Protein choices targeting thermogenesis and metabolism $^{1-3}$


ABSTRACT

Background: Dietary proteins stimulate thermogenesis and satiety more than does carbohydrate or fat; however, less is known about the differences between protein sources.

Objective: The objective was to determine the differential effects of 3 proteins on energy metabolism, satiety, and glucose control.

Design: Energy metabolism, satiety, and glucose control were measured in 23 lean, healthy subjects on separate occasions, before and after consumption of 4 isocaloric test meals in a randomized, double-blind, crossover design. Three meals consisting of 50% protein (whey, casein, or soy), 40% carbohydrate, and 10% fat and a fourth meal consisting of 95.5% carbohydrate were compared with a glucose meal that provided the same glucose load as the protein meals.

Results: The thermic effect was greater after the whey (14.4 ± 0.5% than after the casein (12.0 ± 0.6%; $P = 0.002$) and soy (11.6 ± 0.5%; $P = 0.0001$) meals and was greater after the whey, casein, and soy meals than after the high-carbohydrate meal (6.6 ± 0.5%; $P < 0.0001$). Cumulative fat oxidation tended to be greater after the whey meal (16.2 ± 1.1 g) than after the soy meal (13.7 ± 1.0 g; $P = 0.097$) and was greater after the whey and soy meals than after the high-carbohydrate meal (10.9 ± 0.9 g; $P < 0.05$). The glycemic response to glucose was attenuated 32% by the proteins ($P < 0.001$) at the expense of a greater insulin response after whey than after glucose (154%; $P = 0.02$), casein (143%; $P = 0.07$), and soy (151%; $P = 0.03$). Subjective appetite sensations indicated that casein and soy were more satiating than whey ($P < 0.01$), but whey was more “liked” compared with casein and soy ($P = 0.025$ and $P = 0.09$, respectively).

Conclusion: The results suggest that different protein sources could be used to modulate metabolism and subsequently energy balance. Am J Clin Nutr 2011;93:525–34.

INTRODUCTION

It has been known for many years that the ingestion of dietary proteins stimulates energy expenditure in the postprandial period immediately after meal ingestion. Certainly, on theoretical grounds, the energy cost of digesting, absorbing, and metabolizing proteins ($\approx 23\%$) is greater than that of either carbohydrates ($\approx 6\%$) or fat ($\approx 3\%$) (1), and these theoretical values have been supported and confirmed for protein (2) and carbohydrate/glucose (3) in human clinical trials.

Protein not only increases energy expenditure (2, 4–9) but also decreases energy intake through mechanisms that influence appetite control (4, 10–16). Their addition to foods, meals, and diets decreases the glycemic index and, when exchanged for carbohydrate, the glycemic load as well, with potential benefits for glucose tolerance and insulin sensitivity. Consequently isocaloric diets composed of more protein than habitually consumed should provide potential benefits for those with, or susceptible to, metabolic dysregulations associated with obesity-related disorders.

Recently, results from many medium-term clinical trials have provided evidence that low-carbohydrate, high-fat, and relatively high-protein diets favor weight loss and reduce biomarkers of metabolic disease, at least over 6 mo to 1 y (17–20) or even 2 y (21, 22) and have certain advantages when compared with other more conventional hypocaloric diets. Although a recent long-term study concluded that the macronutrient composition of low-calorie diets does not influence weight loss (23), the experimental differences between the dietary groups, highlighted in an accompanying editorial (24), were much less than those described in the original protocol and were sufficient to have prejudiced the results and conclusions of the authors. Other studies have shown that milk proteins are absorbed and digested at different rates (25–28), that animal and vegetable proteins stimulate energy expenditure differently (29), and that consumption of fish protein improves insulin sensitivity (30, 31). Although milk proteins reduce the postprandial blood glucose response of mixed meals (32, 33) and glucose (34), this occurs at the expense of increased insulin secretion (32, 33). Thus, different proteins appear to have a variety of acute and chronic metabolic effects, which should be investigated further to elucidate their potential health benefits.

The present clinical trial investigated the thermic and metabolic responses and the satiating effects of 4 isocaloric test meals, 3 of which provided 50% of energy as whey, casein, or soy protein and were compared with a high-carbohydrate meal. A fifth hypocaloric glucose test meal providing the same amount of carbohydrate as that in the protein meals was also consumed to investigate how these 3 commonly used proteins influence the glycemic and insulminic index.

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SUBJECTS AND METHODS

Subjects

The study was a single-center, double-blind, placebo-controlled, randomized, 5-treatment trial carried out in accordance with the ethical standards for clinical research, Faculty of Biology and Medicine, Lausanne University, Switzerland, the ethical committee of which reviewed and approved the protocol (reference: protocol 70/2006; recruitment: 1 September 2007).

Potential volunteers, recruited from among the Research Center’s personnel, were invited to an information meeting, during which they received a full explanation and a layperson’s summary of the study. Those who wished to participate in the study had a preliminary medical examination and had blood drawn to ensure normal clinical signs and blood measures. Twenty-three sedentary, lean, healthy men and women, whose physical characteristics are presented in Table 1, agreed to participate by signing the study consent form.

The subjects were studied on 6 different occasions (Figure 1). On the first occasion (Figure 1; visit 1), the subject came to the Metabolic Unit at the Research Center between 0700 and 0800 in a fasting condition. His or her fasting resting metabolic rate was measured continuously for 2 h in a small, ventilated whole-body calorimeter connected to an open-circuit indirect calorimeter (Datatrac II; Datex, Helsinki, Finland). Values recorded during the last 30 min were used to calculate each subject’s mean 24-h basal metabolic rate and 24-h energy requirements by using a physical activity level factor of 1.4 for sedentary individuals (35).

On subsequent occasions, the subjects came to the Metabolic Unit on the day before each test and consumed a controlled menu for breakfast, lunch, and supper (Figure 1; visit 2) that was repeated with exactly the same ingredients on the days before each of the other tests (Figure 1; visits 3–6). After the evening meal, the subjects stayed within the confines of, and spent the night in, the Metabolic Unit.

The subjects were woken at ~0600, and after minimal ablations during which overnight urine was collected, went to the infirmary where his or her body weight was recorded and a catheter was inserted into an antecubital arm vein. The subjects were transferred to the test room and were comfortably installed, in a semirecumbent position, in a reclining chair inside a small whole-body indirect calorimeter chamber. The venous catheter was connected, via a small airtight connecting compartment in the chamber, to a physiologic saline line; the chamber was closed; and the respiratory exchange measurements (Datatrac Metabolic Monitor; Datex) were made continuously for 4 h. After 2 h of resting, fasting measurements, the subjects consumed a glucose test meal that was passed, together with three 500-mg acetaminophen (Panadol; GlaxoSmithKline, Münchenbuechsee, Switzerland) tablets [used as a marker of stomach emptying (36, 37)], through another small airtight connecting compartment in the chamber, and measurements were continued for an additional 2 h. The amount of glucose in this first test meal (Figure 1; visit 2) represented exactly the same amount of carbohydrate as that present in the 3 protein test meals (Table 2). At this time, the subjects exited the chamber for a short period (~5 min) to collect a second urine sample, and, on reentry into the chamber, respiratory exchange measurements were continued for an additional 2.5 h. At the end of the 6.5-h test, the subjects exited the calorimeter chamber and a third urine sample was collected. Water was allowed ad libitum throughout the test. An interval of ≥1–4 wk occurred between tests so that women volunteers could be studied during the follicular phase of their menstrual cycle.

On subsequent occasions, after 2 h of baseline measurements, the subjects consumed 1 of 4 isoenergetic test meals (Table 2) in a randomized crossover design (Figure 1; visits 3–6). Throughout the test, blood samples were collected and subjective indexes of appetite sensations were recorded on a 70-mm, validated (38), visual analog scale (VAS) programmed on a hand-held pocket personal computer (axim 150; Dell Computers, Round Rock, TX). All scores were recorded in millimeters, plus 1 decimal place, by the software, which automatically adjusted the ratings to the equivalent of a 100-mm scale (38).

Blood samples were taken 40 and 5 min before and 15, 30, 45, 60, 90, 120, 180, 240, and 330 min after the beginning of test meal consumption. Appetite sensations were recorded 15 min before and 10, 40, 70, 100, 130, 160, 190, 220, 250, 280, 310, and 330 min after test meal consumption.

Test meals

The test meals were calculated to provide 20% of each subject’s 24-h energy requirements. Three of the meals consisted of 50% protein (0.81 ± 0.07 g/kg), 40% carbohydrate, and 10% fat energy, and one meal consisted almost entirely of carbohydrate (Table 2) and was isoenergetic with the other test meals. For the test meals providing protein, the protein source was whey protein isolate (BiPRO; Davisco Foods International, Le Sueur, MN), micellar casein (MPI 85 MC; Hungarian Dairy Research Institute, Mosonmagyarovar, Hungary), or soy protein isolate (Supro XT 219D; The Solae Co, Ieper, Belgium); the amino acid profiles are presented in Table 3. Dextrose monohydrate (Roferose; Roquette, Lestrem, France) was the carbohydrate source, and sunflower oil accounted for most of the lipid composition of the test meal.

| TABLE 1  |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Subjects Age | Height | Weight | BMI | Fat mass | LBM | BMR |
| n = 23 | | | | | | |
| Men 31 ± 6.4 | 1.79 ± 0.06* | 73.5 ± 5.9* | 23.1 ± 1.6 | 14.9 ± 3.2** | 58.7 ± 5.2*** | 1773 ± 131*** |
| Women 35 ± 5.2 | 1.69 ± 0.05 | 62.2 ± 2.9 | 21.8 ± 1.6 | 18.6 ± 1.8 | 43.6 ± 3.1 | 1456 ± 106 |
| All 32 ± 6.3 | 1.76 ± 0.07 | 70.6 ± 7.3 | 22.7 ± 1.7 | 15.8 ± 3.3 | 54.7 ± 8.2 | 1691 ± 188 |

All values are means ± SDs; n = 23. LBM, lean body mass; BMR, basal metabolic rate. **Significantly different from women (Student’s unpaired t test); *P < 0.002, **P < 0.05, ***P < 0.0001.
The isocaloric carbohydrate test meal consisted of 2 carbohydrate sources: maltodextrin (Glucidex-MD 47; Roquette) and dextrose monohydrate. All of the isoenergetic test meals were flavored with vanilla (Vanilla Bourbon Flavor-75012–32; Givaudan, Kemptthal, Switzerland) and a commercial cocoa powder with the following composition: 19% protein, 9% carbohydrate, and 22% fat by weight.

The protein test meals were prepared by first adding the powdered protein to warm water and then stirring until dissolved, after which dextrose, the flavoring, and the vegetable oil were added. The final product was served at room temperature and had an energy density of $1 \text{ kcal/g}$.

Analyses
Urinary nitrogen was analyzed in timed urine samples (Figure 1) that were collected in the morning after the overnight fast, 2 h after test meal ingestion, and at the end of the test (5.5 h after test meal ingestion).

<table>
<thead>
<tr>
<th>Test meal</th>
<th>Energy</th>
<th>Protein</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>184 kcal</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Whey</td>
<td>459 g/kg</td>
<td>50</td>
<td>50</td>
<td>0.81 ± 0.07</td>
<td>40</td>
</tr>
<tr>
<td>Casein</td>
<td>459 g/kg</td>
<td>50</td>
<td>50</td>
<td>0.81 ± 0.07</td>
<td>40</td>
</tr>
<tr>
<td>Soy</td>
<td>459 g/kg</td>
<td>50</td>
<td>50</td>
<td>0.81 ± 0.07</td>
<td>40</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>459</td>
<td>1.2</td>
<td>0.02 ± 0.001</td>
<td>95.5</td>
<td></td>
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</tbody>
</table>

$^1$ Mean ± SD (all such values).

Blood samples were collected into ice-cold lithium heparin and potassium EDTA-coated monovette syringes (Sarstedt, Nümbrecht, Germany) the latter of which contained Trasytol (Bayer Gmbh, Leverkusen, Germany) and a dipeptidyl peptidase-IV inhibitor, and were immediately centrifuged at $4^\circ\text{C}$ for 15 min at 1500 rpm, portioned into 1.8-mL cryotubes, and stored at $-20^\circ\text{C}$ until analyzed.

<table>
<thead>
<tr>
<th>Amino acid profile of the 3 protein test meals</th>
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<tbody>
<tr>
<td>Whey protein isolate</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Alanine</td>
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<tr>
<td>Arginine</td>
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<tr>
<td>Aspartic acid</td>
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<tr>
<td>Cysteine</td>
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<td>Glutamic acid</td>
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<tr>
<td>Glycine</td>
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<tr>
<td>Histidine</td>
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<tr>
<td>Isoleucine</td>
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<td>Leucine</td>
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<tr>
<td>Lysine</td>
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<tr>
<td>Methionine</td>
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<td>Phenylalanine</td>
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<td>Proline</td>
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<tr>
<td>Serine</td>
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<tr>
<td>Threonine</td>
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<tr>
<td>Tryptophan</td>
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<tr>
<td>Tyrosine</td>
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<tr>
<td>Valine</td>
</tr>
</tbody>
</table>
Heparinized plasma samples were analyzed for glucose, blood urea, acetaminophen, triglycerides, and free fatty acids at all time points and also for total cholesterol, HDL cholesterol, and LDL cholesterol in the fasting samples by using enzymatic, colorimetric techniques adapted for the Xpand Dimension autoanalyzer (Dade Behring, Schwalbach, Germany).

Selected timed samples were also prepared for amino acid analysis by pipetting 500 μL heparinized plasma into a 2-mL Eppendorf microcentrifuge tube containing 50 μL 40% sulfosalicylic acid and vortex-mixed for 1 min. After standing in ice for 15 min, the tubes were centrifuged at 4°C for 15 min at 1500 rpm, and the supernatant fluid was pipetted into sample tubes and stored at −20°C until analyzed. Deproteinized plasma samples were analyzed for amino acids by using cation-exchange chromatography followed by postcolumn derivatization with ninhydrin and photometric detection at 570 and 440 nm with an amino acid analyzer (Biochrom 30 amino acid analyzer; Biochrom Ltd. Cambridges, United Kingdom). EDTA plasma containing protease inhibitors were analyzed for insulin and glucagon by using enzyme-linked immunosorbent assay kits (Linco, St Charles, MO).

### Calculations

Energy expenditure and carbohydrate and fat utilization were calculated from the respiratory exchange data by using standard equations (39). Protein oxidation in the fasting postabsorptive state was calculated from total urinary nitrogen in the overnight urine sample. After test meal ingestion, urinary nitrogen excretion, and subsequently protein oxidation, was corrected for any changes observed in the plasma urea nitrogen pool (40). Protein, carbohydrate, and fat oxidation rates were cumulated after meal ingestion and are expressed as grams oxidized over 330 min.

The postprandial glycemic and insulminemic responses to the meals were calculated as the postprandial incremental area under the curve (IAUC) above baseline at 2 h [to calculate the glycemic and insulin indexes, respectively (41)] and at the end of the test for the glucose (visit 2; 4.5 h) and isoeenergetic (visits 3–6; 5.5 h) test meals.

Individual appetite sensations were recorded for the duration of the experiment, and the mean (±SD) integrated values are expressed. An overall satiety score, the Composite Satiety Score (CSS), was calculated by introducing the results of the individual questions into the following equation:

\[
\text{CSS} = \frac{\text{Fullness} + (100 - \text{desire to eat}) + (100 - \text{hunger}) + (100 - \text{PFC})}{4}
\]

### Statistical analyses

Energy expenditure and satiety-related questions were calculated as the AUC from 0 to 330 min after consumption of the test meals. ANOVA in a mixed model setting with “meal” as a fixed effect, “initial energy expenditure” as a covariate, and “subject” as a random effect was then used to estimate the effect of each meal on the AUC at 330 min.

Postprandial blood variables were calculated as the IAUC at 2 h for glucose and insulin (representative of their respective indexes) and at the end of the test. ANOVA in a mixed model setting with “meal” as a fixed effect, “initial blood value” as a covariate, and “subject” as a random effect was then used to estimate the meal effects.

The maximum concentration and the time to maximum concentration were calculated with ANOVA in a mixed model setting with “meal” as a fixed effect and “subject” as a random effect, to estimate the meal effects. All P values were adjusted according to Tukey-Kramer’s multiple group comparisons procedure. SAS software (version 9.2; SAS Institute Inc, Cary, NC) was used, and the results are expressed as means ± SEMs, unless specified otherwise.

### RESULTS

The physical characteristics of the subjects are presented in Table 1.

### Energy expenditure

Energy expenditure measured before and after meal ingestion is illustrated in Figure 2A. Mean fasting, resting metabolic rates were not significantly different (1.18 ± 0.03 kcal/min) between tests and increased significantly after ingestion of all of the test meals; however, those containing protein stimulated energy expenditure more than did the isocaloric high-carbohydrate meal. It can also be seen that the whey protein meal elicited a larger response than did either the casein or soy meal, which had similar responses.

Energy expenditure calculated as AUC over 5.5 h (Figure 2B) increased after ingestion of all test meals and was higher after the whey meal than after either the casein (mean difference: 10 kcal; P = 0.0029) or the soy (mean difference: 13 kcal; P = 0.0001) meal. Casein and soy were not significantly different (mean difference: 2.6 kcal; P = 0.80). Energy expenditure after all of the protein test meals (whey, casein, and soy) was higher than that after the isocaloric high-carbohydrate meal (mean differences: 34, 24, and 24 kcal, respectively; P < 0.00001).

### Thermic effect

The thermic effects after the different test meals are illustrated in Figure 2C. Because the test meals were isocaloric, the thermic effects were a reflection of the AUC energy expenditure for each of the test meals. The thermic effect of whey (14.4 ± 0.5%) was greater than that of either casein (12.0 ± 0.6%; P = 0.002) or soy (11.6 ± 0.5%; P = 0.001), and the thermic effect of all 3 proteins was significantly greater than that of the high-carbohydrate test meal (mean differences: 7.7%, 5.4%, and 4.9% for whey, casein, and soy respectively; P < 0.0001).

### Substrate utilization

Substrate oxidation rates in the fasting, resting state were not significantly different before each test (carbohydrate: 94.8 ± 5.5 mg/min; fat: 51.9 ± 3.3 mg/min; protein: 62.7 ± 2.9 mg/min).
Carbohydrate oxidation increased after ingestion of all of the test meals, returning to baseline values 240 and 285 min after whey and casein, respectively, but remained higher than baseline at the end of the test after the soy and high-carbohydrate test meals. The changes in fat oxidation after ingestion of the 4 test meals are illustrated in Figure 3. After a slight increase after meal ingestion, fat oxidation decreased with all of the test meals, reaching nadirs at 165 min (whey and casein meals), 195 min (soy meal), and 210 min (high-carbohydrate meal). When cumulated over the 5.5-h test (Figure 3, inset), fat oxidation tended to be higher after whey (16.2 ± 1.1 g) than after soy (13.7 ± 1.0 g; P = 0.098) and higher than that after the high-carbohydrate meal (10.9 ± 0.9 g; P < 0.0001).

Protein oxidation increased almost 2-fold after consumption of the protein-containing test meals, from 62.7 ± 2.9 mg/min in the fasting state to 113.7 ± 8.0 mg/min during the test, and was not significantly different between the 3 conditions. After the high-carbohydrate meal, protein oxidation decreased from 59.9 ± 3.6 mg/min (fasting) to 48.2 ± 6.2 mg/min.

Satiety

The results presented in Table 4 represent the mean integrated value for each question calculated as the AUC/330 min. The desire to eat was significantly greater after the whey meal than after the casein (P < 0.005) and soy (P < 0.01) meals, but was not significantly different from that after the high-carbohydrate test meal. Similarly, hunger and prospective food consumption were greater after the whey meal than after the casein (P < 0.005) and soy (P < 0.01) meals, respectively. Because fullness represents the opposite of the preceding questions, the subjects felt more “full” after the casein (P < 0.005) and soy (P < 0.01) meals than after the whey test meal. There was no significant difference in thirst between the different meals, because water was allowed ad libitum.

After consuming the whey meal, the subjects experienced hunger/emptiness more rapidly, ie, the whey meal was less satiating than either the casein or the soy meal; however, no difference from the high-carbohydrate test meal was observed. Similarly, the scores after the high-carbohydrate test meal were not significantly different from those recorded after the casein and soy test meals.

The CSS reflected the interpretation of the individual satiety questions and indicated that the whey test meal was significantly less satiating than was either the casein (P = 0.0002) or the soy (P = 0.0005) meal.

How much the subjects “liked” the test meal was analyzed once, 10 min after they started consuming the meal. There was a trend for whey to be more “liked” than soy (P = 0.089), which was significantly different from the finding with the casein meal (P = 0.025).

Blood results

No differences were observed in fasting blood concentrations before all of the tests (Table 5).
Postprandial glycemia and insulinemia

The postprandial glycemic and insulinemic responses are presented in Figure 4, A and B, respectively. Although the high-carbohydrate meal contained almost 2.5 times more carbohydrate than did the glucose meal, peak values were not significantly different: 8.5 ± 0.3 and 8.1 ± 0.3 mmol/L, respectively. However, after the peak, glycemia decreased more rapidly after the smaller glucose load (Figure 4A).

Whey, casein, and soy lowered peak glycemia significantly in the presence of glucose (6.1 ± 0.2, 5.9 ± 0.2, and 5.9 ± 0.2 mmol/L, respectively), regardless of whether they were compared with the glucose meal [ie, the same glucose load (P < 0.01)] or with the high-carbohydrate meal [ie, the same amount of energy (P < 0.01)]. As a consequence, the 2-h IAUC (Figure 4A, inset) was significantly lower after the protein meals (72 ± 7, 84 ± 9, and 69 ± 7 mmol · 120 min/L for the whey, casein, and soy meals, respectively) than after the glucose (232 ± 14 mmol · 120 min/L; P < 0.001) and the high-carbohydrate (283 ± 17 mmol · 120 min/L; P < 0.0001) test meals. When expressed as the glycemic index, these values represent 100%, 33 ± 3%, 36 ± 3%, and 32 ± 4% (P < 0.01) and 129 ± 8% (P < 0.01) for the glucose, whey, casein, soy, and high-carbohydrate meals, respectively.

At the end of the test, the blood glucose IAUCs (Figure 4A, inset) were still significantly lower after the protein meals (122 ± 13, 146 ± 21, and 149 ± 17 mmol · 330 min/L for the whey, casein, and soy meals, respectively) than after the glucose (248 ± 15 mmol · 270 min/L) and the high-carbohydrate (411 ± 30 mmol · 330 min/L; P < 0.0001) test meals.

Insulinemia increased after all of the test meals (Figure 4B) and reached peak concentrations between 30 and 45 min. Plasma insulin was 654 ± 70 pmol/L for whey and was significantly higher than that after glucose (413 ± 45 pmol/L; P < 0.05) and the soy (408 ± 58 pmol/L; P < 0.05) test meal. The 2-h IAUC (Figure 4B, inset) was higher after the whey meal (37.5 ± 3.7 nmol · 120 min/L) than after the consumption of equivalent glucose loads as glucose (24.3 ± 2.5 nmol · 120 min/L; P = 0.02) or when combined with casein (26.3 ± 1.7 nmol · 120 min/L; P = 0.07) or soy (24.9 ± 2.3 nmol · 120 min/L; P = 0.03). No differences were observed between the whey and high-carbohydrate (41.4 ± 4.2 nmol · 120 min/L; P = 0.89) test meals. When expressed as the insulinemic index, these values represent 100%, 176 ± 8% (P < 0.01), 118 ± 9% (NS), 108 ± 8% (NS), and 184 ± 17% (P < 0.01) for the glucose, whey, casein, soy, and high-carbohydrate meals, respectively.

Although the 2-h insulin IAUCs after the whey meal were 43% and 51% higher than after the casein and soy meals, respectively, whey consumption did not lower peak glycemia or the incremental 2-h glucose AUC to the same extent. However, glycemia did return toward fasting concentrations more rapidly. Interestingly, at this time, the 2-h insulin IAUC after both casein and soy were very similar to that after the glucose meal, providing the same glucose load and resulting in insulin indexes of 118% and 108% (NS), respectively.

At the end of the test, the incremental AUC insulinemia (Figure 4B, inset) for whey (48.8 ± 4.0 nmol · 330 min/L) was still higher than that for glucose (26.0 ± 2.5 nmol · 270 min/L; 1)
The meals containing protein (\( \text{P} \)) were greater after the whey than after the casein meal (\( \text{P} = 0.026 \)).

After the high-carbohydrate test meal (\( \text{P} = 0.003 \), soy (241 ± 14 min; \( \text{P} = 0.001 \)), and high-carbohydrate (235 ± 11 min; \( \text{P} = 0.004 \)) meals, which indicated that casein was released more rapidly than the other test meals, which emptied from the stomach at similar rates.

Plasma amino acids were analyzed, in selected samples, in a subgroup of 12 subjects (data not shown). Increases were observed after each of the protein test meals, with total amino acids reaching higher peak values 60 min (6313 ± 601 nmol/mL) and 120 min (6376 ± 548 nmol/mL) after the whey meal than after the casein (5557 ± 546 nmol/mL at 60 min; \( \text{P} < 0.02 \)) or soy (5460 ± 394 nmol/mL at 120 min; \( \text{P} < 0.04 \)) meals. The increase after ingestion of the whey test meal was due to large increases of the essential amino acids leucine (+604 ± 82 nmol/mL), lysine (+524 ± 78 nmol/mL), valine (+373 ± 59 nmol/mL), and isoleucine (+278 ± 35 nmol/mL). These amino acids also increased after ingestion of the test meals containing casein and soy, but to a lesser extent. Plasma proline increased more after the casein meal (+285 ± 86 nmol/mL at 120 min) than after the whey (+133 ± 45 nmol/mL; 120 min) or soy (+127 ± 44 nmol/mL; 120 min) meal, which reflected the greater contribution to the amino acid profile of casein than of the other 2 protein sources (Table 3). Five and a half hours after ingestion of the whey and soy test meals, plasma total amino acids had returned to baseline values, but remained elevated after casein.

Acetaminophen was not detected in plasma until 45 min after test meal ingestion. Although the maximum concentration was not significantly different between treatments (range: 98–113 nmol/L), the time to maximum concentration was significantly less after the casein meal (182 ± 12 min) than after the whey (248 ± 14 min; \( \text{P} = 0.0003 \)), soy (241 ± 14 min; \( \text{P} = 0.001 \)), and high-carbohydrate (235 ± 11 min; \( \text{P} = 0.004 \)) meals, which indicated that casein was released more rapidly than the other test meals, which emptied from the stomach at similar rates.

Plasma amino acids were analyzed, in selected samples, in a subgroup of 12 subjects (data not shown). Increases were observed after each of the protein test meals, with total amino acids reaching higher peak values 60 min (6313 ± 601 nmol/mL) and 120 min (6376 ± 548 nmol/mL) after the whey meal than after the casein (5557 ± 546 nmol/mL at 60 min; \( \text{P} < 0.02 \)) or soy (5460 ± 394 nmol/mL at 120 min; \( \text{P} < 0.04 \)) meals. The increase after ingestion of the whey test meal was due to large increases of the essential amino acids leucine (+604 ± 82 nmol/mL), lysine (+524 ± 78 nmol/mL), valine (+373 ± 59 nmol/mL), and isoleucine (+278 ± 35 nmol/mL). These amino acids also increased after ingestion of the test meals containing casein and soy, but to a lesser extent. Plasma proline increased more after the casein meal (+285 ± 86 nmol/mL at 120 min) than after the whey (+133 ± 45 nmol/mL; 120 min) or soy (+127 ± 44 nmol/mL; 120 min) meal, which reflected the greater contribution to the amino acid profile of casein than of the other 2 protein sources (Table 3). Five and a half hours after ingestion of the whey and soy test meals, plasma total amino acids had returned to baseline values, but remained elevated after casein.
DISCUSSION

It is well documented that proteins have a greater thermic effect than do either carbohydrate or fat; however, less information is available concerning the possible effects of different protein sources. It has been suggested that satiety is influenced by different proteins (4, 42–47), and it has also been proposed that an increased thermic effect contributes to the satiating effect of foods (48, 49); however, few studies to our knowledge have investigated these variables together. Although Mikkelsen et al (29) observed that pork-meat protein stimulated 24-h energy expenditure more than that of soy in subjects consuming the diets over a period of 4 d, most studies in the literature have compared protein-rich meals or diets with those rich in fat or carbohydrate. The present findings confirm that protein-rich test meals have a greater thermic effect than that of carbohydrate and extend these results by showing that whey protein elicits a greater thermic response than does protein composed of either casein or soy.

It has been shown that whey and hydrolyzed proteins are more rapidly digested and absorbed than is casein and that this improves nitrogen as well as protein retention (25–28, 46). Similarly, animal protein rather than vegetable protein has been shown to influence protein turnover and favor protein synthesis (50, 51). Increased protein synthesis has been proposed as one possible mechanism responsible for the increased thermogenesis observed after high-protein than after high-carbohydrate diets (52), and the rate of protein synthesis has been observed to be more rapid, 2-fold greater (68% compared with 31%), after the consumption of whey than after that of casein (28). Consequently, differences in the rate of protein synthesis after whey, casein, and soy ingestion may explain the small, but significant, differences in thermic effect observed in the present study.

In addition to the observed effect on thermogenesis, different proteins may influence postprandial fat oxidation (Figure 3). Although fat oxidation cumulated over 5.5 h was not significantly different between the 3 protein meals, there was a trend for it to be less after soy than after whey ($P = 0.098$) consumption, which may have been shown if the study had been powered for fat oxidation rather than thermogenesis. Although this observation is perhaps surprising, Labayen et al (53) also observed that fat oxidation was not suppressed by a high-protein diet in lean and obese women and Alfenas et al (54) observed lower respiratory quotients, indicative of increased fat oxidation, after a breakfast meal containing whey protein than after meals containing casein or soy. Whereas it is tempting to suggest that the elevated glucagon concentrations observed after whey consumption may have interfered with insulin’s antilipolytic effects and permitted fat oxidation to continue at, more or less, fasting concentrations, evidence for the lipolytic effects of glucagon, especially in adipose tissue and in the presence of insulin, are controversial (55). However, the effects of glucagon may be related to hepatic rather than to peripheral adipose tissue lipolysis (56), because short-term high-protein diets have been shown to decrease intrahepatocellular lipids (57).

The addition of protein to carbohydrate-rich meals is known to decrease the postprandial glucose response, because of the insulinogenic effects of some of the component amino acids. This was very evident after whey consumption; however, despite an insulin index of 176%, which was 58% and 68% greater than those after casein and soy, their respective glycemic indexes were very similar (Figure 4A) and certainly not lower after whey. Such results are important when one considers that these 3 protein sources are commonly used as supplements to maintain and/or improve lean body mass in athletes and individuals who wish to lose or gain body weight. Whey supplements have been proposed to increase protein synthesis, and in the presence of increased insulin secretion, one would expect its anabolic effects to favor increased lean body mass. On the other hand, the use of casein or soy might be considered more favorable for blood glucose control, because their addition decreases glycemia with little, if any, effect on insulin secretion above that of the carbohydrate component of the meal. Acetaminophen appearance indicated that gastric emptying was more rapid after casein than after the high-carbohydrate, soy, and whey meals, which had very similar acetaminophen kinetics. Others have also observed that casein empties more rapidly from the stomach than does whey (46, 58), which is unexpected because casein precipitates in the stomach and one would expect it to empty more slowly. When casein protein precipitates, it forms a solid and a liquid phase, the latter of which is released more rapidly from the stomach. Hall et al (46), who also used acetaminophen as a marker of stomach emptying, suggest that acetaminophen remains solubilized in the liquid phase of the meal, which is emptied and absorbed more rapidly after the casein meal than after the whey test meal, in which acetaminophen is distributed more homogeneously. However, because acetaminophen kinetics after test meal ingestion were considerably delayed when compared with other plasma kinetics, it would appear that acetaminophen appearance is not a reliable marker of stomach emptying, at least under the conditions of our experiment.

Although there is general agreement that proteins are more satiating than carbohydrates and fats, the effect of different proteins on satiety is less clear. The present study indicated that whey was less satiating than was casein or soy, whereas others have found that, at habitual protein intakes, whey is more satiating than casein and soy and have suggested that this is due to increased plasma incretins (45, 46, 59), plasma amino acids (46), and thermogenesis (59) after whey consumption. The present results suggest that, although plasma amino acid concentrations rose more rapidly and higher after the consumption of whey, which was also more thermogenic, than after that of casein and soy, it had little effect on satiety. Satiety was greater after casein and soy consumption, which appeared to be related to slower stomach emptying, as indicated by their postprandial plasma amino acid kinetics rather than their acetaminophen kinetics, as discussed above. However, Bowen et al (45) do not believe that gastric emptying and amino acid absorption contribute to acute appetite regulation. The fact that the casein test meal was less “liked” than whey may also have influenced subjective appetite sensations. Interestingly, although many studies from the group of Westerterp-Plantenga have concluded that whey is more satiating than is casein, when the energy contribution of these proteins were increased in their standard breakfast from 10% to 25% they found no differences in appetite ratings (59). In the present study, the proteins provided 50% of the energy in the test meal, and the results suggest, as did those of Veldhorst et al (59), that these proteins have a dose-dependent effect on satiety.

In conclusion, the present study showed that not only do protein-rich meals have greater thermic effects than do isonenergetic high-carbohydrate meals, but also that the thermic
effect after whey consumption was significantly greater than that after casein and soy consumption. All of the proteins reduced postprandial glycemia to the same glucose load; however, casein and soy did so with very little if any increase in insulin secretion. These results for whey, casein and soy, together with those of other proteins, and their well-established satiating properties not only suggest that the protein content of the diet should be increased to more appropriate, or optimal, levels (60, 61), but that specific proteins can be incorporated into the diet to provide specific and desired attributes tailored to take into account the health and metabolic conditions of the individual.

The authors’ responsibilities were as follows—KJA: managed the protocol; KJA, AB-L, SO-A, and MB: recruited and medically screened the subjects and carried out the clinical trial according to GCP; KJA and LB: involved in the development, testing, and safety of the test meal ingredients; CA-Z, IM, SP, and CN-M: analyzed the biological samples; KJA and SE-A: performed the data and statistical analyses; and KJA: wrote the manuscript. All of the authors reviewed and modified the manuscript and participated in the design and development of the protocol. All of the authors are employees of Nestlé Ltd, which is a subsidiary of Nestlé Ltd and provides professional assistance, research, and consulting services for food, dietary, dietetic, and pharmaceutical products of interest to Nestlé Ltd. No other conflicts of interest were reported.

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