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Erratum

Lee YP, Mori TA, Sipsas S, et al. Lupin-enriched bread increases satiety and reduces energy intake acutely. Am J Clin Nutr 2006;85:975–80.

On page 975 (right column), the last sentence of paragraph 3 should read as follows: The incorporation of lupin kernel fiber into processed foods was found to result in higher postmeal satiety up to 4.5 h and lower energy intake ($\approx$15%) over the test day (20). In this article, "LKF" represents "lupin kernel flour" (of which fiber is a major component), not "lupin kernel fiber."

Lupin-enriched bread increases satiety and reduces energy intake acutely[1-3]

Ya P Lee, Trevor A Mori, Sofia Sipsas, Anne Barden, Ian B Puddey, Valerie Burke, Ramon S Hall, and Jonathan M Hodgson

ABSTRACT

Background: Protein and fiber may be important determinants of satiety. Lupin kernel flour is a novel food ingredient that is rich in protein and fiber.

Objective: The objective was to investigate the effects of lupin kernel flour–enriched bread (LB) on satiety and energy intake in humans.

Design: Two randomized controlled crossover trials were performed to compare the acute effects of LB with those of white bread (WB). In study 1, the subjects ($n = 16$) completed 4 treatments 1 wk apart: WB breakfast (as toast) and WB lunch (as sandwiches), WB breakfast and LB lunch, LB breakfast and WB lunch, and LB breakfast and LB lunch. Energy intake at all breakfast meals was matched (1655 kJ), and ad libitum energy intake at lunch, 3 h after breakfast, was measured. In study 2, the subjects ($n = 17$) completed 2 treatments 1 wk apart: WB breakfast and LB breakfast (each 1655 kJ). Blood samples were taken at baseline and at regular intervals for 3 h after breakfast.

Results: In study 1, the LB breakfast resulted in significantly higher self-reported satiety ($P < 0.001$) and lower energy intake (kJ) at lunch (-488; 95% CI: -798, -178) than did the WB breakfast. The LB lunch resulted in a significantly lower within-meal energy intake (kJ) at lunch (-1028; 95% CI: -1338, -727) than did the WB lunch. In study 2, compared with the WB breakfast, the LB breakfast significantly altered the 3-h postmeal plasma ghrelin response ($P = 0.04$) and resulted in significantly lower mean 3-h plasma ghrelin concentrations ($P = 0.009$).

Conclusion: A novel food enriched in protein and fiber derived from lupin kernel flour significantly influences energy intake acutely. *Am J Clin Nutr* 2006;84:975–80.

KEY WORDS Lupin kernel flour, glucose, insulin, ghrelin, satiety, energy intake

INTRODUCTION

Obesity is now a major public health problem worldwide. One possible strategy to combat the obesity epidemic involves understanding the role of dietary components in the control of food intake. This has the potential to prevent weight gain and facilitate weight loss.

Emerging data suggest that the nutrient composition of the diet is an important factor controlling satiety and energy intake, at least in the short term (1–4). Evidence that a high-protein diet is more satiating than is a high-carbohydrate diet (1, 2, 5–7) and that a high-fiber diet is more satiating than is a low-fiber diet (8–11) is convincing. Thus, foods enriched in protein or fiber, replacing energy from carbohydrate, have the potential to increase satiety and reduce energy intake. At present, little information is available on the effects of dietary approaches that increase both protein and fiber. Such diets may influence satiety via effects on appetite-regulating hormones such as ghrelin, a peptide that is released from the stomach and acts on the central nervous system to stimulate food intake (12–14). The protein and fiber contents in the diet may be an important determinant of ghrelin secretion (15–18), thereby influencing postmeal satiety and subsequent energy intake.

A practical approach for increasing the protein and fiber contents of processed foods is to incorporate high-protein and high-fiber ingredients in these foods. Lupin kernel flour (LKF) is a novel food ingredient derived from the endosperm of lupin, which contains 40–45% protein, 25–30% fiber, and negligible sugar and starch (19). The incorporation of LKF into processed foods was found to result in higher postmeal satiety up to 4.5 h and lower energy intake ($\approx 15\%$) over the test day (20).

Partial substitution of wheat flour for LKF in bread increases both the protein and fiber content of the bread. We report herein the results of 2 studies in which we compared the effects of bread enriched in LKF with regular white bread (WB) on satiety and energy intake. The objective of the first study was to assess inter- and intrameal effects on satiety and energy intake, and the objective of the second study was to assess the postmeal effects on plasma ghrelin concentrations.

SUBJECTS AND METHODS

Participants

Nonsmoking healthy men and women were recruited from the general population via newspaper advertisements. Exclusion criteria included a history of cardiovascular or peripheral vascular disease, diabetes, asthma, renal disease, liver disease, gout, psychiatric illness, food allergies, major gastrointestinal problems, or other major illnesses such as cancer, uncontrolled hypertension (systolic blood pressure >150 mm Hg or diastolic blood pressure >95 mm Hg); use of >2 antihypertensive agents; a change in drug therapy within the previous 3 mo; pregnancy or intentions to become pregnant; recent weight loss; and alcohol intake >200 g/wk for women and >300 g/wk for men. In addition, individuals with no history of diabetes but with fasting plasma glucose concentrations ≥5.6 mmol/L were excluded. All procedures followed were in accordance with institutional guidelines. The studies were approved by the University of Western Australia Human Ethics Committee, and all participants provided written informed consent.

Study design

Two randomized controlled crossover trials were performed to compare the acute effects of LKF-enriched bread (LB) with those of WB. Separate trials to investigate effects on satiety and energy intake and on biochemical measurements were considered necessary given the potential for venesection per se to modulate satiety and energy intake and for any effects to change over time as participants become more comfortable with blood sampling. Participants were instructed to maintain their diet, physical activity, and medication regimens for 4 wk before the study began and throughout the intervention. For each study, visits were 1 wk apart on the same day of the week at the same time of day if possible. Each clinic visit took place after the subjects fasted 12 h overnight between 0800 and 1400 in a temperature-controlled room (24 °C). The order of the treatments was randomly assigned by using computer-generated random numbers concealed in opaque envelopes. To avoid a second meal effect the following day, participants consumed the same meal in the evenings before each visit. In addition, participants did not consume alcohol or engage in vigorous physical activity for 24 h before each visit.

Study 1

One treatment was administered at each visit, with a total of 4 clinic visits during the study: *1*) WB breakfast (as toast) and WB lunch (as sandwiches), *2*) WB breakfast and LB lunch, *3*) LB breakfast and WB lunch, and *4*) LB breakfast and LB lunch.

Energy intake at all breakfast meals was matched (1655 kJ). Breakfasts consisted of toast (1400 kJ), consumed with margarine (180 kJ) and jam (75 kJ), and were consumed over 20 min. Participants consumed one cup (237 mL) of water, tea, or coffee with breakfast and with lunch and one cup of water 1.5 h after breakfast. The type and volume of beverages consumed were the same at each visit. Self-reported satiety was measured before breakfast; 15, 30, 45, 60, 90, 120, 150, and 180 min after breakfast; and then 15 min after lunch with the use of 3 different 12-cm visual analogue scales (21). The scales related to the following questions: "How full do you feel?" ("not full at all" = 0 cm to "extremely full" = 12 cm), "How hungry/satisfied do you feel?"

TABLE 1
Energy and nutrient composition of the white bread and the lupin kernel flour–enriched bread provided as part of a fixed-energy breakfast meal

	White bread	Lupin bread
Energy (kJ)	1400	1400
Bread weight (g)	126	158
Total moisture (g)	46	68
Total fat (g)	5.7	5.7
Total protein (g)	12	25
Total ash (g)	2.1	3.3
Total fiber (g)	3.4	15
Total carbohydrate (g)	57	40

("extremely hungry" = 0 cm to "extremely satisfied" = 12 cm), and "How much food do you think you could eat?" ("nothing at all" = 0 cm to "an extremely large quantity" = 12 cm). Participants were requested to place an *X* at any point along the scale, and scores were then converted to continuous variables from 0 to 12 cm.

An ad libitum lunch was provided as sandwiches 3 h after breakfast. The composition of the sandwiches was chosen by the participants from a limited choice of fillings (any combination of margarine, cheese, ham, pressed chicken, tuna, tomato, lettuce, and mayonnaise) before the study began. The sandwich composition was the same for each participant at each visit. Sandwiches providing energy in excess of usual intake at lunch ($\bar{x} \pm$ SD: 4655 ± 336 kJ, depending on sandwich composition) were provided. Instruction was given to "eat until comfortably full." Forty minutes were allocated for lunch, and the total energy intake was then measured as the difference between the energy supplied and the energy remaining.

Study 2

Two clinic visits were made during the study; a WB breakfast was consumed at the first visit and an LB breakfast at the second visit, 1 wk apart. At each clinic visit, a catheter was inserted into the antecubital vein of each subject, and a fasting blood sample was obtained (time: 0 min). Breakfast, as previously described for study 1, was provided as toast. Blood samples were then drawn 15, 30, 45, 60, 90, 120, 150, and 180 min after breakfast.

Bread formulation

The LB was formulated by substituting 40% of the wheat flour usually present in a regular WB with LKF. This resulted in 24% of the final weight of the LB present as LKF. Because this also resulted in a small difference in total fat and wheat protein between the LB and regular WB, a mixture of canola oil and sunflower oil, which had a fatty acid composition similar to that of LKF, was added to the WB, and wheat protein (gluten) was added to the LB. Thus, the LB and WB were matched for fat content and fatty acid composition, and protein content derived from wheat. The objective was to partially substitute energy from wheat carbohydrate with matching energy from protein and fiber from LKF, such that these were the only macronutrient differences in the 1400-kJ of bread provided at breakfast. The nutrient composition of the 2 breads was analyzed by BRI Australia Ltd (North Ryde, Australia) (**Table 1**). All breads used for each study were baked as a single batch at Bodhi's Bakery, Fremantle, WA. The breads were sliced, stored at −20 °C, and defrosted at room temperature 1 h before consumption. The participants rated the

palatability of the WB and LB on the basis of taste, texture, consistency, mouth feel, and smell using a 12-cm visual analogue scale ranging from "dislike very much" to "like very much."

Biochemistry

Venous blood was collected into serum tubes or ice-cold tubes containing EDTA (7.2 mg) and aprotinin [1 TIU (trypsin inhibiting unit)/mL; Sigma-Aldrich, New South Wales, Australia]. After centrifugation at $1500 \times g$ for 10 min at 4 °C, plasma and serum were separated, and aliquots were frozen at −80 °C before being analyzed.

Serum glucose was measured with a hexokinase method, and serum insulin was measured with an enzyme-linked immunosorbent assay (Boehringer Mannheim, Mannheim, Germany) in the Department of Biochemistry at Royal Perth Hospital, Western Australia.

Plasma ghrelin was measured by using an enzyme immunoassay. Briefly, after acidification of 1 mL plasma with 1 mL 1% trifluoroacetic acid and centrifugation at $1500 \times g$ at 4 °C for 10 min, plasma ghrelin was extracted by using solid-phase extraction. A C-18 Bond-Elute column (Varian, Palo Alto, CA) was equilibrated with 1 mL methanol, followed by 1 mL 60% acetonitrile in 1% trifluoroacetic acid and 3 mL of 1% trifluoroacetic acid. The acidified plasma was added to the column and washed twice with 3 mL of 1% trifluoroacetic acid; ghrelin was eluted with 3 mL of 60% acetonitrile in 1% trifluoroacetic acid. Eluent was collected into a polypropylene tube and evaporated to dryness at medium heat in a centrifugal evaporator and reconstituted in 0.5 mL of immunoassay buffer. The extracted ghrelin was analyzed by using the ghrelin (human acylated) enzyme immunoassay kit (SPI-BIO, Montigny le Bretonneux, France). The ghrelin enzyme immunoassay kit specifically measures acylated ghrelin, which is the active isoform of ghrelin. The sensitivity of the assay is 0.3 pg/mL.

Statistics

Statistical analyses were performed by using SPSS 11.5 software (SPSS Inc, Chicago, IL) or SAS 8.2 software (SAS Institute, Cary, NC). Descriptive statistics are presented as means ± SDs. Results are presented as means and 95% CIs in the text or as means ± SEMs in the figures; $P < 0.05$ was the level of significance. Palatability scores and postprandial glucose and insulin area under the curves were compared by using the paired t test in SPSS. Energy intake at lunch, baseline-adjusted post-breakfast self-reported satiety scores, and plasma ghrelin and serum glucose and insulin concentrations were analyzed with random-effects models in SAS by using PROC MIXED. The models included a time-by-treatment interaction term to test whether the slopes of the curves differed, ie, whether the curves were significantly nonparallel to each other. In the random-effects models, participant was treated as the random effect, which accounted for correlated error structures and treatment (WB or LB), period, and treatment order as the fixed effects.

RESULTS

Study 1

Sixteen participants ($n = 8$ men and 8 women) with a mean (±SD) age and body mass index (BMI; in kg/m^2) of 58.6 ± 7.2 y
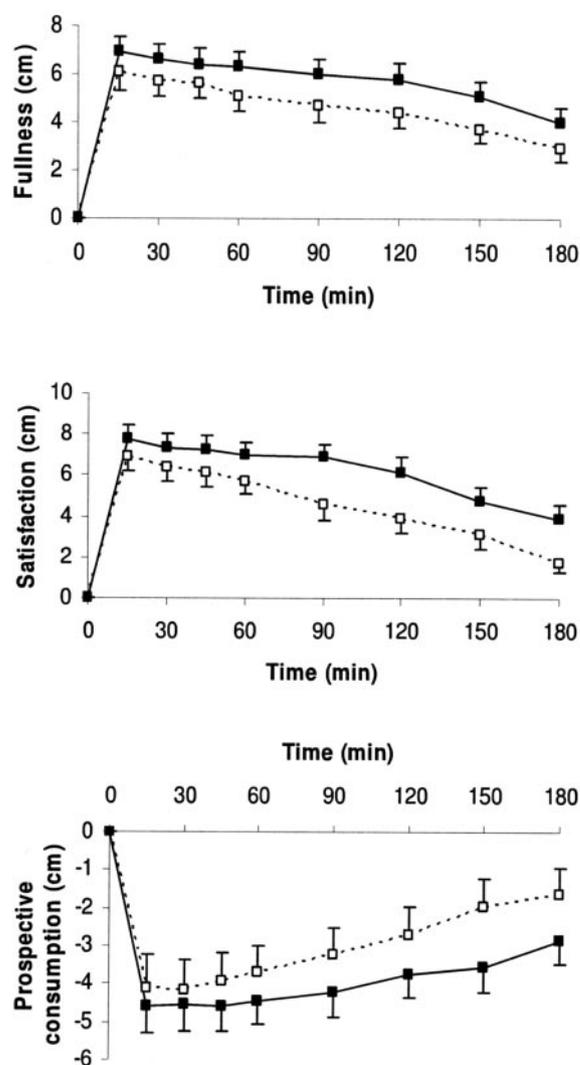

FIGURE 1. Mean (±SEM) difference from baseline in satiety scores for fullness, satisfaction, and prospective consumption ($n = 16$) during the 3 h after consumption of a fixed-energy breakfast consisting of lupin kernel flour–enriched bread (■) or white bread (□). Time-by-treatment interactions in the models were significant for fullness ($P = 0.06$), satisfaction ($P < 0.001$), and prospective consumption ($P < 0.001$). Differences in baseline-adjusted mean scores were significant between breads, $P < 0.001$ (for all satiety scores per random-effects models).

and 31.3 ± 4.5, respectively, were recruited. There was no significant difference in mean palatability (in cm) between the WB (9.1; 95% CI: 7.6, 10.6) and LB (8.7; 95% CI: 6.8, 10.7) at breakfast as toast ($P = 0.73$) or between the WB (8.7; 95% CI: 7.0, 10.5) and LB (7.6; 95% CI: 5.8, 9.4) at lunch as sandwiches ($P = 0.35$).

Compared with the WB, LB at breakfast resulted in significantly higher self-reported satiety (**Figure 1**). Significance values for time-by-treatment interactions in the models for fullness, satisfaction, and prospective consumption were $P = 0.06$, $P < 0.001$, and $P < 0.001$, respectively, which suggested a difference in the slope of the satisfaction and prospective consumption curves. There was also a significant difference (in cm) between the WB and LB, respectively, for baseline-adjusted mean 3-h self-reported fullness [5.38 (95% CI: 4.39, 6.38) and 6.44 (95% CI: 5.45, 7.43); $P < 0.001$], satisfaction [6.50 (95% CI: 5.57,

TABLE 2
Effects of the lupin kernel flour–enriched bread (LB) in comparison with those of the white bread (WB) on energy intake at an ad libitum lunch after a fixed-energy (1655 kJ) breakfast[1]

Treatment	Breakfast (as toast)	Lunch (as sandwiches)	Energy intake at lunch[2]
			kJ
1	WB	WB	3774 (3083, 4465)
2	WB	LB	2504 (2012, 2996)
3	LB	WB	3044 (2415, 3673)
4	LB	LB	2257 (1800, 2715)

[1] The subjects ($n = 16$) completed 4 treatments 1 wk apart and in random order.

[2] All values are means; 95% CI in parentheses. The LB at breakfast resulted in a significantly lower energy intake (in kJ) at lunch (-488; 95% CI: $-798, -178$) than did the WB, and the LB at lunch resulted in a significantly lower within-meal energy intake at lunch (-1028; 95% CI: $-1338, -727$) than did the WB (random-effects models).

7.44) and 8.11 (95% CI: 7.18, 9.05); $P < 0.001$], and prospective consumption [2.85 (95% CI: 2.39, 3.31) and 1.91 (95% CI: 1.45, 2.36); $P < 0.001$].

The energy intake at lunch for each treatment is presented in **Table 2**. LB compared with WB at breakfast resulted in a significantly lower energy intake (in kJ) at lunch (-488; 95% CI: $-798, -178$). That is, energy intake at the lunch meal was significantly lower when the participants consumed a fixed-energy breakfast derived from LB compared with WB. The LB at lunch resulted in significantly lower energy intake at lunch (-1028 kJ; 95% CI: $-1338, -727$ kJ) than did the WB. That is, there was a significant effect of LB, compared with WB, to reduce ad libitum energy intake within a meal.

Study 2

Seventeen participants ($n = 11$ men and 6 women) with a mean age and BMI of 61.0 ± 5.6 y and 27.2 ± 4.3, respectively, were recruited. Four of the 17 participants also took part in study 1. There was no significant difference in palatability (in cm) between the WB (9.5; 95% CI: 8.7, 10.2) and LB (9.5; 95% CI: 8.8, 10.1) at breakfast as toast ($P = 1.00$).

The baseline and postbreakfast plasma ghrelin concentrations are presented in **Figure 2**. There was a significant baseline-
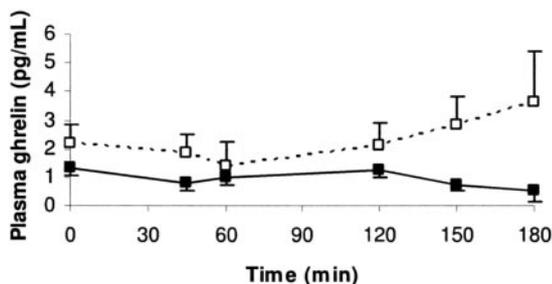
FIGURE 2. Mean ($\pm$SEM) plasma ghrelin concentrations ($n = 17$) at baseline and during the 3 h after consumption of lupin kernel flour–enriched bread (■) or white bread (□) for breakfast. There was a significant baseline-adjusted time-by-treatment interaction ($P = 0.04$) and a significant difference in baseline-adjusted mean 3-h plasma ghrelin concentrations ($P = 0.009$, random-effects models).
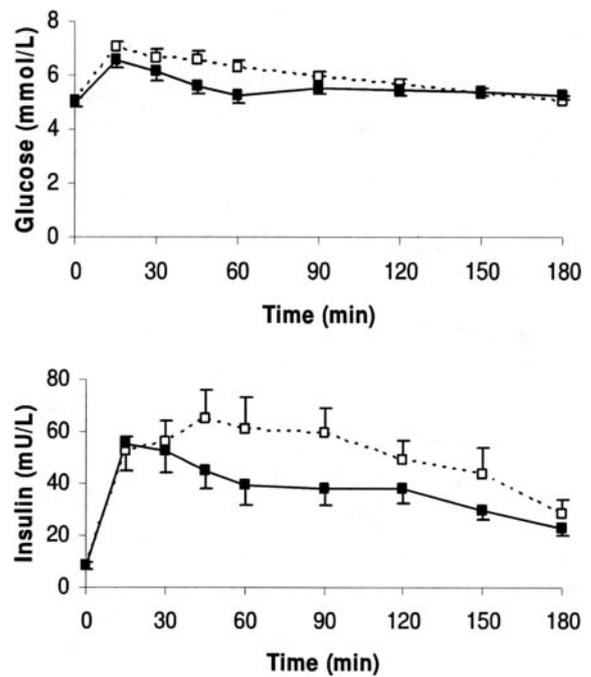
FIGURE 3. Mean ($\pm$SEM) serum glucose and insulin concentrations ($n = 17$) at baseline and during the 3 h after the consumption of lupin kernel flour–enriched bread (■) or white bread (□) for breakfast. There was a significant time-by-treatment interaction for glucose response ($P = 0.01$). There was no significant time-by-treatment interaction for insulin response ($P = 0.06$). The main effect of treatment for insulin was -11.6 (95% CI: $-16.3, -6.9$) mU/L ($P < 0.001$, random-effects models). There was a significant difference between breads in the area under the curve for glucose and insulin ($P < 0.01$, paired t test).

adjusted time-by-treatment interaction ($P = 0.04$), which suggests a difference in the slope of the curves and an altered postmeal ghrelin response. In addition, baseline-adjusted mean 3-h plasma ghrelin concentrations (in pg/mL) were significantly lower ($P = 0.009$) with the LB (1.93; 95% CI: 0.71, 3.14) than with the WB (0.97; 95% CI: -0.25, 2.19).

Postprandial serum glucose and insulin concentrations are presented in **Figure 3**. There was a significant time-by-treatment interaction for glucose response ($P = 0.01$), which suggests a difference in the slope of the curves. There was no significant time-by-treatment interaction for insulin response ($P = 0.06$). The main effect of treatment was -11.6 (95% CI: $-16.3, -6.9$) mU/L ($P < 0.001$), and the main effect for period was 2.9 (-1.8, 7.6) mU/L ($P = 0.22$). The incremental glucose area under the curve (in mmol · h/L) for the LB was significantly lower (1064; 95% CI: 998, 1131) than that for the WB (999; 95% CI: 932, 1065) ($P = 0.006$). Similarly, the incremental insulin area under the curve (in mU · h/L) for the LB was significantly lower (8995; 95% CI: 5940, 12051) than that for the WB (6530; 95% CI: 4423, 8636) ($P = 0.002$).

DISCUSSION

This study reports the effect of protein and fiber-enriched bread on satiety and energy intake. The source of protein and fiber was LKF, which partially replaced wheat carbohydrate. Incorporation of LKF into bread resulted in higher satiety and a

lower energy intake. Both inter- and intrameal effects were observed. There was also a significant effect of the LB on postprandial ghrelin, glucose, and insulin responses. These results suggest that protein and fiber enrichment of bread with LKF has the potential to influence appetite and reduce energy intake, at least in the short term.

All measures of self-reported satiety suggested greater satiety after the LB than after the WB. The observed higher fullness and satisfaction and the lower prospective food intake are consistent with results of previous studies, which showed that high-protein diets increase postmeal self-reported satiety more than do high-carbohydrate diets (1, 17, 22–25) and high-fiber diets increase postmeal self-reported satiety more than do low-fiber diets (20, 26, 27). Consistent with the findings for self-reported satiety, the LB at breakfast resulted in up to a 20% lower energy intake at lunch than did the WB breakfast. These results are supported by previous studies that showed that high-protein diets reduce energy intake at subsequent meals more so than do high-carbohydrate diets (23, 28) and high-fiber diets reduce energy intake at subsequent meals more so than do low-fiber diets (26, 29).

The pattern of postprandial ghrelin secretion was investigated as a factor that might modulate any effect of LKF-enrichment of bread on intermeal satiety. Ghrelin is an orexigenic hormone released from the stomach that acts centrally to regulate appetite by binding to the growth hormone secretagogue receptor in the hypothalamic nuclei (30, 31). The LB breakfast significantly altered the 3-h postmeal plasma ghrelin response ($P = 0.04$) and resulted in significantly lower mean 3-h plasma ghrelin concentrations ($P = 0.009$) than did the WB breakfast.

Our results are supported by those of previous studies that have shown that postprandial plasma ghrelin concentrations may be mediated by high-protein (15, 16, 32–34) and high-fiber (18) diets. However, some studies suggest that protein increases, rather than decreases, plasma ghrelin acutely (16). The type of protein may play a role in modulating postmeal plasma ghrelin responses. For example, meat protein has been shown to increase (16) and whey protein has been shown to decrease (34) plasma ghrelin in the first hour after a meal. In our study, the major difference observed was in the pattern of ghrelin secretion over time after breakfast. After the WB, ghrelin secretion was suppressed for up to 2 h and then increased before lunch, whereas ghrelin secretion appears to remain suppressed for up to 3 h after the LB. A delayed plasma ghrelin response was observed in previous studies that investigated the effects of high-protein meals (34, 35). This finding is consistent with ghrelin mediating a late satiety response and, perhaps, ultimately energy intake at the lunch meal.

Our data also showed a significant intrameal (within meal) effect on energy intake at lunch. Energy intake was up to 34% lower with the LB than with the WB at lunch. Fewer studies have investigated the effects of macronutrients on intrameal energy intake. Consistent evidence shows that high-fiber meals can lead to a longer chewing time and increased gastric distension resulting in earlier sense of fullness (4, 36) and earlier meal cessation and reduced energy intake (10, 38, 39). The fiber content of the LB was ≈ 6 g/1000 kJ greater than that of the WB. Previous studies have found both acute and long-term reductions in energy intake with a high-fiber diet (29). The effect of protein, compared with that of carbohydrate, on energy intake within a single meal is less clear. However, ad libitum energy intake was lower when high-protein meals were consumed than when high-fat meals were consumed (38).

In addition to the nutrient composition of food, many other factors could influence satiety, including energy intake, energy density, food weight and volume, and palatability (39–41). At the breakfast meal, the energy intake was matched between breads and there was little difference in energy density. However, there was a difference in the weight and volume of the breads, which may have contributed to the differences in satiety. Our findings are unlikely to be influenced by differences in palatability, because no significant difference were observed in palatability between breads when eaten as toast or as sandwiches.

The present study showed a significant reduction in postprandial glucose and insulin responses. This reduction was expected given that energy intake at breakfast was matched and the total load of glycemic carbohydrate was reduced by $\approx 30\%$ by partial replacement of wheat carbohydrate with lupin protein and fiber from LKF. The reduction in the carbohydrate load is likely to be the primary contributor to the observed differences in glucose and insulin responses. The importance of circulating postprandial concentrations of insulin and glucose in the control of appetite and energy intake remains uncertain (16, 42, 43).

In conclusion, increasing the protein and fiber contents of bread with the use of LKF results in higher satiety and lower energy intakes. Significant effects were found both during a meal and at a subsequent meal. The effect of LKF on the pattern of postprandial plasma ghrelin secretion is consistent with the observed effect on energy intake at a subsequent meal. These results indicate that LKF-enriched foods influence satiety and energy intake. Thus, LKF is a novel food ingredient that could be incorporated into a range of products that might benefit appetite regulation. ▨

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