Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate[1–3]

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ABSTRACT

Background: Single-meal tests have shown that protein has greater thermogenic and satiating effects than does carbohydrate, which may be relevant for the prevention and treatment of obesity if these effects can be maintained over 24 h.

Objective: The effects of pork-meat protein, soy protein, and carbohydrate on 24-h energy expenditure were compared.

Design: Twelve young, healthy, overweight and mildly obese [body mass index (in kg/m^2): 26–32] nonsmoking men participated in a randomized, single-blind, 3-way crossover study lasting 4 d. The intervention had a 1–10-wk washout period. The 3 isoenergetic intervention diets were as follows: pork diet (29% of energy as fat and 29% as protein, mainly from pork meat), soy diet (29% of energy as fat and 28% as protein, mainly from soy), and carbohydrate diet (28% of energy as fat and 11% as protein). Twenty-four–hour energy expenditure was measured in a respiratory chamber at baseline and on day 4 of each intervention period.

Results: Twenty-four–hour energy expenditure was higher with the pork than with the soy (248 kJ/d, 1.9%; $P = 0.05$) or carbohydrate (492 kJ/d, 3.9%; $P < 0.0001$) diet and higher with the soy than with the carbohydrate (244 kJ/d, 1.9%; $P < 0.05$) diet. However, because of a higher satiating effect, energy intake was 10–15% lower during the chamber stay than at baseline ($P > 0.05$) with all 3 diets. The differences in energy expenditure remained unchanged after adjustment for differences in 24-h energy balance.

Conclusions: Substitution of carbohydrate with 17–18% of energy as either pork-meat or soy protein produced a 3% higher 24-h energy expenditure. The animal protein in pork meat produced a 2% higher 24-h energy expenditure than did the vegetable protein in soy. *Am J Clin Nutr* 2000;72:1135–41.

KEY WORDS Protein, carbohydrate, thermogenesis, obesity, energy expenditure, diet, pork, soy protein, men

INTRODUCTION

The prevalence of obesity is high and rapidly increasing in most parts of the world, and the comorbidities associated with obesity are a major health concern (1). Ad libitum, fat-reduced diets seem effective in preventing weight regain after weight loss (2) and can, without initial energy restriction, induce a weight loss of ≈5–10 kg in the obese (3). Recently, we showed that substitution of carbohydrate with protein in ad libitum, fat-reduced diets improves weight loss (4). On the basis of the results of short-term studies, protein seems to be superior to carbohydrate in promoting both satiety (5–16) and diet-induced thermogenesis (DIT) (17–22). The effects of different dietary proteins on 24-h energy expenditure (EE) have not been studied. The amino acid compositions of animal pork protein and vegetable soy protein differ substantially. With the use of these protein sources, two 4-d isoenergetic, protein-rich diets were created to compare their effects on 24-h energy expenditure with those of an isoenergetic, carbohydrate-rich diet.

SUBJECTS AND METHODS

Subjects

Twelve young, healthy, overweight-to-moderately obese [body mass index (in kg/m^2): 26–32] nonsmoking men participated in the study. Baseline characteristics of the subjects are shown in **Table 1**. Subjects were recruited by advertisements at local educational institutions. None of the subjects was taking any medication and all subjects were certified as being in good health by a staff physician on the basis of a physical examination. All subjects had stable weights at inclusion (ie, ±2 kg within the previous 3 mo) and were instructed to remain such throughout the study period. Informed consent was obtained according to the Declaration of Helsinki II, and the study was approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg. Subjects were informed that the purpose of the study was to compare the effects of 3 different diet compositions on EE and appetite and that the diets consisted of natural foods.

Study design

The study had a randomized, single-blind, 3-way crossover design. Each intervention lasted 4 d and was followed by a

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TABLE 1
Clinical characteristics of the subjects at baseline[1]

Characteristic	
Age (y)	26 ± 3.2
Body weight (kg)	101.4 ± 10.1
Height (m)	1.87 ± 0.07
BMI (kg/m^2)	28.9 ± 1.7
Fat-free mass (kg)[2]	73.0 ± 6.8
Fat mass (kg)[2]	29.1 ± 7.6
Fat mass (%)[2]	28.3 ± 5.8
$\dot{V}O_2$max[3]	
(mL/min)	4056 ± 608
(mL·min^{-1}·kg^{-1})	39.8 ± 6.5

[1] $\bar{x}$ ± SD; n = 12.
[2] Body composition estimated by dual-energy X-ray absorptiometry.
[3] Maximum oxygen uptake.

washout period of 1–10 wk. The 3 isoenergetic interventions were as follows: *1*) low-fat, high pork-meat protein diet (pork diet); *2*) low-fat, high-soy-protein diet (soy diet); and *3*) low-fat, high-carbohydrate diet (carbohydrate diet). The subjects were outpatients on the first 3 d of the intervention but spent day 4 of the intervention in a respiratory chamber. All subjects completed all interventions.

All subjects underwent a physical examination before inclusion, which included measurements of height, body weight, hip circumference, waist circumference, blood pressure, and heart rate. Further baseline measurements included estimation of body composition by dual-energy X-ray absorptiometry (DXA) (DPX-IQ, slow mode; Lunar, Madison, WI), assessment of maximal oxygen uptake by using a computer-controlled bicycle ergometer (ER 900; Jaeger Gmbh, Wuerzburg, Germany) in combination with a ventilated-hood system (Oxygen Champion; Jaeger Gmbh) for indirect calorimetry, assessment of habitual diet composition by using food diaries, and evaluation of habitual physical activity by using questionnaires. The energy intake during the baseline measurement of 24-h EE in the whole-body calorimeter was based on results from DXA scanning and previously published equations for predicting 24-h EE (23). The macronutrient composition of the subjects' diets during their stay in the respiratory chamber at baseline (screening period) was 50% of energy as carbohydrate, 35% as fat, and 15% as protein. The energy content of all 3 intervention diets was based on the EE measured during this screening period.

Each intervention lasted 4 d and was performed in pairs of 2 subjects consuming the same diet because of the limited number (n = 2) of respiratory chambers at the department. The subjects met at the department at 0800 on day 1, after fasting overnight, for measurements of the meal-induced effects on appetite and blood indexes. At 0830, the subjects were served a breakfast providing 3 MJ with an intervention-specific composition. Subjective appetite sensations were recorded and blood samples were collected until 1400, at which time a meal with a composition that was standard for all interventions was served to measure ad libitum food intake. Intervention diets consumed from the evening of day 1 until the evening of day 3 were served to the subjects with detailed instructions on preparing food, recording leftovers, and the importance of maintaining physical activity levels during the 3 intervention periods. The subjects remained as outpatients until 2200 on day 3, at which time they returned to the department and spent the night in the respiratory chamber. Measurements were performed from 0900 on day 4 until 0900 on day 5. Meals were served at fixed times and the subjects were instructed to follow a fixed exercise program and to spend the rest of the time engaged in sedentary activities.

Diets

The 3 interventions consisted of diets with different isoenergetic compositions and that varied in protein type and in the ratios of protein to carbohydrate (**Table 2**). The diets were matched for energy density and fat and fiber contents over the 4-d intervention period. The carbohydrate diet contained more carbohydrate and less protein than did the 2 high-protein diets (ie, the pork and soy diets). Most of the protein (20% of energy) in the pork diet was pork meat and in the soy diet was soy. The diets were prepared in the kitchen at the Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Copenhagen. The energy content of the individual isoenergetic intervention diets was based on baseline 24-h EEs. Leftovers during the first intervention were registered so that the same type and amount of food could be removed from subsequent diets, thereby maintaining a uniform energy content during all interventions. The subjects were blinded to the different compositions of the 3 intervention diets by use of the same menu for each (**Table 3**), the aim of which was that each diet have the same organoleptic quality. Samples of the 3 diets were chemically analyzed for macronutrients, with the protein content estimated on the basis of nitrogen analysis and a protein-nitrogen ratio of 6.25 for all diets (Table 2). The diets were analyzed for concentrations of individual amino acids as well (**Table 4**). Actual intakes of energy, protein, and nitrogen after subtractions of leftovers, both during the chamber stay (baseline) and during the total 4-d intervention period, are shown in **Table 5**.

TABLE 2
Composition of the 3 intervention diets and the diet at baseline[1]

	Diet			
	Pork	Soy	Carbohydrate	Baseline
Energy (kJ/100 g)	433	439	424	515
Fat				
(g/100 g)	3.3	3.4	3.1	4.8
(% of energy)	29.0	29.4	27.8	35.0
Carbohydrate				
(g/100 g)	10.6	11.0	15.2	15.2
(% of energy)	41.6	42.6	60.9	50.0
Nitrogen (g/100 g)	1.20	1.15	0.45	0.72
Protein[2]				
(g/100 g)	7.5	7.2	2.8	4.5
(% of energy)	29.4	27.9	11.2	15.0
Fiber				
(% of energy)	1.4	1.6	1.6	1.1
(g/MJ)	3.2	3.6	3.8	2.1
Pork protein[3]				
(g/100 g)	5.0	0	0	0.5
(% of energy)	19.6	—	—	1.6
Soy protein[3]				
(g/100 g)	0	4.8	0	0
(% of energy)	—	18.6	—	—

[1] Values based on chemical analysis of one sample of each diet.
[2] Protein content based on nitrogen analysis and a protein-nitrogen ratio of 6.25.
[3] Protein type based on distribution of ingredients in the diets.

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TABLE 3
Menus on the 4 d of the intervention diets

	Day 1	Day 2	Day 3	Day 4
Breakfast	Omelet with bread	Potato mix with bread, butter, and orange juice	Potato mix with bread, butter, and orange juice	Potato mix with bread, butter, and orange juice
Snack	—	Bread with leek paté	Bread with leek paté	—
Lunch	Casserole with pasta and meat	Meat loaf with potato salad	Meat loaf with potato salad	Meat loaf with potato salad and bread with leek paté
Snack	—	Bread with leek paté	Bread with leek paté	—
Dinner	Vegetable casserole with bread, peas, and corn	Vegetable-based pie, melon slice, and orange soda	Rice soup with bread and feta cheese	Pasta dish

Measurement of energy expenditure

EE was measured by indirect calorimetry at baseline during a 24-h stay in a respiratory chamber and on day 4 of each intervention. The respiratory chamber used consists of 2 open-circuit chambers, which were described in detail previously (24). The 2 chambers work independently, each having a floor area of 6.5 m^2 and a volume of 14.7 m^3, and are equipped with facilities (eg, writing desk, chairs, bed, stationary bicycle, telephone, toilet, and television) to provide a "pleasant" stay for the subjects. The gas exchange of the subjects was calculated from measurements of oxygen and carbon dioxide concentrations (Hartman and Braun analyzers, Frankfurt, Germany) at the outlet of the chamber and from measured air flow through the chambers. Urine was collected during the 24-h period and protein oxidation was calculated from the urinary nitrogen (UN) content. The room temperature was kept constant at 24 °C during the day and at 18 °C during the night. EE and utilization rates of lipid and carbohydrate were calculated as described by Livesey and Elia (25). The spontaneous physical activity in each of the respiratory chambers was assessed by microwave radar (Sisor Mini-Radar; Static Imput System, Lausanne, Switzerland) Zettler Ghz-Doppler Mime) and stored in the computer.

The within-subjects CV for 24-h EE measurements were reported previously to be 2–3% (24, 26). Calibration of the respiratory chamber was performed every 6 mo by comparing a known volume of carbon dioxide and nitrogen entering the chamber with the volume of carbon dioxide measured and the volume of oxygen displaced by the unit. The estimated DIT was 3% of energy as fat, 8% as carbohydrate, and 30% as protein. The measured DIT of the 3 diets was expressed relatively as differences in measured 24-h EE between diets.

Primary-effect measures

The primary-effect measures were 24-h EE measured from 0900 on day 4 to 0900 on day 5 and during the 4 subperiods: *1*) basal metabolic rate (BMR) measured from 0800 to 0900 on day 5, *2*) sleeping EE (SEE) measured from 0100 to 0600 on day 5, *3*) DIT measured from 1800 to 2300 on day 4, and *4*) EE measured from 0900 to 1800 (daytime EE) on day 4. EE are average values for the specific time period. Twenty-four–hour UN excretion (24-h UN) during the chamber stay, 24-h spontaneous physical activity during the chamber stay, total energy and fiber intakes on days 1–4, energy and fiber intakes during the chamber stay, energy balance during the chamber stay, duration of washout period, and body weight after the chamber stay were investigated as explanatory variables of differences in EE.

Statistics

All values are expressed as means ± SDs unless otherwise specified. A P value <0.05 was chosen as the level of significance. Effects were analyzed by analysis of variance followed by pairwise comparisons (paired samples t tests) of individual means of the 4 dietary conditions during the chamber stays: pork diet, soy diet, carbohydrate diet, and baseline. Possible determinants of differences in EE were investigated by using correlation analysis. A P value <0.10 was chosen as the criterion for including the determinant in an analysis of covariance. A general linear model was used with EE as the dependent variable, diet and subject as fixed factors, and the determinants as covariates. The effects of covariates on differences in 24-h EE were further investigated by using stepwise linear regression. All P values underwent a Bonferroni correction. All statistical analyses were done by using SPSS (version 8.0 for WINDOWS; SPSS Inc, Chicago).

RESULTS

Twenty-four–hour EE was higher after the pork diet than after the soy diet (248 kJ/d, 1.9%), than after the carbohydrate diet

TABLE 4
Amino acid compositions of the intervention diets

	Diet		
	Pork	Soy	Carbohydrate
	% of total		
Aspartic acid	9.1	10.1	9.1
Threonine[1]	4.1	3.4	3.3
Serine	3.8	4.4	4.3
Glutamic acid and glutamine	18.6	21.5	25.5
Proline[1]	5.2	6.3	7.3
Glycine	3.7	3.5	3.2
Alanine[1]	4.9	3.9	4.1
Cysteine and cystine	1.2	1.4	1.7
Valine	5.6	5.3	5.3
Methionine[1]	3.0	1.9	2.1
Isoleucine	5.1	4.9	4.3
Leucine	8.5	8.1	7.6
Tyrosine	3.8	4.0	3.7
Phenylalanine	4.7	5.4	5.0
Histidine[1]	4.1	2.9	2.5
Tryptophan	0.3	0.3	0.5
Lysine[1]	7.8	5.8	4.7
Arginine	6.5	7.0	5.9
Total	100	100	100

[1] There was a >20% relative difference between the pork and soy diets.

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TABLE 5
Dietary intakes and energy balance during the intervention diets and at baseline[1]

	Diet			
	Pork	Soy	Carbohydrate	Baseline
Baseline (day 4)				
Energy (MJ/d)	10.6 ± 2.2[a]	10.6 ± 2.0[a]	10.8 ± 2.2[a,b]	12.2 ± 0.9[b]
Protein (g/d)	183.4 ± 38.0[a]	174.5 ± 33.5[a]	71.1 ± 14.7[b]	107.4 ± 8.3[c]
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	1.81 ± 0.39[a]	1.71 ± 0.35[a]	0.70 ± 0.16[b]	1.05 ± 0.07[c]
Nitrogen (g/d)	42.2 ± 8.5[a]	39.1 ± 7.4[a]	16.2 ± 3.4[b]	—
Fiber (g/d)	33.9 ± 7.0[a]	38.1 ± 7.3[b]	40.9 ± 8.4[b]	25.6 ± 2.0[c]
Total intervention (days 1–4)				
Energy (MJ/d)	11.4 ± 1.4[a]	11.8 ± 1.4[a]	11.6 ± 1.6[a]	—
Protein (g/d)	196.0 ± 24.1[a]	193.5 ± 23.6[a]	76.2 ± 10.2[b]	—
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	1.94 ± 0.24	1.90 ± 0.24	0.75 ± 0.11	—
Nitrogen (g/d)	44.1 ± 5.4[a]	43.3 ± 5.3[a]	17.5 ± 2.3[b]	—
Fiber (g/d)	36.4 ± 4.4[a]	42.5 ± 5.2[b]	43.9 ± 5.8[b]	—
Energy balance				
Chamber stay (day 4) (MJ/d)	−3.63 ± 2.96[a]	−3.20 ± 2.57[a]	−2.67 ± 2.93[a]	−0.50 ± 0.86[b]
Total intervention period (days 1–4) (MJ/d)	−2.52 ± 1.53[a]	−1.54 ± 1.55[a,b]	−1.54 ± 1.91[b]	—

[1] $\bar{x} \pm$ SD; $n = 12$. All values based on chemical analysis. Means with different superscript letters are significantly different, $P < 0.05$ (paired-samples t test).

(492 kJ/d, 3.9%), and than at baseline (595 kJ/d, 4.8%) and higher after the soy diet than after the carbohydrate diet (244 kJ/d, 1.9%) (**Table 6**). BMR was higher after the pork diet than after the carbohydrate diet (0.3 kJ/min, 4.5%) and after the pork diet than after the baseline diet (0.3 kJ/min, 5.0%). SEE was higher after the pork diet than after the carbohydrate diet (0.2 kJ/min, 3.2%) and after the soy diet than after the carbohydrate diet (0.2 kJ/min, 3.1%). DIT was higher after the pork diet than after the carbohydrate diet (0.5 kJ/min, 5.5%) and higher after the soy diet than after the carbohydrate diet (0.2 kJ/min, 1.9%). Daytime EE was higher after the pork diet than after the carbohydrate diet (0.4 kJ/min, 3.0%). The 24-h UN values were similar after the pork and soy diets and higher after the pork and soy diets than after the carbohydrate diet (14.5 g UN/d, 128%) and at baseline (10.0 g UN/d, 63%). There was a trend toward a 4% lower 24-h spontaneous physical activity after the soy diet than after the pork and carbohydrate diets (Table 6).

All subjects completed all 3 interventions, and energy intakes were similar during the 3 interventions (Table 5). However, energy intakes at baseline during the chamber stay were significantly lower after the pork and soy diets than at baseline (by 1600 kJ/d, 13.1%). Energy intakes during the carbohydrate diet were not significantly different from those during the pork diet, during the soy diet, or at baseline (Table 5). Absolute protein intake was similar during the pork and soy diets. Total protein intake was 108 g/d (152%) higher on average during the 2 high-protein diets than during the carbohydrate diet. Protein intake at baseline was intermediary between the pork and soy diets and the carbohydrate diet. The daily fiber intake was significantly higher (4.2 g/d, 12.4%) with the soy diet than with the pork diet at baseline and during the other days of the intervention period. Fiber intakes during all interventions were higher than those at baseline (Table 5). Differences in fiber intake did not correlate with differences in 24-h EE or 24-h UN, except for the correlation between 24-h UN and baseline and between 24-h EE and baseline.

Energy balances during the chamber stays were similar with the 3 intervention diets but energy balance was significantly lower (6-fold) at baseline than during the 3 diets (on average by 2667 kJ/d). Estimated energy balance for the total intervention period was more negative with the pork diet than with the carbohydrate diet (980 kJ/d) but there was no significant difference between the soy and carbohydrate diets (Table 5).

Differences in 24-h UN and in 24-h spontaneous physical activity correlated with differences in 24-h EE between the pork and soy diets ($r = 0.62$ and $r = 0.59$, respectively). A trend toward covariation between differences in 24-h UN and differences in 24-h spontaneous physical activity between the pork and soy diets was also found ($r = 0.52$, $P < 0.1$). Differences in 24-h EE between the pork and soy diets did not persist after adjustment for 24-h UN, 24-h spontaneous physical activity, or both. Stepwise linear regression analysis showed that differences in 24-h UN explained 32% of the differences in 24-h EE between the pork and soy diets, whereas differences in 24-h spontaneous physical activity did not explain the variation in differences in 24-h EE.

Differences in energy intake, energy balance, or washout duration did not correlate with differences in 24-h EE. Differences in 24-h UN between the pork and soy diets did not correlate with differences in energy balance or in intakes of nitrogen, protein, fiber, or energy; adjustment for 24-h energy balance reduced the difference in 24-h EE between these 2 diets (145 kJ/d, 1.6%).

Pairwise comparisons between measured and predicted differences in DIT showed that measured differences were higher than predicted between all diets, except for the differences between the soy and carbohydrate diets. Measured DIT was 211 kJ/d, which was 6-fold higher than the predicted difference between the pork and soy diets (**Table 7**). There was a trend toward a lower (0.8 kg, 0.8%; NS) fasting body weight after the chamber stay with the pork diet than with the soy diet (Table 6). Adjustment for differences in body weight increased the differences in 24-h EE between the pork and soy diets significantly to 2.8%.

DISCUSSION

The main finding of the present study was that the isoenergetic substitution of carbohydrate with either pork or soy protein produced a nearly 3% higher 24-h EE on day 4 of the intervention despite a slightly lower energy intake with these 2 high-protein diets. This finding was observed consistently for EE,

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TABLE 6
Measurements of energy expenditure (EE) and other indexes during the respiratory chamber stays[1]

	Diet			
	Pork	Soy	Carbohydrate	Baseline
24-h EE (MJ/d)	13.11 ± 1.0[a]	12.86 ± 0.89[b]	12.62 ± 0.98[c]	12.52 ± 1.06[b,c]
Basal metabolic rate (kJ/min)	6.90 ± 0.58[a]	6.73 ± 0.58[a,b]	6.60 ± 0.37[b]	6.57 ± 0.61[b]
Sleeping EE (kJ/min)	6.04 ± 0.44[a]	6.03 ± 0.33[a]	5.85 ± 0.40[b]	5.90 ± 0.43[a,b]
Diet-induced thermogenesis (kJ/min)	9.08 ± 0.83[a]	8.77 ± 0.58[a]	8.61 ± 0.65[b]	8.75 ± 0.64[a,b]
Daytime EE (kJ/min)	12.10 ± 1.12[a]	11.91 ± 1.07[a,b]	11.75 ± 1.15[b]	11.47 ± 1.38[a,b]
24-h Urinary nitrogen excretion (g/d)	26.2 ± 4.4[a]	25.4 ± 4.0[a]	11.3 ± 1.5[b]	15.8 ± 3.2[c]
24-h Spontaneous physical activity (%)	9.53 ± 1.18[a]	9.05 ± 0.95[a]	9.33 ± 0.97[a]	9.38 ± 1.19[a]
Body weight (kg)[2]	101.9 ± 10.6[a]	102.7 ± 10.2[a]	102.6 ± 10.2[a]	102.3 ± 10.1[a]

[1] $\bar{x} \pm$ SD; $n = 12$. Means with different superscript letters are significantly different, $P < 0.05$ (paired-samples t test).
[2] Measured after the stay in the respiratory chamber.

BMR, SEE, and DIT. The finding of a higher thermogenic effect of protein agrees with the results of previous short-term studies (17–22) and may partly explain the larger weight loss shown previously in obese subjects who consumed a high-protein diet ad libitum for 6 mo than in those who consumed a high-carbohydrate diet with the same reduced-fat content (4). The fact that EE was greater with the high-protein diets (soy and pork diets) than at baseline, despite a lower energy intake during the high-protein diets, further supports the observation that high-protein diets increase EE. The findings of the present study further suggest that animal protein in the form of pork meat induces a nearly 2% higher increase in 24-h EE than does a vegetable protein source in the form of soy. The measured difference in DIT between the pork and soy diets was 6-fold higher than the predicted difference, suggesting that the thermogenic effect of protein depends on the protein type.

Energy intake during the chamber stay was significantly lower after the 2 high-protein diets than after the baseline diet. This finding was probably due to a high satiating effect of the protein-rich diets, which made it impossible for most of the subjects to eat the served amount of food. The average energy density of the 3 intervention diets was 432 kJ/100 g, or 0.23 kg food/MJ. The energy density of the baseline diet was 515 kJ/100 g, 19% higher than that of the intervention diets, demanding only 0.19 kg food/MJ. Differences in energy intake between the 3 intervention diets and the baseline diet may be explained by differences in energy density, but the difference in energy intake between the 2 high-protein diets and the baseline diet but not between the carbohydrate and baseline diets suggests a more satiating effect of protein than of carbohydrate. This finding agrees with the results of previous short-term studies, suggesting a more satiating effect of protein than of carbohydrate and fat (5, 6, 9–11, 15, 16). Differences in energy intake between the 3 intervention diets and the baseline diet probably were due mainly to the higher fiber content and lower energy density of the intervention diets. Fiber content and energy density have large effects on energy intake, at least in the short-term (27). Differences in energy intake did not correlate with differences in EE.

Estimated energy balance during the total 4-d intervention period was nearly 1 MJ/d more negative with the pork diet than with the carbohydrate diet, whereas energy balance with the soy and carbohydrate diets was not significantly different from that with the pork diet. This indicates that the pork diet had the highest potential to induce negative energy balance. The biological rationale behind the higher thermogenic effect of protein than of carbohydrate may be that the body has no storage capacity to cope with high intakes of protein and therefore has to process it metabolically, which readily increases thermogenesis (28, 29). The thermogenic effect of protein may be mediated through increased protein synthesis and the high ATP costs of peptide-bound synthesis as well as other aspects of the increased protein turnover during increased protein intake (18, 30–32).

Urea production and gluconeogenesis are other energetically costly pathways of protein metabolism (33). Increased protein turnover can explain most (68%) of the acute thermogenic effect of protein feeding (21), and increased protein turnover generally results in increased EE (34). High-protein meals may also increase proton-pump activity in liver membranes and thereby induce uncoupled respiration (35). A hypothesis has been proposed concerning different hormonal and neural effects of the protein-carbohydrate ratio of the diet [ie, thyroid hormones, androgens, catecholamines, and growth hormone (24, 36–38)] that may influence EE, and controlled studies indicated that an increase in the protein-carbohydrate ratio of the diet will increase the postprandial glucagon-insulin ratio (39–42). Short-term studies indicated that protein intake does not increase sympathetic activity as measured by norepinephrine concentrations postprandially (43) and it is not known whether any protein-induced effects on sympathetic activity are dependent on the amino acid composition of the ingested protein. The main limitations of the present study were that protein absorption was not measured and that the actual protein-nitrogen ratios of the 2 high-protein diets (ie, soy and pork diets) were unknown. Soy protein may have a lower protein-nitrogen ratio than does pork protein.

TABLE 7
Predicted and measured differences in diet-induced thermogenesis (DIT)[1]

Diet	Measured DIT	Predicted DIT	Measured − predicted DIT
		kJ	
Pork-soy	250	39	211
Pork-carbohydrate	490	401	89
Pork-baseline	590	215	375
Soy-carbohydrate	240	362	−122
Soy-baseline	340	176	164
Carbohydrate-baseline	100	−186	286

[1] Values are differences between diets pairwise. DIT was predicted from macronutrient intake on the basis of the following assumptions for DIT: 3% of energy as fat, 8% as carbohydrate, and 30% as protein. Differences in measured DIT were defined as differences in 24-h EE between diets.

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Twenty-four–hour UN was identical with the pork and soy diets, which indicates that protein absorption is the same after consumption of animal- and vegetable-based protein, as shown previously in one study (44) but not in another (45). The latter study was performed in ileostomy patients with a generally low protein absorption with both diets. Despite a higher fiber intake with the soy diet, differences in fiber intake between the pork and soy diets did not correlate with differences in 24-h UN. Nitrogen intake differed by 1.9% between the pork and soy diets, whereas nitrogen excretion differed by 3.1%. This suggests that protein absorption probably was the same during the 2 high-protein diets despite the 16.8% difference in fiber intake between the 2 diets. Furthermore, differences in neither the nitrogen intake on day 4 nor in the total nitrogen intake on days 1–4 between the pork and the soy diets correlated with differences in 24-h UN ($r = -0.23$, $P = 0.5$ and $r = 0.08$; NS), indicating that factors other than nitrogen intake were responsible for the difference in 24-h UN between the pork and soy diets.

Differences in 24-h UN correlated significantly with differences in 24-h EE and could explain 32% of the total variation. Adjustment for 24-h UN eliminated the difference in 24-h EE between the pork and soy diets; 24-h UN can be considered a marker of effects related to 24-h EE. The observed correlation between differences in 24-h UN and differences in 24-h EE could thus be a consequence of physiologic changes affecting both EE and UN, eg, increased rates of urea synthesis, gluconeogenesis, and protein breakdown. Differences in physical activity level could also have induced differences in both amino acid oxidation (ie, UN) and total EE. Twenty-four–hour spontaneous physical activity tended to be lower with the soy diet than with the pork and carbohydrate diets, indicating that consumption of soy protein may lower physical activity. The reason for this is unknown; however, a difference in amino acid–specific effects on sympathetic activity is a possible explanation.

Animal studies indicate that the amount of protein synthesis after protein intake depends on how well the composition of essential amino acids in the dietary protein matches the optimum requirements for protein synthesis in the body (46, 47). Likewise, human studies indicate that a well-balanced amino acid mixture produces a higher thermogenic response than does an amino acid mixture with a lower biological value, ie, a different amino acid composition than is used for protein synthesis (48). This may partly explain why intake of soy protein results in less protein synthesis than does intake of animal protein (49). A comparison of the essential amino acid composition (mg/g crude protein) of the pork and soy diets with that of the WHO-recommended pattern (50) showed that histidine, methionine + cysteine, and tryptophan were the limiting amino acids in both protein sources. Analytic problems may have been the reason for the relatively low tryptophan content in both proteins. The protein in the pork diet contained 27% more histidine and 14% more methionine and cysteine than that in the soy diet. This may have induced a higher protein synthesis and turnover rate during the pork diet, and thus a higher EE. Animal studies further suggest that leucine is especially thermogenic compared with other amino acids and that the effect is mediated through increased protein synthesis (51). The pork diet contained ≈5% more leucine than did the soy diet. Differences in the protein-specific heat equivalent and in the protein-nitrogen ratio between the pork and soy diets did not explain the differences in 24-h EE between these 2 diets calculated from the indirect calorimetry equations.

Low EE is a risk factor for obesity (52), which is thought to be genetically determined (53). Modest stimulation (≈2–5%) of 24-h EE may improve the treatment and prevention of obesity (54). This study showed that replacement of fat with pork-meat protein increased 24-h EE by ≈4.7%, in concert with a decrease in energy intake. Neither the soy nor the carbohydrate diet resulted in 24-h EE values that were higher than those after the baseline diet, which had a micronutrient composition equivalent to that of the average Danish dietary intake. Further studies are needed to show whether a higher intake of pork-meat protein than of vegetable protein and carbohydrate can improve reductions in body weight and fat mass in ad libitum diets with a recommended fat content in overweight and obese individuals.

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