; van Hall et al., 2000; Jentjens et al., 2001). Since fenugreek is high in amino acids, especially 4-hydroxyisoleucine, it is possible that this may exert an insulinotropic effect on glycogen storage. Our laboratory has recently shown that muscle glycogen resynthesis was increased 67% during a four-hour recovery period after exhaustive exercise when fenugreek was added to a carbohydrate bolus. Additionally, there were no differences in the circulating insulin and glucose concentrations during the recovery period (Ruby et al., 2005). These results indicate that fenugreek may act by a mechanism independent of insulin.

Research to date has focused on fenugreek supplementation in the hyperglycemic sedentary state and in a rodent model. To the authors' knowledge there has been only one published report in normoglycemic humans. This study demonstrated lower blood glucose and blood potassium just four hours after acute fenugreek supplementation (Abdel-Barry et al., 2000). The impacts of fenugreek induced hypoglycemia and hypokalaemia on health and performance are unknown and merit further investigation. Evidence in mice suggests that fenugreek supplementation is beneficial to exercise performance in the normoglycemic animal. In these mice, swim time to exhaustion was increased with four weeks of fenugreek supplementation (Ikeuchi et al., 2006). Thus, fenugreek may not only be effective for the treatment of diabetes, but may also serve as a potential ergogenic aid for the improvement of performance in athletes and healthy individuals.

Given the previous research, it is uncertain if increased muscle glycogen resynthesis during the early phase of recovery, with the addition of fenugreek during post-exercise carbohydrate feeding, would continue into the next day. Additionally, it is uncertain if increased muscle glycogen and/or other potential fenugreek actions would affect exercise performance on subsequent days. Thus, the purpose of this study was to investigate the effects of the addition of fenugreek to post-exercise carbohydrate feeding on muscle glycogen and subsequent exercise performance in normoglycemic trained male subjects.

Materials and methods

Subjects

Eight trained male cyclists participated in this study (see Table 1 for descriptive data). All subjects were given oral and written information about the experimental procedures and potential risks before giving written consent. All procedures conformed to the standards set forth by the *Declaration of Helsinki*, and the procedures were first approved by the University Institutional Review Board.

Preliminary testing

Peak VO_2 was measured for each subject using a graded exercise protocol (starting at 95 watts and increasing by 35 watts every 3 min) on an

Table 1. Subject descriptive data ($n = 8$)

Age (yr)	28.6 ± 9.6
Height (cm)	180.0 ± 6.2
Weight (kg)	75.2 ± 8.2
Body fat (%)	14.2 ± 3.4
Peak VO_2 ($ml \times kg^{-1} \times min^{-1}$)	63.1 ± 5.9
VO_2 at LT ($ml \times kg^{-1} \times min^{-1}$)	34.7 ± 4.6

Data are mean ± SD

Table 2. Amino acid profile and dose of the Fenugreek Supplement. Data were supplied by Technical Sourcing International, Missoula, MT and are relevant to the batch used in the current study

Amino acid	Relative dose ($mg \cdot kg^{-1}$ BW)	Absolute dose (mg)
Arginine	0.07 ± 0.007	5.18
Aspartate	0.11 ± 0.011	8.14
Threonine	0.02 ± 0.002	1.48
Serine	0.07 ± 0.007	5.18
Glutamate	0.16 ± 0.016	11.84
Glycine	0.06 ± 0.006	4.44
Alanine	0.10 ± 0.010	7.40
Cyseine	0.06 ± 0.006	4.44
Valine	0.03 ± 0.003	2.22
Methionine	0.01 ± 0.001	0.74
Isoleucine	0.02 ± 0.002	1.48
Leucine	0.02 ± 0.002	1.48
Phenylalanine	0.05 ± 0.005	3.70
Ornithine	0.01 ± 0.001	0.74
Lysine	0.01 ± 0.001	0.74
Histidine	0.01 ± 0.001	0.74
Tyrosine	0.04 ± 0.004	2.96
4-OH-Ile	1.99 ± 0.202	148.00

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%). Data are mean ± SD

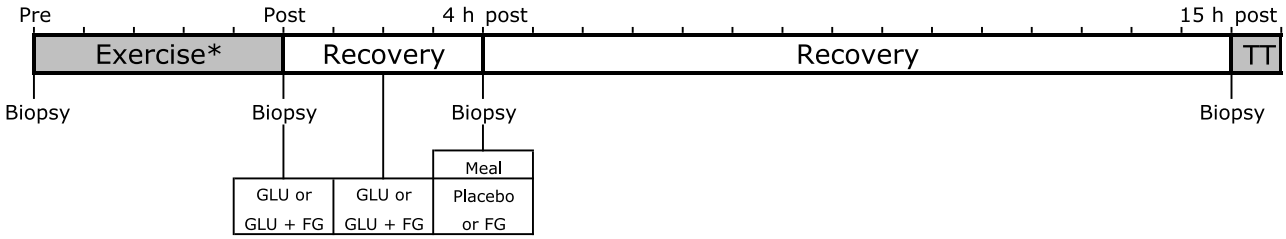

Fig. 1. Schematic overview of the study design. * Supplemented with sports drink at beginning of each hour and expired gases collected at end of each hour, *GLU* feedings of dextrose ($1.8\,g \cdot kg^{-1}$ BW) plus placebo capsules, *GLU + FG* feedings of dextrose ($1.8\,g \cdot kg^{-1}$ BW) and the experimental supplement extracted from Fenugreek seeds. *Meal* Comprised a standardized meal; *Placebo* placebo capsule; *FG* fenugreek capsule; *TT* simulated 40 km cycling time trial

electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA). Expired gases were collected during the test using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT) and analyzed at 15-second intervals. Body density was determined using hydrodensitometry and corrected for estimated residual lung volume. Net underwater weights were recorded using a digital scale (Exertech, Dresbach, MN). Body density was then converted to body composition using the Siri equation (Siri, 1993).

Design

Subjects completed a placebo controlled, double blind crossover design. The placebo trial (GLU) included feedings of dextrose ($1.8\,g \cdot kg^{-1}$ BW per feeding) plus placebo capsules (micro crystallized cellulose), and the experimental trial (GLU + FG) included feedings of dextrose ($1.8\,g \cdot kg^{-1}$ BW) and the experimental supplement (see Table 2) extracted from Fenugreek seeds (Technical Sourcing International, Missoula, MT). The feeding schedule was identical for GLU and GLU + FG trials and were isocaloric. Trial order was randomly assigned and counter-balanced with at least seven days between trials.

Experimental protocol

On the day of the trial, subjects arrived at the laboratory at 07.30 and consumed a standard breakfast containing 2854 kJ of energy (16.5 g fat, 117.1 g carbohydrate, and 17.1 g protein). The subjects relaxed in the lab until 10.00 to allow food to digest at which time a needle muscle biopsy from the *vastus lateralis* was performed (Pre). The subjects then cycled for 5 h at an intensity equal to 50% of their peak power output (52.1 ± 3.3% of VO_2 peak). During the exercise subjects were allowed to consume water *ad libitum* and were required to consume a volume of commercially available sports drink containing $0.12\,g \cdot kg^{-1}\,BW \cdot h^{-1}$ of carbohydrate at the beginning of each hour in an effort to better maintain a euglycemic state. During the final 10 min of each hour, expired gases were collected in order to monitor exercise intensity relative to individual VO_2 peak.

Immediately following the five hour cycling exercise bout a second biopsy of the *vastus lateralis* was obtained from a separate incision made approximately 2 cm proximal to the initial biopsy site on the same leg (Post). Immediately after and again 2 h following the post biopsy, each subject received either GLU or GLU + FG. Four hours following the post biopsy a third biopsy was taken (4 h Post) from a separate incision approximately 2 cm from the previous incision. The subjects then took another dose of placebo or experimental capsules along with a meal containing 3707 kJ of energy (22.3 g fat, 134.5 g carbohydrate, and 36.8 g protein). Subjects were allowed to consume provided snacks (3401 ± 1661 kJ of energy). The snacks consumed were recorded so that these same snacks were consumed during the subsequent trial. Therefore, each trial within an individual subject was isocaloric. Subjects were required to spend the night in the lab in order to closely control activity and diet.

On the following morning (15 h after the completion of the 5 h exercise bout), subjects awoke and a fourth muscle biopsy was obtained (15 h Post) from a separate incision on the same leg 2 cm proximal from the previous biopsy. At this time subjects completed a simulated flat 40 km time trial on the same Velotron cycle ergometer utilizing Racer Mate custom course software (RacerMate Inc., Seattle, WA). See Fig. 1 for a schematic overview of the study design.

Biopsies

For each trial, biopsies were taken from the *vastus lateralis* muscle of the same leg in a randomized order using a 4 mm Bergstrom percutaneous muscle biopsy needle (Bergstrom, 1962). Each successive biopsy on the same leg was obtained from a separate incision 2 cm proximal to the previous biopsy. After any excess blood and connective tissue or fat were removed, tissue samples were immersed in liquid nitrogen and stored at −80 °C for later analysis.

Muscle glycogen analysis

Muscle glycogen was analyzed using an enzymatic spectophotometric method. Samples were weighed (12.8 ± 4.6 mg wet weight) upon removal from a −80 °C freezer and placed in 0.5 ml, 2N HCl solution. The sample solutions were weighed, incubated for 2 h at 100 °C in an oven, re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5 ml of 0.67 NaOH was added. A volume of this muscle extract (20 µl) was added to 1 ml of infinity glucose (HK) liquid stable reagent (ThermoTrace Ltd) and read on a spectrophotometer at 340 nm. Muscle glycogen was then calculated using the extinction co-efficient of NADH. Muscle glycogen concentrations are expressed in $mmol \cdot kg^{-1}$ wet weight of muscle.

Statistics

Muscle glycogen was analyzed using a repeated measure ANOVA (trial × time). Time trial performance was analyzed using a two-tailed paired *t*-test. A probability of type I error less than 5% was considered significant ($p < 0.05$). All data are reported as means ± SD.

Results

Muscle glycogen

There was no trial × time interaction for the measure of muscle glycogen ($p > 0.05$), indicating that the rate of muscle glycogenolysis during the 5 h exercise and the rate of muscle glycogen resynthesis after exercise was similar between the GLU and GLU + FG trials. A main effect for

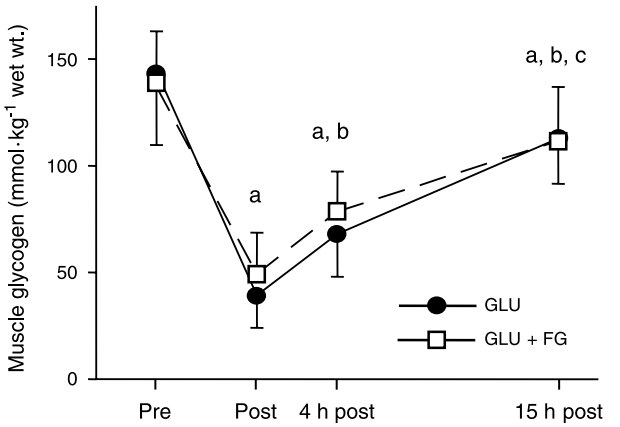

Fig. 2. Changes in muscle glycogen content with 5 h of cycling exercise (Pre-Post) and recovery (4 h Post, 15 h Post) in response to post exercise glucose (*GLU*) and glucose with fenugreek (*GLU + FG*) post exercise feedings. *a* $p < 0.05$ from pre (main effect for time); *b* $p < 0.05$ from post (main effect for time); *c* $p < 0.05$ from 4 h post (main effect for time). Data are mean ± SD

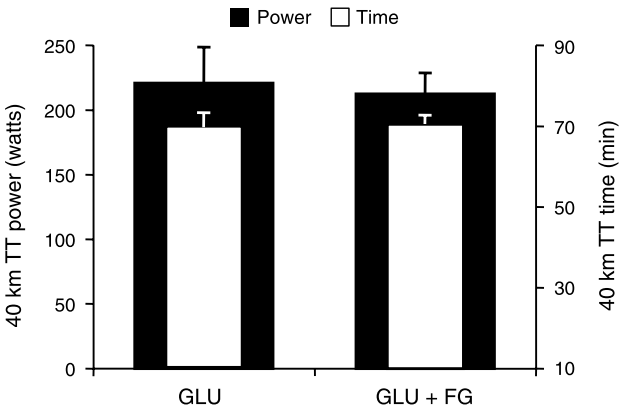

Fig. 3. Simulated 40 km time trial performance (left axis power, right axis time) with glucose (*GLU*) and glucose with fenugreek (*GLU + FG*) post exercise feedings. Data are mean ± SD

time was observed ($p > 0.05$), demonstrating that muscle glycogen decreased with the 5 h exercise bout and then was increased at 4 h post exercise and on the next morning (15 h post) after exercise. See Fig. 2.

Time trial performance

There was no difference ($p > 0.05$) in average power output (221 ± 28 vs. 213 ± 16 watts) or time to completion (69.7 ± 3.7 vs. 70.5 ± 2.2 min) of the simulated flat 40 km time trial between GLU and GLU + FG, respectively (Fig. 3).

Discussion

The current study was designed to simulate a common challenge that endurance athletes encounter. Time for optimal recovery is often not available during peak training periods and multi-day races. Our previous research indicated that the combination of fenugreek with carbohydrate promoted a 63% higher rate of post-exercise muscle glycogen resynthesis compared to carbohydrate alone (Ruby et al., 2005). The resynthesis of muscle glycogen is an important component of recovery for endurance athletes, thus our previous results would suggest that subsequent performance would also be increased. However, no marker of exercise performance was included in this earlier study. The purpose of the current study was to investigate the addition of fenugreek to post-exercise carbohydrate feedings on glycogen resynthesis and subsequent exercise performance in normoglycemic trained males. In light of the previous research on the beneficial effects of fenugreek, including our own, the novel findings of the current study were that fenugreek supplementation had no effect on glycogen resynthesis or on subsequent exercise performance in response to this exercise protocol.

It is not obvious to the authors why the current results differ from that of our previous study (Ruby et al., 2005). While the initial 4 h after exercise were identical between the studies, the exercise bout itself was quite different. The previous study included 90 min of high intensity cycling intervals, while the current study involved 5 h of low intensity steady state cycling. Additionally, the current study allowed subjects to consume small amounts of carbohydrate during exercise. It is interesting to note that, despite these differences in exercise protocol, the post exercise and the 4 h post-exercise muscle glycogen concentrations are similar in response to the placebo trial for each of the two studies.

The actions of fenugreek, when combined with high intensity exercise, may produce a synergistic effect to increase the rate of muscle glycogen resynthesis. Due to the broad range of reported effects of fenugreek, the mechanism of action is likely multi-faceted. A series of experiments by Vijayakumar et al. (2005) have characterized a mechanism of action for fenugreek aided glucose uptake. These experiments demonstrated that fenugreek led to increased tyrosine phosphorylation of the p85 subunit of phosphatidylinositol 3-kinase (PI3-K). The downstream target of PI3-K, protein kinase C (PKC), was further activated. However, no effect on the other downstream target of PI3-K, protein kinase B (Akt), was noted. In this model, fenugreek leads to increased glucose transport independent of Akt via increased fusion of GLUT4 vesicles that are within 250 nm of the plasma membrane (Gonzalez and McGraw, 2006). While little is known about the mechanism associated with contraction mediat-

ed GLUT4 translocation, insulin stimulated GLUT4 translocation is regulated by Akt (Gonzalez and McGraw, 2006). Akt phosphorylation and/or activity has been shown to increase as a result of exercise (Thorell et al., 1999; Turinsky and Damrau-Abney, 1999; Nader and Esser, 2001; Sakamoto et al., 2004). Moreover, the magnitude of the exercise induced increase in Akt may be a factor of exercise intensity (Sakamoto et al., 2004). Thus, increased Akt activity during high intensity exercise may increase GLUT4 translocation and thus increase glucose transport. Additionally, Akt has been implicated as a possible candidate for mediating glycogen synthesis (Peak et al., 1998). These factors lead to the possibility that increased Akt activity as a result of high intensity exercise, but not low intensity exercise, may be necessary to induce fenugreek promoted glucose transport and subsequent rate of muscle glycogen resynthesis. More studies are needed to clarify exercise induced glucose uptake signaling and the possible effects of fenugreek supplementation.

The current research used normoglycemic healthy male athletes given acute post-exercise doses of fenugreek. The majority of research involving fenugreek includes: 1) rodent studies, 2) chronic dosing, 3) the use of hyperglycemic (diabetic) models, and 4) no exercise intervention. Other than the previous report from our laboratory (Ruby et al., 2005), the only investigation in an acute normoglycemic human model reports a 13% decrease in blood glucose and a 14% decrease in potassium levels 4 h after fenugreek ingestion (Abdel-Barry et al., 2000). These authors noted that approximately one third of the subjects experienced symptoms such as feelings of hunger, increased micturition frequency, and dizziness. Furthermore, the decrease in blood potassium levels at rest may have ominous effects on the heart, nerves, and skeletal muscle. During exercise, the redistribution of potassium out of the blood and back into the muscle cells would be beneficial. Under normal exercise conditions, there is a net potassium release from contracting muscles. The resulting increased extracellular potassium and decreased intracellular potassium contributes to muscle fatigue by depolarizing the resting membrane potential (Clausen and Everts, 1991; Lindinger and Heigenhauser, 1991). If fenugreek exerts an effect on the Na/K ATPase to redistribute potassium to intracellular compartments muscular fatigue may be reduced. Swim time to exhaustion in mice has been shown to increase with fenugreek supplementation (Ikeuchi et al., 2006). While potassium levels were not measured, it was concluded that swim time to exhaustion was improved due, at least partially, to increased fat utilization.

In the current study, performance was not improved with fenugreek supplementation. The performance trial was conducted 11 h after the last dose of fenugreek was administered. This lapse of time may be too long in order for the acute reduction in potassium to have an effect, while the acute doses of fenugreek supplementation may not have been continued long enough for the chronic effect of increased fat utilization to affect exercise performance. We had hypothesized that increased glycogen availability in the GLU + FG group would lead to improved exercise performance. However, since muscle glycogen stores were similar between GLU and GLU + FG, exercise performance did not improve.

In summary, the current research demonstrated no effect with the addition of fenugreek to post-exercise glucose feeding on muscle glycogen resynthesis or exercise performance. The contrast of this with previous work (Ruby et al., 2005) may be partially explained by the differences in exercise intensity and duration and their effect on cellular signaling cascades, ultimately leading to glucose transport and subsequent glycogen synthesis. Alternatively, the outcome of this or our previous study may have been fortuitous. However, the impressive array of reported effects of fenugreek are difficult to ignore and it is possible that effects should be interpreted with specificity and not generalized. More research is needed in order to understand the interactions that fenugreek may have in the normoglycemic exercising human and the conditions required to induce physiological effects.

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