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A fenugreek seed extract selectively reduces spontaneous fat intake in overweight subjects

Hugues Chevassus[1,2], Jean-Baptiste Gaillard[3], Anne Farret[1,2], Françoise Costa[1,2], Isabelle Gabillaud[1,2], Emilie Mas[3], Anne-Marie Dupuy[3], Françoise Michel[3], Caroline Cantié[1,2], Eric Renard[4,5], Florence Galtier[1,2,5], Pierre Petit[1,2,5].

[(1)]*CHRU Montpellier, Centre d'Investigation Clinique, Montpellier, France*

[(2)]*INSERM, CIC 0001, Montpellier, France*

[(3)]*CHRU Montpellier, Laboratoire de Biochimie, Montpellier, France*

[(4)]*CHRU Montpellier, Service des Maladies Endocriniennes, Montpellier, France*

[(5)]*Université Montpellier I and CNRS UMR 5232, Centre de Pharmacologie et Innovation dans le Diabète, Montpellier, France*

Corresponding author: Hugues Chevassus, h-chevassus@chu-montpellier.fr

Centre d'Investigation Clinique INSERM-CHRU, Hôpital Saint-Eloi, 80 avenue Augustin Fliche, 34295 Montpellier Cedex 5, France

Telephone number: +33 (0)4 67 33 23 25 Fax number: +33 (0)4 67 33 23 35

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Abstract

Purpose - Fenugreek seeds (*Trigonella foenum-graecum* L.) have long been used as herbal medicine for metabolic and nutritive dysfunctions. They were shown to modulate feeding behaviour in animals. We have recently observed a selective decrease in fat consumption in normal weight healthy volunteers after treatment by a hydro-alcoholic seed extract. However, strong clinical data about the effects of fenugreek seeds on energy intake is lacking, especially in overweight individuals. The present study aimed at investigating the effects of a repeated administration of a fenugreek seed extract on eating behaviour in overweight subjects.

Methods - Thirty-nine overweight healthy male volunteers completed a 6-week double-blind randomized placebo-controlled parallel trial of a fixed dose of a fenugreek seed extract. Main endpoints were energy intake (dietary records and meal test), weight, fasting and post-absorptive glucose and insulin, appetite/satiety scores and oxidative parameters.

Results - Daily fat consumption, expressed as the ratio fat reported energy intake / total energy expenditure (fat-REI/TEE) was significantly decreased by the fenugreek seed extract in overweight subjects (fat-REI/TEE: 0.26 ± 0.02 *vs* 0.30 ± 0.01; $P = 0.032$). We also observed a significant decrease of the insulin/glucose ratio in the subjects treated with fenugreek seed extract (0.89 ± 0.09 *vs* 1.06 ± 0.10 mUI/mmol; $P = 0.044$). No significant effect was observed concerning weight, appetite/satiety scores, or oxidative parameters.

Conclusion - The repeated administration of a fenugreek seed extract slightly but significantly decreased dietary fat consumption in healthy overweight subjects in this short-term study.

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an herbaceous annual plant from the family of *Leguminosae*, cultivated in Mediterranean countries and India. Its seeds have been known for a long time as herbal medicine for various pathological conditions. A number of pharmacological and clinical studies have shown glucose and lipid lowering properties of either the seed itself, seed extracts or purified components (see [1] for review). In addition, antioxidant properties have been observed in animal studies [2, 3].

Fenugreek-based preparations are used in humans to stimulate appetite and promote weight gain [4]. However, these properties are supported only by animal data, which show some inconsistencies. We have previously demonstrated that a repeated administration of a hydro-alcoholic seed extract enhanced motivation to eat and food consumption in rats, with a slight weight increase [5]. Appetite and food intake were also increased by a subchronic treatment with a purified steroid saponin fraction from the seed, with a modified circadian rhythm of feeding behaviour [6]. In contrast, in a safety study using a dietary supplement with fenugreek seeds in animals [7], no effect was observed on food consumption. More recently, it was found that a fenugreek seed extract reduced the body weight gain induced by a high-fat diet in obese mice [8]. However, no clinical data on body weight and food intake was available since our recent report showing a reduction of fat consumption in free-living conditions in healthy normal weight volunteers, after a 14-day repeated administration of a fenugreek seed extract [9].

As the latter property could be of interest in overweight subjects, we undertook the present study to investigate the effects of this fenugreek seed extract on fat intake in overweight patients. Secondary objectives included weight, glycaemia, plasma insulin, lipid profile, as well as oxidative stress and antioxidant capacities, which improvements with fenugreek seeds have been demonstrated in animals, but never shown in humans.

Materials and methods

Study design

The study was designed as a 6-week double-blind randomized placebo-controlled parallel trial.

Subjects

Thirty-nine healthy overweight male volunteers, aged 18-59 years (mean: 38 years), completed this study. All were of stable weight (mean weight: 85.4 kg; range: 75.2-105.5; mean body mass index: 27.3 $kg.m^{-2}$; range:

24.9-29.4). One subject of the 40 included was withdrawn of the study before the first experimental drug administration because of the consumption of a non-authorized treatment.

Ethical aspects

This study was approved by the Ethics Committee "Comité de Protection des Personnes Montpellier-Saint-Eloi" and conducted in accordance with the Helsinki declaration and the ICH guideline for Good Clinical Practice. All the subjects gave written informed consent to participate.

Test compound

The test compound was a marketed dry hydro-alcoholic fenugreek seed extract administered three times daily as oral coated tablets coming from the batch used in our previous study conducted in healthy normal weight volunteers [9]. The total daily dose of 1176 mg (approximately 14 $mg.kg^{-1}$) is the double of the extract's daily dose commonly prescribed in human. It was selected because it appeared to be the active dose in healthy volunteers [9]. This dose is also in accordance with the two active doses used in the animal study testing the same extract [5], corresponding to 5 and 53 $mg.kg^{-1}$ human equivalent doses. The extract contained diosgenine, steroid saponins, 1.38% trigonelline and 1.50% 4-hydroxyisoleucine, as characterized by thin-layer chromatography and high performance liquid chromatography. Placebo tablets were manufactured using the same excipients, the same process and the same packaging as for active tablets making them indistinguishable. The similarity between the seed extract and the placebo tablets was verified on 5 criteria (aspect, size, weight, colour and smell).

Investigations

Diet and physical activity were assessed in free-living conditions before and at the end of the ambulatory treatment period, using a 7-day record, accurately reviewed by a trained dietician and a physician. Reported energy intake (REI) was determined with Enkal-Pro® software (Loging Software, Lille, France). Total energy expenditure (TEE) was calculated as basal metabolic rate (BMR) multiplied by physical activity level (PAL) according to Black *et al* [10]. Energy intake was expressed as the ratio REI/TEE. Underreporting was defined as a ratio REI/BMR < 1.1 [11].

The day following the first 7-day record completion, subjects attended the study centre for a baseline visit in fasting condition. Body weight was recorded while the subjects were dressed in underwear and after they had

urinated, using the same balance Seca® 861 (Seca, Hamburg, Germany) with an accuracy of ± 0.1 g. Body composition was measured by a multi-frequency bioelectrical impedance analysis. Blood was drawn and urine was collected for determination of plasma glucose, serum insulin and lipid profile, oxidative stress and antioxidant capacities. They were then discharged and treatment was taken ambulatory. During the last week of treatment, subjects completed a second 7-day record, and then attended the study centre after an overnight fast for their final visit, which comprised all the baseline evaluations. In addition, they had to consume completely in a maximum of 30 min a standardized breakfast (13% protein, 35% fat, 52% carbohydrate) providing 20% of their daily energy requirements, and to describe their appetite, satiety and palatability feelings on visual analogue scales [12, 13] before and every 30 min for 270 min after starting eating breakfast. At 12:30 a.m., they were served an *ad libitum* mixed meal consisting of 13% protein, 35 % fat, 52 % carbohydrate, and the amount of food ingested was weighed with an accuracy of ± 0.1 g.

Analytical methods

Plasma glucose was assayed by the glucose oxidase method. Serum insulin was measured by RIA (BI-INSULIN IRMA, CIS Bio-international, Gif-sur-Yvette, France). Serum triglycerides, total cholesterol and high-density lipoprotein (HDL)-cholesterol were assayed with an enzymatic method (lipase/glycerokinase, cholesterol oxidase and cholesterol oxidase after separation respectively).

Antioxidant parameters were total plasma antioxidant capacity (quantitative colorimetric technique), low density lipoproteines (LDL) susceptibility to oxidation, serum and LDL vitamin E (HPLC), and plasma vitamin C (HPLC). LDL were obtained by plasma sequential ultracentrifugation with a potassium bromide-adjusted density of 1.063. LDL oxidation was initiated with Copper [Cu (II), 5 µM] and continuously monitored during 6 hours by measuring the increase in absorbance at 234 nm due to conjugated diene formation. LDL susceptibility to oxidation was expressed by the reaction lag phase time, the maximal propagation rate, the time of half oxidation and the slope of the propagation phase. Oxidative stress was assessed by plasma malonyldialdéhyde [14], plasma advanced oxidation protein products [15], plasma advanced glycation end products (fluorescence spectroscopy) and the urinary ratio free 8-iso PGF_2 (LC-MS/MS)/creatinine.

Data analysis and statistics

The sample size (40 subjects to be enrolled in 2 groups of 20) was calculated using data obtained in our previous study [9], with an expected mean difference for energy consumption (main outcome) between fenugreek seed

extract and placebo of 216 kcal per day, a common SD of 238 kcal par day, a two-sided alpha of 0.05 and a statistical power of 80%.

Results are expressed as the mean ± standard error of the mean (SEM). 95% Confidence intervals for the differences (95% CI) are given where necessary.

Means were compared using analysis of covariance (ANCOVA) with baseline data as covariate, after logarithmic transformation if necessary. Non parametric data were compared using the Mann-Whitney test.

Calculations and statistical analysis were performed using the Systat® 10.0 software for Windows® (SPSS, Chicago, IL). The level of significance was set at $P < 0.05$.

Results

Underreporting was similar between fenugreek and placebo groups (38.9% (7/18) *vs* 40% (8/20), NS). As a result, total-REI was lower than daily TEE in both groups (basal ratio total-REI/TEE: 0.74 ± 0.05 (n = 18), and 0.73 ± 0.03 (n = 20) respectively, NS). At baseline, macronutrient intake was similar between fenugreek and placebo groups (Figure 1, panel A). After 6 weeks of treatment with the fenugreek seed extract, a significant decrease in TEE-adjusted reported fat intake was observed (post treatment fat-REI/TEE: 0.26 ± 0.02 (n = 18) *vs* 0.30 ± 0.01 (n = 20), $P = 0.032$), while protein and carbohydrate intakes were not significantly modified (Figure 1, panel B). Total-REI was not significantly modified by the treatment (post treatment total-REI/TEE: 0.68 ± 0.05 (n = 18) *vs* 0.73 ± 0.03 (n = 20), NS). Spontaneous food intake during the *ad libitum* meal test, weight, body composition, appetite and satiety scores did not differ between treated and untreated subjects.

The main biological parameters are described in tables I and II. The ratio of fasting serum insulin/plasma glucose was significantly decreased in subjects treated with fenugreek seed extract (0.89 ± 0.09 (n = 19) *vs* 1.06 ± 0.10 (n = 19) $mUI.mmol^{-1}$, $P = 0.044$) (Table I). No effect on plasma lipid profile (Table I), antioxidant parameters and oxidative stress markers (Table II) could be observed.

No serious adverse events occurred. Among the 35 non serious adverse events reported (17 with fenugreek, 18 with placebo), only 5 might be related to the experimental treatment: 4 cases of mild gastro-intestinal symptoms and 1 case of specific urine and sweat smell.

Discussion

The present report shows for the first time that a treatment with a fenugreek seed extract selectively reduces spontaneous fat consumption in overweight male subjects. We also show that the fenugreek seed extract does not

increase food consumption. This latter result differs from the traditional use of fenugreek-based preparations for appetite stimulation and weight gain [4]. It also contrasts with our previous observations of enhanced food consumption in animals treated by a hydro-alcoholic crude extract or a purified steroid saponin fraction [5, 6]. However, this observation is in accordance with the results of our previous investigation in normal weight healthy subjects [9].

This effect has been observed with a reliable evaluation of energy intake in free-living conditions i.e. baseline and post-treatment 7-day records, adjustment on physical activity, systematic dietician review. While self-reported dietary records may underreport energy intake, they remain an important tool for nutrition researchers and a record of 7 consecutive days is considered reliable to quantify diet for outpatients [16]. As expected in an overweight population [17], underreporting of energy intake was frequent (≈ 40%), but similar in treated and control groups, which were initially comparable.

The effect on fat consumption could not appear in standardized conditions because the meal test used was composed of a single course that didn't allow any dietary choice.

Such a specific effect, without any significant change in the intake of other nutrients, and without any alteration of appetite or palatability, has already been observed in pharmacological studies undertaken in rats by activation of the hypothalamic serotonin pathway [18], and in human after a methylphenidate challenge activating the brain dopamine pathway by blocking its reuptake [19].

The moderate magnitude of this effect (- 10%) made it unlikely to induce a decrease in weight over short periods, and indeed we did not observe any modifications in body weight or body composition. It is also worthy to notice that no significant change in body weight was observed by Sharma et al. [20] in a clinical study evaluating the safety of fenugreek seeds consumed in diabetic patients, although their study was not designed to assess eating behaviour or weight modifications. Nevertheless, it can be hypothesized that fenugreek seed extract may favour a weight reduction in the long term, particularly in overweight or obese patients for whom a low-fat diet is recommended, and thus improve the metabolic status of these subjects.

The lower ratio of fasting serum insulin/plasma glucose may reflect an improved insulin sensitivity, as previously described with fenugreek seed preparations in type 2 diabetes [21]. This effect may be related to 4-hydroxyisoleucine, an amino acid present in fenugreek seeds, known for its insulin-sensitizing effect [22]. However, even if this biological mechanism would support the hypothesis of an improved insulin sensitivity in our sample, fasting glucose and insulin values (and related calculated indexes) are insufficient to draw such a conclusion, and specific investigations such as clamp studies would be required. As fenugreek is traditionally

used to treat type 2 diabetic patients in Mediterranean and Asian countries, our results provide a rationale for conducting further research on the potential usefulness of fenugreek in disorders of carbohydrate metabolism.

In spite of an extensive exploration, we observed no significant effect on plasma antioxidant capacity or oxidative stress, which were ancillary biological objectives. This result is in contrast with previously reported data in rats [2] and mice [3]. This might be related to our experimental conditions. First, our population was characterized by a normal baseline oxidative/antioxidant profile, and antioxidant properties are easier to detect in conditions of increased oxidative stress. Second, our sample size may have been insufficient to show a significant difference on these parameters. Alternatively, it is possible that the antioxidant properties shown in animal studies are not present in human.

Finally, it has to be noticed that the treatment with the fenugreek seed extract did not modify the subjects' lipid profile, which also may be explained by the healthy status of the subjects.

Although fenugreek seed extract is coloured and displays a specific smell, active and placebo tablets could not be identified by the investigators and the subjects, thanks to the coating process making them indistinguishable. Nevertheless, one subject detected an unusual smell in his urine and sweat during the treatment period without having expressly been interpreted as treatment-related. This unique case of possible identification of the treatment received by the subject did not conduct to any de-blinding for the rest of the volunteers. Considering this problem, the occurrence of de-blinding has to be systematically tested in clinical studies investigating fenugreek seed preparations.

Conclusion

The repeated administration of a fenugreek seed extract slightly but significantly decreased dietary fat consumption in human in this short-term study; this new property, together with a potential favourable effect on insulin sensitivity, may be helpful in subjects who need to decrease their fat intake, and may improve the metabolic status of overweight subjects. The fenugreek seed's component(s) involved and the mechanism(s) of this effect remain to be elucidated.

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Table I

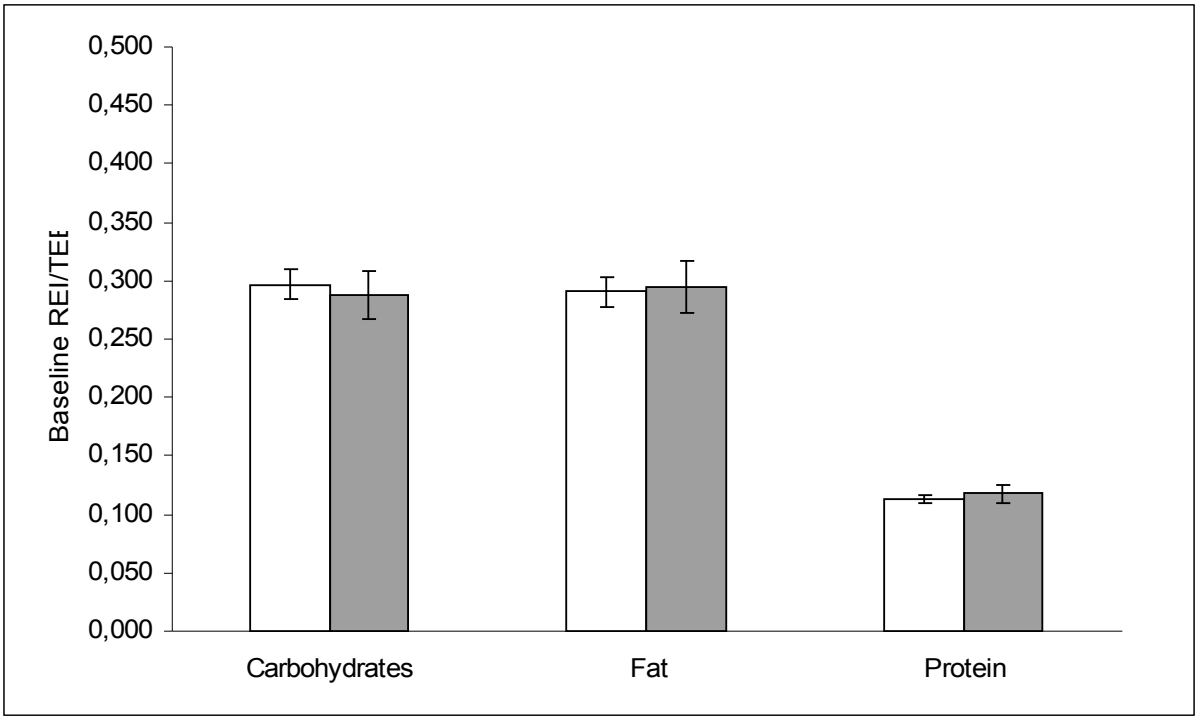
Main metabolic parameters	Fenugreek	Placebo	P
Fasting plasma glucose ($mmol.L^{-1}$)			
- Baseline	4.61 ± 0.21	4.87 ± 0.19	0.355
- Post-treatment	5.38 ± 0.10	5.26 ± 0.16	0.545
Fasting serum insulin ($mU.L^{-1}$)			
- Baseline	5.10 ± 0.41	5.02 ± 0.31	0.887
- Post-treatment	4.73 ± 0.43	5.38 ± 0.36	**0.057**
Fasting insulin/glucose ratio ($mU.mmol^{-1}$)			
- Baseline	1.17 ± 0.13	1.07 ± 0.08	0.708
- Post-treatment	0.89 ± 0.09	1.06 ± 0.10	**0.044**
Total cholesterol ($mmol.L^{-1}$)			
- Baseline	4.82 ± 0.26	5.19 ± 0.19	0.254
- Post-treatment	4.88 ± 0.25	5.06 ± 0.20	0.207
HDL-cholesterol ($mmol.L^{-1}$)			
- Baseline	1.27 ± 0.05	1.22 ± 0.08	0.555
- Post-treatment	1.30 ± 0.05	1.17 ± 0.07	0.067
Triglycerides ($mmol.L^{-1}$)			
- Baseline	1.25 ± 0.15	1.15 ± 0.11	0.732
- Post-treatment	1.27 ± 0.16	1.41 ± 0.14	0.148

Table II

Antioxidant status	Fenugreek	Placebo	P
Total plasma antioxidant capacity (mmol.L^{-1})			
- Baseline	1.48 ± 0.03	1.51 ± 0.03	0.572
- Post-treatment	1.51 ± 0.04	1.49 ± 0.04	0.138
LDL susceptibility to oxidation : lag phase time (min)			
- Baseline	16.2 ± 1.7	15.5 ± 0.8	0.826
- Post-treatment	15.3 ± 0.6	14.8 ± 0.7	0.546
LDL susceptibility to oxidation : slope of the propagation phase (OD.min^{-1})			
- Baseline	0.017 ± 0.001	0.018 ± 0.001	0.419
- Post-treatment	0.018 ± 0.001	0.020 ± 0.001	0.264
LDL susceptibility to oxidation : maximal propagation rate (OD.min^{-1})			
- Baseline	0.019 ± 0.001	0.020 ± 0.001	0.339
- Post-treatment	0.020 ± 0.001	0.021 ± 0.001	0.647
LDL susceptibility to oxidation : half-time of maximum diene formation (min)			
- Baseline	35.4 ± 2.6	34.9 ± 1.2	0.788
- Post-treatment	33.9 ± 1.0	34.1 ± 1.4	0.877
Serum vitamin E (µmol.L^{-1})			
- Baseline	27.9 ± 1.6	27.3 ± 0.9	0.752
- Post-treatment	28.1 ± 1.8	27.7 ± 1.2	0.870
LDL vitamin E (mg.L^{-1} of LDL)			
- Baseline	10.9 ± 0.9	12.3 ± 0.6	0.210
- Post-treatment	12.4 ± 1.1	11.8 ± 0.77	0.220
Plasma vitamin C (µmol.L^{-1})			
- Baseline	42.2 ± 4.0	52.7 ± 4.1	0.071
- Post-treatment	43.9 ± 4.0	52.1 ± 4.3	0.762
Plasma malonyldialdehyde (µmol.L^{-1})			
- Baseline	0.89 ± 0.07	0.85 ± 0.05	0.758
- Post-treatment	0.85 ± 0.06	0.82 ± 0.05	0.841
Urine F2-isoprostanes/creatinine (pmol.$mmol^{-1}$)			
- Baseline	307 ± 31	478 ± 55	0.004
- Post-treatment	357 ± 54	510 ± 82	0.704
Plasma advanced glycation end products			
- Baseline	13887 ± 982	12302 ± 445	0.261
- Post-treatment	12737 ± 697	12204 ± 478	0.804
Plasma advanced oxidation protein products(µmol.L^{-1})			
- Baseline	57.9 ± 5.5	61.4 ± 3.4	0.592
- Post-treatment	54.0 ± 6.9	61.6 ± 5.3	0.504

Figure 1

A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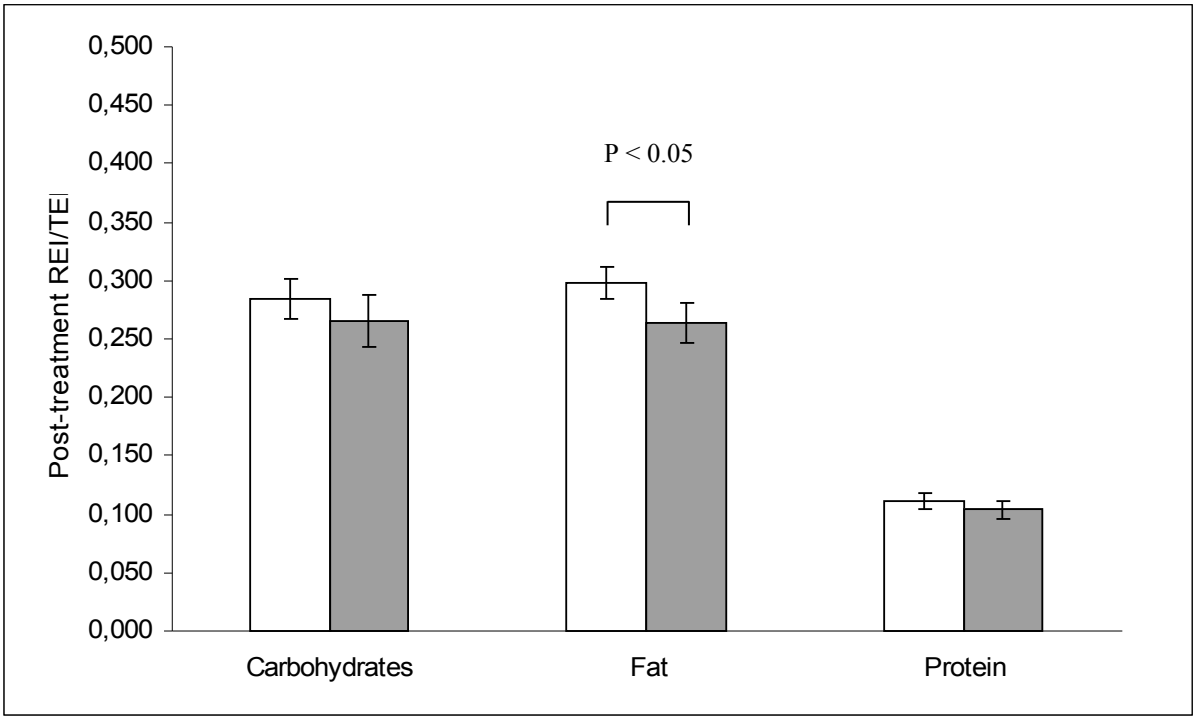
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Post-treatment REI/TEI
P < 0.05
Carbohydrates
Fat
Protein

Legends

Figure 1

Ratio energy intake/energy expenditure (REI/TEE) recorded by overweight healthy subjects in free-living conditions, before treatment (baseline data, panel A), and during the 7 last days of the 6-week treatment period (post-treatment data, panel B) for placebo group (white bars) and fenugreek seeds extract 1176 mg/day group (striped bars)

Table I

Comparison of fasting data of plasma glucose, serum insulin and lipid profile between fenugreek seed extract 1176 mg/day and placebo in overweight subjects

Table II

Comparison of antioxidant status between fenugreek seed extract 1176 mg/day and placebo in overweight subjects