Carob Pulp Preparation Rich in Insoluble Dietary Fiber and Polyphenols Enhances Lipid Oxidation and Lowers Postprandial Acylated Ghrelin in Humans

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ABSTRACT Ghrelin is an orexigenic hormone that may affect substrate utilization in humans. Ghrelin is influenced by macronutrients, but the effects of insoluble dietary fiber and polyphenols are unknown. We investigated the effects of a polyphenol-rich insoluble dietary fiber preparation from carob pulp (carob fiber) on postprandial ghrelin responses and substrate utilization. Dose-dependent effects of the consumption of carob fiber were investigated in a randomized, single-blind, crossover study in 20 healthy subjects, aged 22–62 y. Plasma total and acylated ghrelin, triglycerides, and serum insulin and nonesterified fatty acids (NEFA) levels were repeatedly assessed before and after ingestion of an isocaloric standardized liquid meal with 0, 5, 10, or 20 g of carob fiber over a 300-min period. The respiratory quotient (RQ) was determined after consumption of 0 or 20 g of carob fiber. Carob fiber intake lowered acylated ghrelin to 49.1%, triglycerides to 97.2%, and NEFA to 67.2% compared with the control meal ($P < 0.001$). Total ghrelin and insulin concentrations were not affected by consumption of a carob fiber–enriched liquid meal. Postprandial energy expenditure was increased by 42.3% and RQ was reduced by 99.9% after a liquid meal with carob fiber compared with a control meal ($P < 0.001$). We showed that the consumption of a carob pulp preparation, an insoluble dietary fiber rich in polyphenols, decreases postprandial responses of acylated ghrelin, triglycerides, and NEFA and alters RQ, suggesting a change toward increased fatty acid oxidation. These results indicate that carob fiber might exert beneficial effects in energy intake and body weight. J. Nutr. 136: 1533–1538, 2006.

KEY WORDS: • *carob* • *insoluble dietary fiber* • *polyphenols* • *ghrelin* • *respiratory quotient*

Dietary fiber consumption has been suggested to influence hunger, satiety, and energy intake in humans (1,2). Dietary fiber is a complex group of substances, commonly divided into soluble and insoluble fibers. Soluble fiber prolongs gastric emptying and macronutrient absorption, which is linked to delayed hunger feelings and decreased energy intake (1,3). Soluble fiber was shown to improve glucose homeostasis and lipid profile (4,5) and has been associated with short-term reduced energy intake in obese adults (3). Insoluble dietary fiber increased the rate of glucose disappearance (6) and improved carbohydrate handling (7). The effects of dietary fiber consumption on body weight management may be related to gut hormones, which regulate satiety and energy intake (1). Ghrelin is a peptide hormone produced and excreted mainly in the stomach (8), and circulating in 2 major forms, acylated ghrelin and desacyl ghrelin (9,10). Acylated ghrelin acts as an orexigenic signal to the central nervous system, with increased levels during fasting, and suppressed levels postprandially (11,12). The administration of ghrelin induces body weight gain in rodents by promoting food intake and decreasing fat utilization (8,13). In rodents, ghrelin administration increased the respiratory quotient (RQ),[2] suggesting decreased fatty acid oxidation and increased glycolysis (11,13). Carob pulp preparation (carob fiber), derived from the bean-like fruits of *Ceratonia siliqua*, is rich in insoluble dietary fiber and polyphenols. In humans, consumption of carob fiber was shown to have a high antioxidant capacity (14) and to lower serum cholesterol and serum triglycerides (15). Furthermore, other studies showed that polyphenols may increase fat oxidation and energy expenditure in humans (16) and in mice (17). Therefore, carob fiber may exert beneficial effects on postprandial lipid metabolism and substrate utilization potentially related to the secretion of gut hormones.

Hence, the aim of this study was to evaluate the postprandial effects of polyphenol-rich insoluble dietary fiber on circulating ghrelin levels, plasma triglycerides, nonesterified fatty acids (NEFA), and substrate utilization in humans.

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[2] Abbreviations used: IAUC incremental area under the curve; NEFA, nonesterified fatty acids; RQ, respiratory quotient.

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MATERIALS AND METHODS

Study design and subjects. The study was designed as a randomized crossover trial consisting of 4 sessions lasting 300 min each, and separated by intervals of at least 1 wk. Healthy adults ($n = 20$; 9 men, 11 women) were recruited for the study. Exclusion criteria included a history of chronic diseases, dyslipidemia, impaired glucose tolerance, intentional weight loss within 3 mo before the study, extreme sports, history of chronic alcohol abuse, and the use of any medication influencing glucose or lipid metabolism. The ethics committee of the University of Potsdam approved the study protocol. All volunteers gave written informed consent before starting the study. In the morning of every study day after a 10-h overnight fast, subjects consumed 400 mL of a standardized liquid meal (Pfrimmer-Nutricia) within <5 min. The energy content of the meal was 2520 kJ with a nutrient content of 73.6 g carbohydrates, 24 g protein, and 23.2 g fat; the meal was free of dietary fiber. Liquid meals were enriched with 0, 5, 10, or 20 g of carob fiber and provided in random order. The carob fiber contained 5.8 g simple carbohydrates, 5.2 g protein, and 0.2 g fat/100 g. The total fiber content was 74.6 g/100 g carob fiber corresponding to 68.4 g insoluble and 6.2 g soluble fiber. The total polyphenol content of the preparation was 2.8 g/100 g. The major polyphenol compounds of carob fiber are gallic acid, gallotannins, and flavonol glycosides (18). The energy contribution of the carob fiber enrichment was negligible with respect to the total energy intake of the liquid meal (0.4% for 5 g, 0.8% for 10 g, and 1.2% for 20 g carob fiber), with no differences among treatments. Participants were unaware of the fiber content of the test meals. However, it was possible to distinguish by taste between meals containing carob fiber vs. placebo. In a substudy, substrate utilization (oxygen consumption and carbon dioxide production) after consumption of a liquid meal with or without 20 g carob fiber was assessed in random order in 9 women.

Habitual alcohol intake. Alcohol intake of the study participants was assessed 3 times by a semiquantitative, self-administered food record for 4 consecutive days before and during the study. The results of the 3 food records were averaged. Trained staff delivered the record and subjects were instructed to record their entire food intake at the time of the consumption. The coding of alcohol intake was done on the basis of the German Food Code and Nutrient Data Base BLS II.3 (19). The accuracy and validity of energy intake estimated by the food record was validated against total energy expenditure estimated by doubly labeled water technique (20).

Body composition. Anthropometric data of the participants were collected at the beginning of the study. Body weight was assessed using an electronic scale calibrated to the nearest 0.1 kg (Soehnle). Body height was determined with a GPM anthropometer (Siber and Hegner) to the nearest 0.1 cm. BMI was calculated as body weight (kg)/height $(m)^2$.

Blood sampling and analyses. In the main study, an indwelling catheter was inserted into an antecubital vein, and blood samples were collected before and 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 270, and 300 min after administration of the test meal. During each session, a total of 180 mL blood was collected. Postprandial measurements were made within a 300-min time period to ensure complete meal absorption independently of the carob fiber enrichment as a routine procedure comparable to other studies (21). For plasma separation, blood was collected in EDTA-containing tubes. Tubes were immediately centrifuged at 1500 × g for 10 min at 4°C; the plasma supernatant was obtained and stored at −40°C until analyzed. For the measurement of total and acylated ghrelin, plasma was collected in tubes containing EDTA and 500 kU aprotinin/L whole blood (Bayer). For stabilization, the plasma supernatant was acidified with 1 mol/L HCl (Merck) and stored at −40°C. To obtain serum, blood was collected in tubes containing a serum clot activator. After clotting, samples were centrifuged at 1500 × g for 10 min at 4°C, and the serum was collected and stored at −40°C.

Plasma total ghrelin was measured by a commercial RIA, which utilizes ^{125}I-labeled ghrelin and a ghrelin antiserum to determine the level of total ghrelin in plasma by a double antibody/polyethylene glycol technique (GHRT-89HK, Linco Research). The sensitivity of the method was 93 ng/L. There was no cross-reaction with ghrelin 1–10, motilin-related peptide, glucagon, leptin, or insulin. The intra- and interassay CV were 10.0 and 14.7%, respectively. The percentage recovery was 96%. Plasma active ghrelin was determined using a RIA that utilizes ^{125}I-labeled ghrelin as a tracer and an antibody (raised in guinea pigs) that is specific for the biological active form of ghrelin with the octanoyl group on serine 3 (GHRA-88HK, Linco Research). The sensitivity of the method was 7.8 ng/L. There was no cross-reaction with ghrelin 14–28, motilin-related peptide, glucagon, leptin, or insulin. The intra- and interassay CV were 6.7 and 9.6%, respectively. The percentage recovery was 114%. Plasma glucose was measured in duplicate by a hexokinase method using commercially available colorimetric reagents (ADVIA® 1650 Chemistry System, GLUH, Bayer) with an intra-assay CV of 1.2%. Serum insulin was determined by ADVIA Centaur Immunoassay (Bayer) with an intra-assay CV of 2.6%.

Plasma triglycerides were analyzed using enzymatic colorimetric agents TRIG for ADVIA Chemistry System 1650 (Bayer). The intra-assay CV was 1.2%. Serum NEFA were analyzed using an enzymatic colorimetric method (ASC-ACOD method, Wako Chemicals) with an interassay CV of 2.7%. Serum total cholesterol was analyzed using CHOD/PAP method (ABX Diagnostics Cholesterol, ABX Diagnostics) with an intra-assay CV of 0.82%. Serum HDL cholesterol was analyzed using a colorimetric test (ABX Diagnostics HDL Cholesterol Direct, ABX Diagnostics) with an intra-assay CV of 1.29%. LDL cholesterol was calculated according to Friedewald (22).

Indirect calorimetry. Energy expenditure and substrate oxidation were assessed by indirect calorimetry using a respiratory chamber (23). Oxygen consumption and carbon dioxide production were measured. After a run-in period of at least 20 min, resting energy expenditure was determined for 60 min. After ingestion of 400 mL of the liquid meal, containing either 0 or 20 g of carob fiber, measurement was continued for another 240 min. Throughout the measurements, the subjects remained seated. Energy expenditure and substrate oxidation rates were calculated according to Ferrannini (24).

Statistical analyses. All statistical analyses were performed using SPSS for Windows 11.5. Baseline characteristics are shown as means ± SD; figures show means ± SEM. Time series data for all variables and for each subject were normalized to baseline values, which was defined as time = 0 min. Differences in responses to the liquid meals were tested by parametric ANOVA with time, carob concentration, and carob concentration × time as fixed factors and subject as a random factor. Statistical differences in the response of the blood variables during the examinations were analyzed using ANOVA. Total postprandial energy expenditure and RQ after consumption of liquid meals with or without carob were compared using paired Student's t test. A probability $P < 0.05$ was considered to be significant. The percentage effects of liquid meals enriched with carob were calculated using liquid meals without carob as a reference. The mean percentage increase or decrease was calculated for the highest or lowest time

TABLE 1

General characteristics and fasting blood variables of the study population[1]

Characteristic	
n	20
Age, *y*	29.4 ± 11.5
BMI, *kg/m²*	23.0 ± 2.1
Plasma glucose, *mmol/L*	4.9 ± 0.4
Serum insulin, *pmol/L*	42.4 ± 18.7
HOMA-IR	1.4 ± 0.6
Plasma triglycerides, *mmol/L*	1.1 ± 0.4
Serum total cholesterol, *mmol/L*	4.5 ± 1.0
Serum HDL cholesterol, *mmol/L*	1.3 ± 0.3
Serum LDL cholesterol, *mmol/L*	2.7 ± 0.8
Serum NEFA, *μmol/L*	404.7 ± 178.6
Plasma total ghrelin, *ng/L*	1102.0 ± 206.7
Plasma acylated ghrelin, *ng/L*	73.2 ± 34.1
Plasma nonacylated ghrelin, *ng/L*	1028.8 ± 206.9
Acylated:total ghrelin	0.07 ± 0.03

[1] Values are means ± SD.

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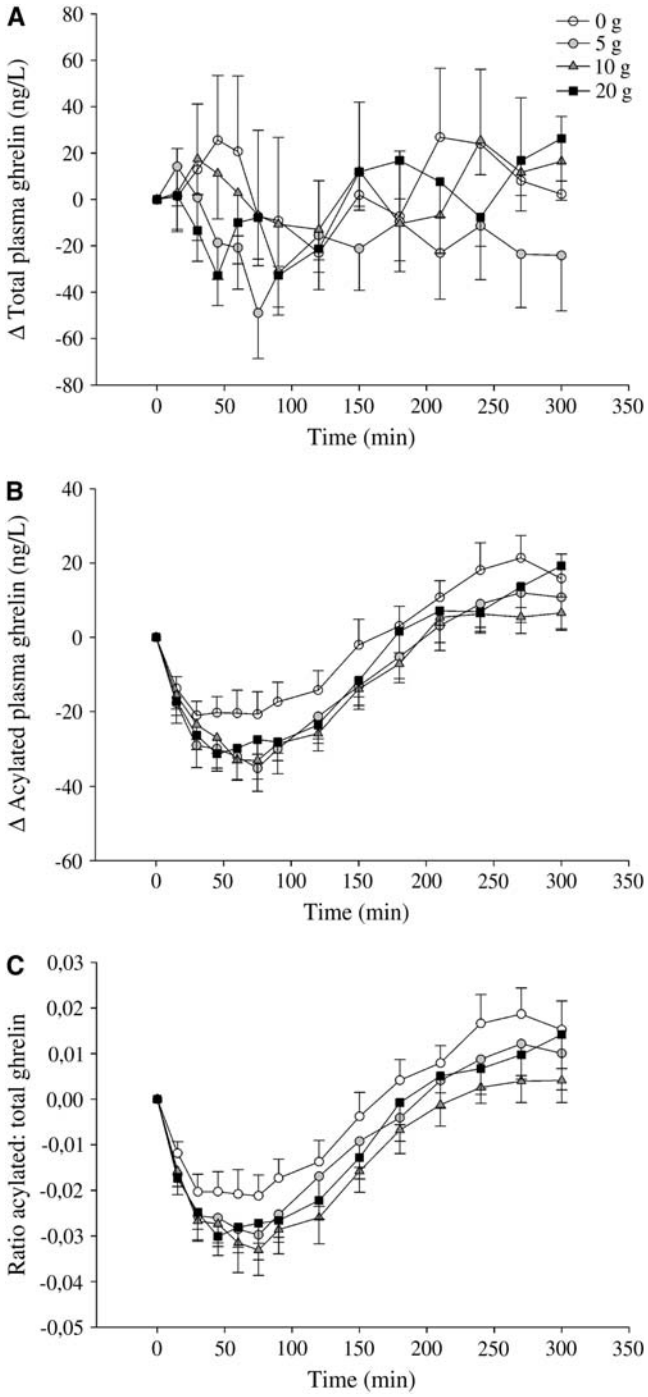

FIGURE 1 Changes in plasma ghrelin [total (*A*), acylated (*B*), and the ratio of acylated:total ghrelin (*C*)] relative to baseline after a liquid meal challenge test in healthy subjects 22–62 y old. Carob fiber consumption significantly decreased acylated ghrelin levels ($P < 0.001$). The ratio between acylated and total ghrelin was significantly lower after consumption of liquid meals enriched with 10 ($P = 0.021$) and 20 g ($P = 0.046$) compared with the control.

points of the curves. The area under the energy expenditure curve was calculated as the incremental area under the curve (IAUC).

Pearson correlation coefficients were calculated for each subject between total and acylated plasma ghrelin and other biomarkers with and without placebo treatment. Individual coefficients of correlation were averaged and 5% CI were calculated after correction with Fisher's z transformation.

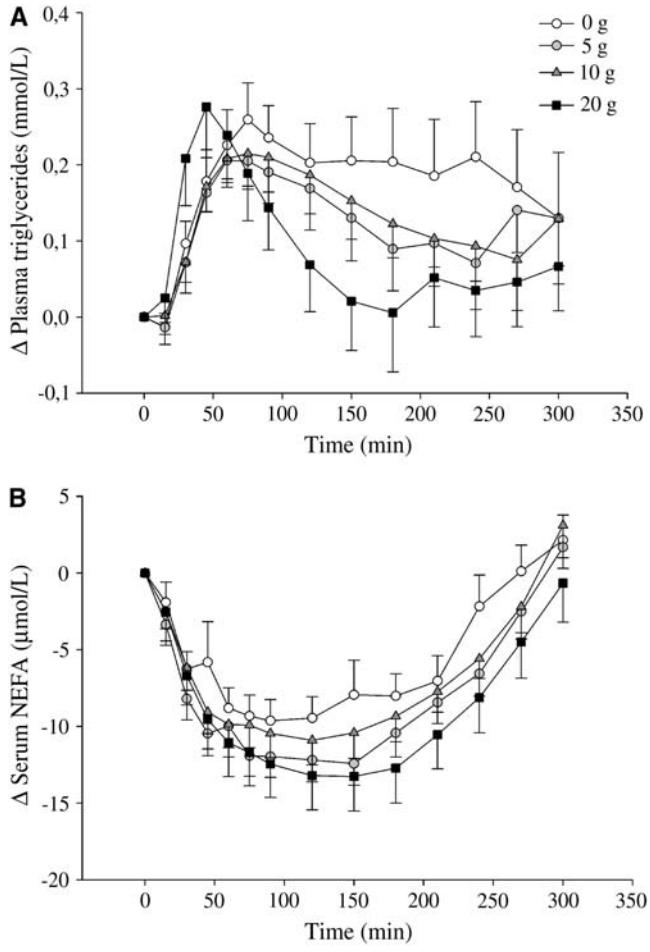

FIGURE 2 Changes in plasma triglycerides (*A*) and serum NEFA (*B*) relative to baseline after a liquid meal challenge test in healthy subjects 22–62 y old. Carob fiber consumption significantly lowered plasma triglycerides ($P = 0.033$) and serum NEFA ($P < 0.001$).

RESULTS

All participants had a normal BMI (18.5–25 kg/m^2). Fasting plasma glucose, serum insulin, serum total cholesterol, LDL cholesterol, and HDL cholesterol concentrations were also within normal ranges (**Table 1**). Five participants were smokers. However, all participants avoided smoking before the study and habitual smoking behavior did not change the effects of carob fiber consumption. The habitual alcohol intake did also not change the effects of carob fiber consumption. After consumption of liquid meals, plasma glucose peaked after 30 min and then declined ($P < 0.001$), with no dose-dependent differences in postprandial plasma glucose responses after 0, 10, or 20 g of carob fiber. After consumption of a liquid meal enriched with 5 g carob fiber, the plasma glucose response was slightly but significantly lower compared with the control meal ($P = 0.022$; results not shown).

Serum insulin increased after consumption of liquid meals with a peak after 30 min and then declined ($P < 0.001$) independent of 0, 5, 10, or 20 g carob enrichment (results not shown). These results were similar when only changes in plasma glucose and serum insulin for up to 90 min were included in the analyses.

Acylated but not total ghrelin decreased rapidly after meal consumption with a nadir after 60 min ($P < 0.001$; **Fig. 1**). The carob fiber enrichment did not affect the responses of total and

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nonacylated plasma ghrelin after the consumption of liquid meals. After the intake of enriched liquid meals, acylated ghrelin concentrations decreased compared with the control meal after 60 min ($P < 0.001$) without dose-dependent effects between 5, 10, and 20 g of carob fiber. The ratio between acylated and total ghrelin was lower after consumption of liquid meals containing 10 ($P = 0.021$) and 20 g ($P = 0.046$) compared with the control meal.

After consumption of the test meals, plasma triglycerides increased without differences between fiber enrichments. At 60 min after the test meal consumption, carob intervention decreased triglyceride levels in a dose-dependent manner ($P < 0.001$; **Fig. 2***A*).

Serum NEFA concentrations decreased after consumption of enriched test meals compared with the control ($P < 0.001$). The decrease was more pronounced after consumption of a liquid meal containing 20 g of carob fiber ($P < 0.001$) than after consumption of 10 g ($P = 0.026$) and 5 g of carob fiber ($P < 0.001$) compared with the control meal. NEFA suppression during 120 min after liquid meal consumption was more pronounced after carob consumption compared with control ($P < 0.001$; **Fig. 2***B*).

RQ at baseline was 0.79 ± 0.03. After consumption of the liquid meal with 20 g of carob fiber, RQ decreased up to 99.9% compared with the control meal ($P < 0.001$; **Fig. 3***A*).

Resting energy expenditure was 4.3 ± 0.5 kJ/min. Energy expenditure increased significantly after consumption of all liquid meals. However, the increase was more pronounced after carob fiber intake than after control ($P < 0.001$). Postprandial energy expenditure over 240 min was 229 ± 28 kJ (IAUC) after the liquid meal with carob fiber and 201 ± 23 kJ after the control meal ($P = 0.006$; Fig. 3*B*).

Substrate utilization changed markedly from glucose oxidation toward lipid oxidation ($P < 0.001$; Fig. 3 *C*,*D*).

Plasma acylated ghrelin was negatively correlated with plasma insulin, triglycerides, energy expenditure, and fat oxidation and positively with NEFA (**Table 2**).

DISCUSSION

This study shows for the first time that consumption of an insoluble dietary fiber rich in polyphenols obtained from carob pulp reduces postprandial NEFA and triglyceride concentrations and affects substrate utilization toward lipid oxidation. These observations are associated with a marked decrease in acylated ghrelin concentrations after consumption of the fiber-enriched liquid meal compared with the control meal.

The decline in plasma ghrelin after food intake is related to postgastric feedback signals (13,25) and depends on the kind of macronutrients ingested and the energy density of the meals (26); however, it is suggested to be independent of the texture of the food ingested (27). A high-fat diet increased stomach ghrelin expression in mice (28), whereas a high-carbohydrate

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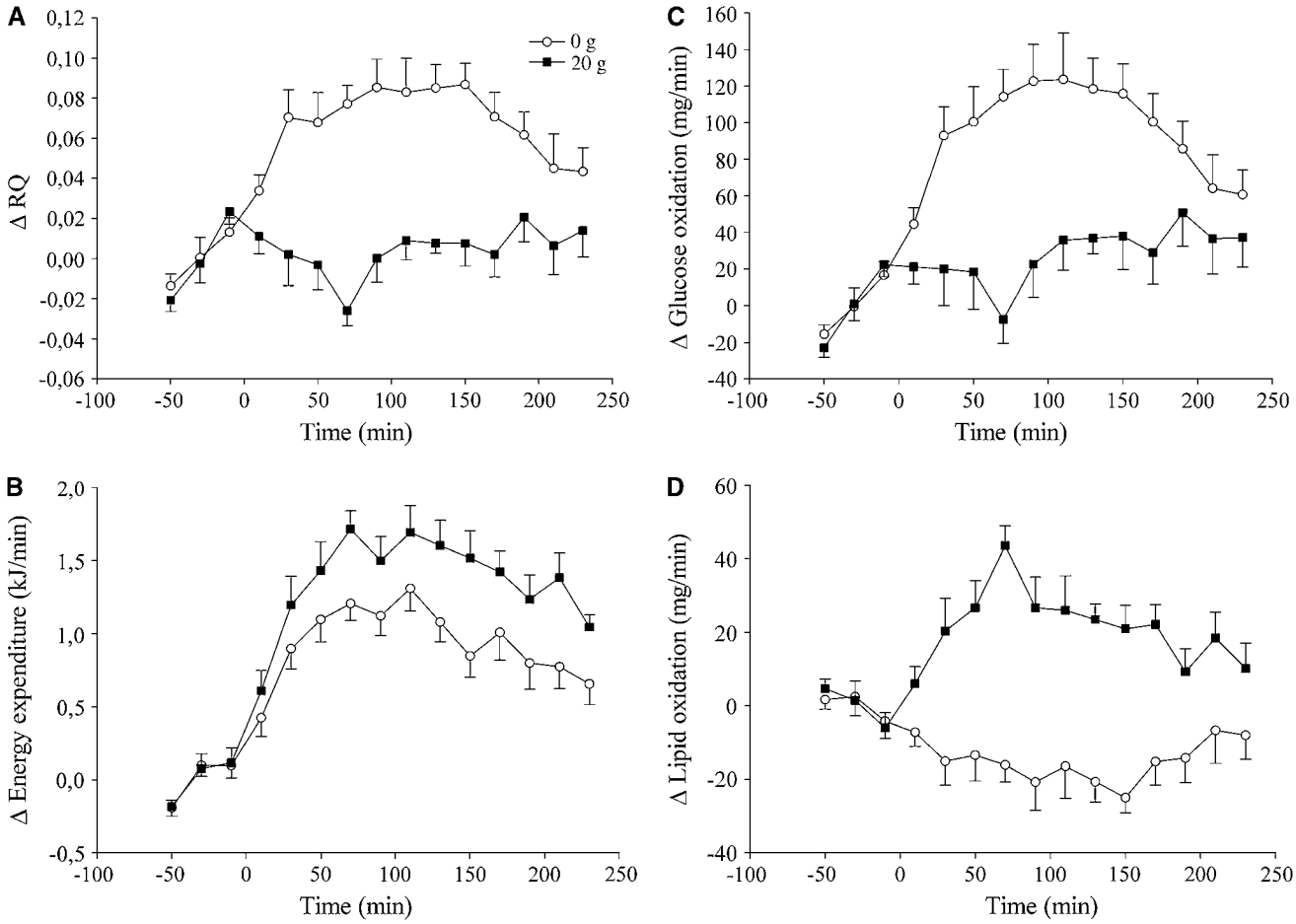

FIGURE 3 Changes in RQ (*A*), energy expenditure (*B*), glucose oxidation (*C*), and lipid oxidation (*D*) relative to baseline after a liquid meal challenge test in healthy subjects 22–62 y old. Carob fiber consumption significantly lowered respiratory quotients ($P < 0.001$) and increased energy expenditure ($P < 0.001$). This is due to significantly decreased glucose oxidation ($P < 0.001$) and increased lipid oxidation rate ($P < 0.001$) compared with the control meal.

TABLE 2

Coefficients of correlation between acylated plasma ghrelin and other biomarkers after consumption of carob fiber in healthy subjects 22–62 y old[1]

	r (95% CI)
Plasma glucose[2]	−0.173 (−0.450 – 0.134)
Serum insulin[2]	−0.699 (−0.797 to −0.566)
Plasma triglycerides[2]	−0.268 (−0.447 to −0.069)
Serum NEFA[2]	0.366 (0.089 – 0.591)
RQ[3]	−0.075 (−0.495 – 0.373)
Energy expenditure[3]	−0.347 (−0.533 to −0.128)
Glucose oxidation[3]	−0.136 (−0.543 – 0.322)
Fat oxidation[3]	−0.337 (−0.528 to −0.114)

[1] Values are Pearson coefficients of correlation (95% CI).
[2] Correlations for all treatments are based on 56 (14 time points, 4 treatments) and correlations for carob fiber treatments are based on 42 (14 time points, 3 treatments) pairs of data for plasma glucose, triglycerides, serum insulin and NEFA.
[3] For RQ, energy expenditure, glucose, and fat oxidation, correlations for all treatments are based on 22 (11 time points, 2 treatments) and for carob fiber only on 11 (11 time points, 1 treatment) pairs of data.

meal resulted in a more pronounced decline in ghrelin levels than a fat-rich meal (29).

A possible innergastric pathway of fiber-dependent ghrelin suppression may operate through gastric somatostatin, which was shown to increase after dietary fiber intake in humans (30), to suppress ghrelin secretion in rats (31), and to lower circulating ghrelin levels in humans (32). Our results do not indicate that the postprandial decline in ghrelin levels is mediated by insulin, which is in accordance with some (33,34), but not all (35) previously performed studies.

Carob fiber intake also had beneficial effects on serum NEFA and plasma triglyceride concentrations. Carob fiber was shown to bind bile acids, which may result in decelerated lipid absorption (36). However, in the present study, the postprandial peaks in serum triglyceride concentrations did not differ between meals with and without carob fiber, indicating that triglyceride-lowering effects are mediated by other factors.

Ghrelin has been recognized as a regulator of lipid metabolism. In rodents, exogenous ghrelin induces a reduction in fat utilization (37), which is most likely mediated by a network of hypothalamic cells (10,38). Lower acylated ghrelin levels may mediate a decline in triglycerides and NEFA concentrations through elevated fat utilization and increased uptake of circulating NEFA by the muscle (13,39). This hypothesis is supported by a decrease in RQ, indicating a switch in substrate utilization toward fatty acid oxidation. Comparable results were shown after consumption of resistant starch, which led to increased lipid oxidation (40). A further hypothesis to explain our results may be related to the recently reported action of free fatty acid synthase to suppress ghrelin secretion from the stomach and hypothalamus (41). The reduction in NEFA after carob fiber supplementation due to increased oxidation of lipids may have resulted in a reduction in NEFA synthesis and suppression of free fatty acid synthase, and, thus, in the suppression of ghrelin secretion. Lower postprandial levels of acylated ghrelin and the lower ratio of acylated vs. total ghrelin in the present study may also mediate the decrease observed in RQ. In rodents, RQ increased after ghrelin administration (11,13). Recently, desacyl ghrelin, the major circulating isoform of total ghrelin, was shown to induce a negative energy balance by decreasing food intake and delaying gastric emptying (42). A shift of the acylated vs. total ghrelin ratio may have similar effects. However, in other studies, reduced postprandial thermogenesis after consumption of a high-fiber meal was observed (43,44), indicating that the effects of dietary fiber may differ depending on the type of fiber and the type of meal enriched with dietary fiber.

In addition, the presence of active substances may contribute to the metabolic effects observed in our study. Carob fiber contains a remarkable amount of condensed tannins and other polyphenols (18). Polyphenols in green tea were shown to promote fat oxidation via sympathetic activation of thermogenesis, fat oxidation, or both (16). Therefore, the phenolic content of carob fiber may also account for the decrease in RQ after consumption of the fiber. A synergetic effect of both dietary fiber and polyphenols contained in carob pulp may also have contributed to the effects observed.

Our results suggest that carob fiber is able to increase fat utilization in healthy young subjects. However, further studies are required to clarify whether the effects are due to dietary fiber, polyphenol content, or to a synergetic action of both. The present study also shows short-term effects after consumption of a single meal, which might be less pronounced after long-term consumption due to adaptation mechanisms.

In conclusion, intake of carob fiber decreases postprandial acylated ghrelin levels and decreases the acylated vs. total ghrelin ratio. This effect might account for the observed increase in postprandial energy expenditure and the decrease in RQ after carob consumption. The results indicate that carob fiber enhanced fat oxidation and might exert beneficial effects in the prevention and therapy of the metabolic syndrome.

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