

# Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake<sup>1-3</sup>

Anne Raben, Lisa Agerholm-Larsen, Anne Flint, Jens J Holst, and Arne Astrup

## ABSTRACT

**Background:** It has been suggested that the satiating power of the 4 macronutrients follows the oxidation hierarchy: alcohol > protein > carbohydrate > fat. However, the experimental evidence for this is still scarce.

**Objective:** The goal was to investigate the effects on appetite, energy intake and expenditure, and substrate metabolism of meals rich in 1 of the 4 macronutrients.

**Design:** Subjective appetite sensations, ad libitum food intake, energy expenditure, substrate metabolism, and hormone concentrations were measured for 5 h after breakfast meals with similar energy density and fiber contents but rich in either protein (32% of energy), carbohydrate (65% of energy), fat (65% of energy), or alcohol (23% of energy). Subjects were normal-weight, healthy women ( $n = 9$ ) and men ( $n = 10$ ) studied in a crossover design.

**Results:** There were no significant differences in hunger or satiety sensations or in ad libitum energy intake after the 4 meals. Diet-induced thermogenesis was larger after the alcohol meal (by 27%;  $P < 0.01$ ), whereas protein produced an intermediary response (17%; NS) compared with carbohydrate and fat (meal effect:  $P < 0.01$ ). After the alcohol meal, fat oxidation and leptin concentrations were greatly suppressed (meal effects,  $P < 0.0001$  and  $P < 0.05$ ) and triacylglycerol concentrations were as high as after the fat meal.

**Conclusion:** Intake of an alcohol-rich meal stimulates energy expenditure but suppresses fat oxidation and leptin more than do isoenergetically dense meals rich in protein, carbohydrate, or fat. Despite differences in substrate metabolism and hormone concentrations, satiety and ad libitum energy intake were not significantly different between meals. Our data, therefore, do not support the proposed relation between the macronutrient oxidation hierarchy and the satiety hierarchy. *Am J Clin Nutr* 2003;77:91-100.

**KEY WORDS** Satiety, hunger, macronutrient oxidation, obesity, glucose, insulin, lactate, triacylglycerol, glucagon, nonesterified fatty acids, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1, glucagon-like peptide 2, leptin

## INTRODUCTION

The prevalence of obesity is increasing dramatically, creating a growing global health problem (1). Obesity is thought to be a multifactorial disorder. Two major causes of the development of overweight and obesity are a reduction in physical activity and an increase in energy intake, primarily from fat-rich, energy-dense foods (1). In the energy balance equation, energy intake is probably

much more sensitive to dietary changes than is energy expenditure (2, 3). The mechanisms involved in the regulation of food intake are, however, complex and imply several different mechanisms, involving internal as well as external factors. Some are macronutrient related (eg, glucostatic and lipostatic mechanisms) and others are related to hormones and afferent signals [eg, insulin, glucagon-like peptide 1 (GLP-1), and leptin]. Furthermore, energy density (kJ/g) probably has a strong preabsorptive effect on food intake and thereby on energy intake (4).

The measured thermic effect of separate nutrients is highest and most prolonged for protein (20-30%), followed by carbohydrate (5-10%) and fat (0-3%) (5, 6). The position of alcohol is still unclear. There also seems to be a line of evidence suggesting a hierarchy in macronutrient oxidation rate during the postprandial state with the sequence alcohol > protein > carbohydrate > fat (3). Furthermore, protein may create higher satiety sensations than carbohydrate, which may be more satiating than fat (7-11). This has therefore led to the suggestion that a high satiating efficiency of a macronutrient is related to its oxidation rate—with the position of alcohol still being unclear.

To our knowledge, all 4 macronutrients have not before been included in a study investigating their effects on appetite, energy intake and expenditure, and macronutrient metabolism at the same time. The purpose of the present study was therefore to investigate the effects of meals rich in either protein, carbohydrate, fat, or alcohol on postprandial energy and substrate metabolism, subjective appetite sensations, and ad libitum energy intake. Blood samples were taken in one-half of the subjects for substrate and hormone analyses to explain possible differences in the primary outcome variables. Young, normal-weight women and men were included.

<sup>1</sup> From the Research Department of Human Nutrition, Center for Advanced Food Studies, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark (AR, LA-L, AF, and AA), and the Department of Medical Physiology, The Panum Institute, University of Copenhagen (JJH).

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<sup>3</sup> Address reprint requests to A Raben, Research Department of Human Nutrition, Center for Advanced Food Studies The Royal Veterinary and Agricultural University, 30 Rolighedsvej, 1958 Frederiksberg C, Denmark. E-mail: ar@kv1.dk. Received November 5, 2001.

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**TABLE 1**  
Characteristics of the subjects enrolled<sup>1</sup>

	Women (n = 9)	Men (n = 10)	All subjects (n = 19)	Subgroup (n = 5 M, 5 F)
Age (y)	22.9 ± 0.9	23.7 ± 0.5	23.3 ± 0.5	22.4 ± 0.5
Weight (kg)	60.9 ± 1.6	75.7 ± 2.6	68.7 ± 2.3	67.1 ± 3.5
Height (m)	1.69 ± 0.02	1.82 ± 0.02	1.76 ± 0.02	1.76 ± 0.03
BMI (kg/m <sup>2</sup> )	21.3 ± 0.5	22.9 ± 0.4	22.1 ± 0.4	21.5 ± 0.5
Fat mass (%)	22.1 ± 1.1	15.8 ± 1.1	18.8 ± 1.1	17.6 ± 1.4
Energy requirement (MJ/d) <sup>2</sup>	9.5 ± 0.2	13.7 ± 0.3	11.7 ± 0.5	11.5 ± 0.7

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . Subgroup, subjects for whom blood samples were taken.

<sup>2</sup>Estimated energy requirements based on tables from FAO/WHO/UNU (16).

## SUBJECTS AND METHODS

### Experimental design

The study used a crossover design in which baseline and postprandial variables were measured over a period of 5 h after consumption of the 4 test meals. The test meals were given in random order to the subjects on separate days with  $\geq 4$  wk and no more than 8 wk separating the test days. The subjects were not allowed to engage in strenuous physical activity for 2 d before the test day. The day before each test day, subjects were given an individual standardized, weight-maintenance, carbohydrate-rich diet for 1 d. These measures were taken to ensure equally filled glycogen stores and similar macronutrient balance in each individual on the 4 different test days (12).

### Subjects

According to our previous reproducibility study on appetite scores, 8–15 subjects are needed to obtain statistically significant differences in mean postprandial scores when using a crossover design (13). To account for dropouts, 20 healthy subjects (10 female and 10 male) were enrolled after announcement at the Royal Veterinary and Agricultural University and the University of Copenhagen. Inclusion criteria were as follows: aged 20–30 y; normal weight (body mass index, in kg/m<sup>2</sup>, > 18.5 but < 25.0); nonsmoker; not an elite athlete; normal alcohol habits; no history of obesity, diabetes, or liver diseases; normal blood pressure; and no regular use of medications (except for birth control pills). Before entering the study, the subjects were screened to ensure that they fulfilled the inclusion criteria. The women were screened for a regular menstrual cycle, and during the study the 4 separate test days were placed at the same time of the women's cycle (between days 7 and 11 in the follicular phase) so that hormonal fluctuations would not affect the measurements (14, 15). Blood samples were collected during the test days from 10 randomly chosen subjects (5 women, 5 men).

All subjects gave written consent after the experimental procedure had been explained to them. The study was approved by the Municipal Ethical committees of Copenhagen and Frederiksberg to be in accordance with the Helsinki-II declaration.

Of the 20 enrolled subjects, 1 subject (a woman) was excluded because of illness (migraine) on a test day. Thus, 19 subjects completed the study and carried out 4 test days each. The subject characteristics are presented in **Table 1**.

### Diets

The standard diet contained 49.2% of energy as carbohydrate, 37.4% of energy as fat, 13.2% of energy as protein, and 1.7 g dietary fiber/MJ, and had an energy density of 8.5 kJ/g. The diet was prepared at the Research Department of Human Nutrition from normal Danish food items according to each subjects' individual

energy requirements, adjusted to the nearest 0.5 MJ. The subjects' energy requirements for the standard diet and the test meals were determined by using World Health Organization tables according to age, weight, and sex (16). We used the multiplication factors of 1.64 for women and 1.78 for men to account for the medium physical activity of the subjects. The subjects were instructed to adhere strictly to the standard diet.

Four different test meals rich in protein, carbohydrate, fat, or alcohol, respectively, were tested in a randomized order (**Table 2**). Dietary fiber content and energy density were identical in all 4 meals. The test meals offered contained 2500 kJ (26% of energy expenditure) for women and 3000 kJ (22% of energy expenditure) for men. The protein-rich meal consisted of crisp bread with cheese, yogurt with muesli, boiled egg, and skim milk. The carbohydrate-rich meal consisted of corn flakes with skim milk, white bread with butter, cheese, jam, and honey. The fat-rich meal consisted of yogurt mixed with double cream and grated apple, honeydew melon, rye bread with butter, cream cheese, and whole milk. The alcohol-rich meal consisted of rye bread and whole-grain bread with butter and cheese, yogurt with muesli, honeydew melon, and orange juice with vodka. Men were given 24 g and women 20 g alcohol. The same time (a maximum of 15 min) was spent on the meal on each test day. At the end of the test day, the subjects were offered a hot lunch meal ad libitum in a dining room at the department. The meal consisted of pasta and meat sauce with vegetables and was offered in 6- and 8-MJ versions for women and men, respectively. The test meal contributed 13.3% of energy as protein, 50.0% of energy as carbohydrate, 36.8% of energy as fat, 0.7 g dietary fiber/MJ, and 8.2 kJ/g. Subjects were instructed to eat as much as they wanted to feel comfortably satiated. The instructor registered the amounts eaten by using a digital food weight. The computer database of foods from the National Food Agency of Denmark (Dankost 2.0) was used in the calculations of energy and nutrient composition of the diets.

**TABLE 2**  
Energy composition of the 4 test meals<sup>1</sup>

	Protein meal	Carbohydrate meal	Fat meal	Alcohol meal
Protein (% of energy)	31.8	12.2	11.6	12.1
Carbohydrate (% of energy)	37.2	65.4	23.9	42.9
Fat (% of energy)	31.1	23.7	64.6	24.3
Alcohol (% of energy)	0	0	0	23.0
Energy density (kJ/g)	4.8	4.8	4.8	4.8
Dietary fiber (g/MJ)	1.8	1.8	1.8	1.8

<sup>1</sup>Calculation of energy composition was based on a computerized food database from the National Food Agency of Denmark (DanKost 2.0).

### Experimental protocol

On the test day, the subjects arrived at the department at 0800 with a minimum of activity (by car or bus) and after having fasted for 10 h from the evening before. After the subjects voided, body weight was measured (to the nearest 100 g) with subjects wearing underwear and by using the same digital scale each time (SECA model 707; Seca, Copenhagen). Body composition was subsequently estimated by bioelectrical impedance with an Animeter (HTS-Engineering Inc, Odense, Denmark). For one-half of the subjects, a Venflon catheter (Viggo, Gothenburg, Sweden) was inserted in an antecubital arm vein. The subjects then rested for 30 min in a supine position on a bed covered with an antidecubitus mattress and with a slight elevation of the head, and resting metabolic rate was measured for 45 min (0845–0930). A fasting blood sample was taken at 0935–0940.

The meal was served at 0945 (time = 0) and was consumed within 15 min. Exactly the same time was spent on the 4 test meals for each individual subject. Before, after (at 1000), and every 30 min until 5 h after the meal (at 1500), questionnaires to assess hunger, satiety, fullness, prospective food consumption, and desires for something salty, sweet, or fatty or for meat or fish were filled out by each subject. Ratings were made on 10-cm visual analogue scales (VAS scores) presented as a small folder with one question per page. Words were anchored at each end of the VAS, expressing the most positive (ie, good, pleasant) or the most negative (bad, unpleasant) rating (13, 17). Immediately after the test meal, subjects recorded their evaluation of taste, aftertaste, smell, visual appeal, and overall impression of the test meals by VAS scores.

Postprandial blood samples were taken at 15, 45, 75, 105, 135, 165, 195, 255, 285, and 315 min from meal initiation. During the postprandial phase (15–315 min), energy expenditure was measured continuously. The subjects were allowed to watch light entertainment movies and to have small breaks of  $\approx 5$  min once every hour if needed. During the break the subjects could lie down in a supine position without the hood, sit, walk quietly, or go to the toilet if needed. All urine, except morning urine, was pooled in the same container during the total 7-h measurement period (800–1500) for later nitrogen analyses. A minor amount of water consumption was allowed during the test day (maximum: 200 mL), but the total amount consumed was noted together with the breaks, and both conditions were repeated on the next 3 test days.

### Energy expenditure and oxidation rates

Energy expenditure was measured by indirect calorimetry with an open-air-circuit, computerized, ventilated hood system (18). Ventilation through the system was determined by a Hastings mass flow meter (type HFM 201-100; Teledyne Hastings-Raydist, Hampton, VA). Carbon dioxide was measured by a Uras 10P infrared analyzer (Hartmann & Braun, Frankfurt, Germany) and oxygen by a Servomex 1100A paramagnetic analyzer (Servomex Ltd, Crowborough, Sussex, United Kingdom). Energy expenditure (EE) and oxidation rates of protein (P-OX), carbohydrate (C-OX), fat (F-OX), and alcohol (A-OX) were calculated from the gas exchange and urinary nitrogen measurements with the use of the constants of Elia and Livesey (19):

$$EE \text{ (kJ)} = 15.913 \times O_2 \text{ (L)} + 5.207 \times CO_2 \text{ (L)} \\ - 4.646 \times N \text{ (g)} + 1.380 \times \text{alcohol (g)} \quad (1)$$

$$DIT \text{ (kJ)} = \text{area under the EE curve above and below fasting level (kJ/min} \cdot \text{min)} \quad (2)$$

where DIT is diet-induced thermogenesis.

$$DIT \text{ (\%)} = 100 \times DIT \text{ (kJ)} / \text{energy in test meal (kJ)} \quad (3)$$

$$P\text{-OX (kJ)} = N \text{ (g)} \times 116 \text{ (kJ/g)} \quad (4)$$

$$P\text{-OX (g)} = P\text{-OX (kJ)} / 18.56 \text{ (kJ/g)} \quad (5)$$

$$A\text{-OX (kJ)} = \text{alcohol (g)} \times 29.68 \text{ (kJ/g)} \quad (6)$$

$$A\text{-OX (g)} = A\text{-OX (kJ)} / 29.68 \text{ (kJ/g)} \quad (7)$$

$$EE_{\text{npnal}} \text{ (kJ)} = EE \text{ (kJ)} - P\text{-OX (kJ)} - A\text{-OX (kJ)} \quad (8)$$

where  $EE_{\text{npnal}}$  is nonprotein, nonalcohol energy expenditure.

$$RQ_{\text{npnal}} = [\text{CO}_2 \text{ (L)} - 4.97 \text{ (L/g)} \times N \text{ (g)} \\ - 0.973 \text{ (L/g)} \times \text{alcohol (g)}] / [\text{O}_2 \text{ (L)} \\ - 5.95 \text{ (L/g)} \times N \text{ (g)} - \text{alcohol (g)} \\ \times 1.460 \text{ (L/g)}] \quad (9)$$

where  $RQ_{\text{npnal}}$  is the nonprotein, nonalcohol respiratory quotient.

$$C\text{-OX (\%)} = [21.12 \text{ (kJ/L)} \times (RQ_{\text{npnal}} \\ - 0.710)] / [21.12 \text{ (kJ/L)} \\ \times (RQ_{\text{npnal}} - 0.710) + (19.61 \text{ (kJ/L)} \\ \times (1 - RQ_{\text{npnal}}))] \times 100 \quad (10)$$

$$C\text{-OX (kJ)} = EE_{\text{npnal}} \text{ (kJ)} \times C\text{-OX (\%)} \quad (11)$$

$$C\text{-OX (g)} = C\text{-OX (kJ)} / 17.52 \text{ (kJ/g)} \quad (12)$$

$$F\text{-OX (kJ)} = EE_{\text{npnal}} \text{ (kJ)} - C\text{-OX (kJ)} \quad (13)$$

$$F\text{-OX (g)} = F\text{-OX (kJ)} / 39.40 \text{ (kJ/g)} \quad (14)$$

The rate of protein oxidation was assumed to be constant throughout the test day. One gram nitrogen was assumed to correspond to 6.25 g protein, and  $\approx 81\%$  of the ingested nitrogen was assumed to be excreted in the urine (20). Protein oxidation should have been calculated for each individual test period. However, because we did not have complete nitrogen data from all subjects ( $n = 16\text{--}18$  per meal), we decided to use the same mean value of nitrogen for an individual for the 4 test meals. The error was probably small, because the average nitrogen recovered for the 4 meals was similar (range: 2.89–3.09 g; NS). Alcohol was assumed to be totally oxidized during the 5-h postprandial measurements (19, 21, 22), and its oxidation rate was estimated to be constant with 1.89 kJ/min (totaling 595.4 kJ/315 min) for women and 2.26 kJ/min (totaling 711.9 kJ/315 min) for men.

### Laboratory analysis

Blood was sampled without stasis through an indwelling catheter into iced syringes. Within 30 min, samples were centrifuged for 10 min at  $3000 \times g$  and  $4^\circ\text{C}$ , and the supernatant fluid was stored at  $-80$  or  $-20^\circ\text{C}$  until analyzed. Blood for determination of plasma glucose and lactate was collected in fluoride-EDTA prepared tubes (Vacurette; Greiner laborotechnik, Kremsmoenster, Austria) and was analyzed by standard end-point enzymatic methods (MPR3 Gluco-Quant Glucose/HK and MPR3 Hexokinase/G&P-DH test kits; Boehringer Mannheim GmbH Diagnostica, Copenhagen) (23, 24). Serum was extracted for the analysis of nonesterified fatty acids, and the extracts were stored at  $-20^\circ\text{C}$  until later analysis by an enzymatic,



**TABLE 3**  
Subjective evaluations of the test meals<sup>1</sup>

	Protein meal	Carbohydrate meal	Fat meal	Alcohol meal	<i>P</i> for meal effect (ANOVA)
Visual appeal (cm) <sup>2</sup>	3.6 ± 0.6	2.4 ± 0.5	3.8 ± 0.6	3.0 ± 0.5	0.26
Smell (cm) <sup>2</sup>	4.5 ± 0.4	3.8 ± 0.5	3.9 ± 0.4	4.5 ± 0.5	0.27
Taste (cm) <sup>3</sup>	3.1 ± 0.5 <sup>a,b</sup>	1.6 ± 0.3 <sup>a</sup>	3.1 ± 0.6 <sup>a,b</sup>	4.7 ± 0.6 <sup>b</sup>	<0.001
Aftertaste (cm) <sup>4</sup>	7.0 ± 0.5 <sup>b</sup>	8.4 ± 0.2 <sup>b</sup>	7.3 ± 0.5 <sup>b</sup>	4.8 ± 0.6 <sup>a</sup>	<0.0001
Overall impression (cm) <sup>2</sup>	3.4 ± 0.6 <sup>a,b</sup>	2.0 ± 0.3 <sup>a</sup>	3.6 ± 0.5 <sup>a,b</sup>	4.8 ± 0.6 <sup>b</sup>	<0.01

<sup>1</sup> $\bar{x} \pm \text{SEM}$ ;  $n = 19$ . Means within a row with different superscript letters are significantly different,  $P < 0.05$ .

<sup>2</sup>0 = Appetizing.

<sup>3</sup>0 = Good.

<sup>4</sup>0 = Much.

quantitative colorimetric method (Wako NEFA test kit, NEFA C, ACS-ACOP method; Wako, Interkemi, Denmark). Serum triacylglycerol was extracted and measured by an enzymatic endpoint method [Test-Combination Triacylglycerol (GPO-PAP) kit, Boehringer Mannheim, GmbH-Diagnostica] (25). Insulin was measured against standards of mouse monoclonal antibodies by immunoassay (DAKO insulin, enzyme-linked immunosorbent assay method; DAKO, Cambridgeshire, United Kingdom) (26). A Cobas Mira (Roche Diagnostica System, Basel, Switzerland) was used to analyze these samples. For determination of the concentrations of the hormones glucagon, glucose-dependent insulinotropic polypeptide (GIP), GLP-1, glucagon-like peptide 2 (GLP-2), and leptin, blood was sampled in syringes containing EDTA and aprotinin. Plasma was stored at  $-20^{\circ}\text{C}$ . Before analysis the samples were extracted by ethanol. The concentration of GIP was determined by using human GIP and  $^{125}\text{I}$ -labeled human GIP as standards and tracer (27). GLP-1 was measured against standards of a synthetic GLP-1 amide specific for the carboxyl-terminal end of GLP-1, which reacts with GLP-1 of intestinal origin (28). GLP-2 was measured by using an antiserum against the amino terminus of human GLP-2, which measures only fully processed, nondegraded GLP-2 (29). Leptin was determined in serum with a human leptin radioimmunoassay kit (DRG Diagnostic, Marburg, Germany) (30, 31). Urine volume was determined, and nitrogen concentration was measured with a nitrogen analyzer (NA 1500; Carlo Erba Strumentazione, Milan, Italy) (32).

### Statistical analyses

All results are given as means  $\pm$  SEMs. Postprandial values are expressed as changes from fasting values. Response curves after the 4 test meals were compared by analysis of variance (ANOVA) with the GLM procedure in SAS (version 6.12; SAS Institute Inc, Cary, NC). We tested for meal  $\times$  time  $\times$  sex, meal  $\times$  sex, and meal  $\times$  time interactions and the effects of time, sex, and meal separately. Subject (meal) was used as an error term for meal effects. Post hoc tests were done on least-squares means by using Tukey adjustments for multiple comparisons. Area under (or above) the curves,  $\Delta$ -AUC, was calculated separately for each subject as the difference between the integrated area of the response curve and the rectangular area determined by the basal values. Negative (or positive) areas were included. For appetite scores, the mean change in postprandial scores from fasting level,  $\Delta$ -mean, was calculated. The level of statistical significance was set at  $P < 0.05$ . STATGRAPHICS software (version 4.2; Graphic Software Systems, Inc, Rockville, MD) and SAS (SAS Institute Inc) were used for the statistical calculations.

## RESULTS

### Evaluation of the test meals

The questionnaire, given to the subjects immediately after they consumed the test meals, showed that both the women and the men evaluated the test meals alike. The scores for overall appearance and smell were not significantly different between the 4 test meals (Table 3). However, the subjects found that the alcohol meal had a more unpleasant taste and was less appetizing overall than was the carbohydrate meal and had more aftertaste than did the other 3 meals.

### Satiety scores

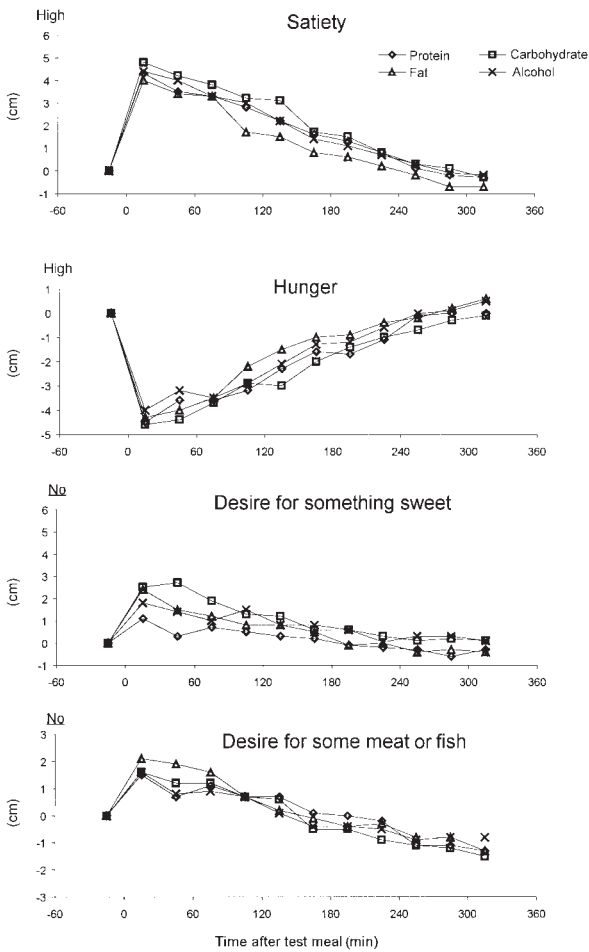
There were no significant differences in postprandial sensations of hunger or satiety (Figure 1) or in prospective food consumption and fullness (data not shown) after the 4 meals. However, desire for something sweet showed a significant interaction between meal and time ( $P < 0.05$ ), with an initially lower desire after the carbohydrate meal and a higher desire after the protein meal (Figure 1). The subjects' desire for some meat or fish showed an interaction between time, sex, and meal ( $P < 0.0001$ ; Figure 1). The  $\Delta$ -mean ratings were not significantly different between the meals (Table 4), but the women reported to be less hungry and more full than did the men (sex effect:  $P < 0.05$ ), and the men had a higher desire for something fatty than did the women (sex effect:  $P < 0.05$ ).

### Ad libitum energy intake

Because the evaluations of the breakfast meals were different, these evaluations were included as covariates in the statistical analysis of the subsequent ad libitum intake. There was a meal  $\times$  sex effect for ad libitum energy intake ( $P < 0.05$ ; Figure 2), but post hoc tests showed no significant differences between meals for each sex separately. On average, energy intake was twice as high in the men as in the women (sex effect:  $P < 0.001$ ).

### Energy expenditure and substrate oxidation

Postprandial energy expenditure was initially highest after the alcohol meal, but at the end, energy expenditure was highest for the protein meal (meal  $\times$  time effect:  $P < 0.001$ ; Figure 3). Average DIT was 17% higher after the protein meal (NS) and 27% higher after the alcohol meal ( $P < 0.01$ ) than after the carbohydrate and fat meals (meal effect:  $P < 0.01$ ; Figure 3). When expressed as a percentage of energy intake, DIT averaged 8.3% after the protein meal, 9.0% after the alcohol meal, and 7.1% after both the fat and carbohydrate meals (meal effect:  $P < 0.01$ ). There were no sex or sex  $\times$  meal effects.



**FIGURE 1.** Subjective appetite sensations in 19 normal-weight subjects after meals rich in protein, carbohydrate, fat, or alcohol. Data are means of changes from fasting levels. By ANOVA, there was a significant time effect for hunger and satiety ( $P < 0.0001$ ), a significant meal  $\times$  time effect for desire for something sweet ( $P < 0.05$ ), and a significant time  $\times$  sex  $\times$  meal effect for desire for some meat or fish ( $P < 0.0001$ ).

The carbohydrate oxidation curve was highest and lasted the longest after intake of carbohydrate (meal  $\times$  time effect:  $P < 0.0001$ ; Figure 3). Likewise, the  $\Delta$ -AUC was 2–3 times larger after the carbohydrate meal than after the other 3 meals (meal effect:  $P < 0.0001$ ; Figure 3).

Postprandial fat oxidation was initially greatly suppressed after both the carbohydrate and alcohol meals compared with the fat and protein meals, and remained suppressed after the alcohol meal (meal  $\times$  time effect:  $P < 0.0001$ ; Figure 3). The  $\Delta$ -AUC was significantly lower after the alcohol meal than after the carbohydrate meal and was almost unchanged after the fat and protein meals (meal effect:  $P < 0.0001$ ; Figure 3).

**Glucose, lactate, insulin, and glucagon**

Postprandial plasma glucose increased more after the carbohydrate meal than after the other 3 meals (meal  $\times$  time effect:  $P < 0.0001$ ; **Figure 4**). Glucose decreased slightly below fasting concentrations after the protein, fat, and alcohol meals but not after the carbohydrate meal. The  $\Delta$ -AUC was also highest after the carbohydrate meal compared with the other meals (meal effect:  $P < 0.001$ ). There was a tendency toward a difference in response between the sexes, with a 3 times higher mean concentration in the women than in the men (sex effect:  $P = 0.053$ ; data not shown).

The postprandial lactate response showed an interaction between meal and time ( $P < 0.0001$ ; Figure 4), with the highest concentration after the carbohydrate meal followed by the alcohol meal. The  $\Delta$ -AUCs were also different between meals, with a higher  $\Delta$ -AUC after both the carbohydrate and alcohol meals than after the fat and protein meals (meal effect:  $P < 0.0001$ ).

The plasma insulin response showed the highest increase after the carbohydrate meal followed by the alcohol meal (meal  $\times$  time effect:  $P < 0.01$ ; Figure 4). There was also a significant time  $\times$  sex effect ( $P < 0.05$ ), with steeper increases in the women than in the men (data not shown). The insulin  $\Delta$ -AUCs were highest after the carbohydrate meal and lowest after the fat meal (meal effect:  $P < 0.0001$ ). In general,  $\Delta$ -AUCs were twice as high in the women as in the men (sex effect:  $P < 0.01$ ; data not shown).

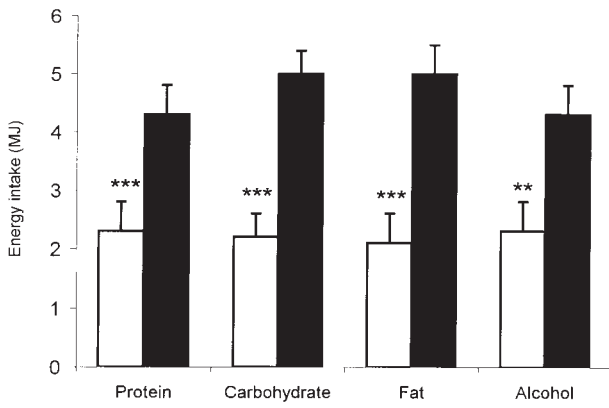
Glucagon concentrations were found to be almost unchanged from fasting concentrations after the carbohydrate meal, but increased after the 3 other meals, although with different time

**TABLE 4**  
Subjective appetite sensations for 19 normal-weight men and women<sup>1</sup>

	Protein meal	Carbohydrate meal	Fat meal	Alcohol meal	<i>P</i> for sex effect (ANOVA)
Hunger (cm)					0.03
Women	-2.5 $\pm$ 0.3	-3.0 $\pm$ 0.5	-1.9 $\pm$ 0.4	-2.5 $\pm$ 0.6	
Men	-1.2 $\pm$ 0.6	-1.1 $\pm$ 0.6	-1.1 $\pm$ 0.3	-0.5 $\pm$ 0.4	
Satiety (cm)	1.6 $\pm$ 0.4	1.9 $\pm$ 0.4	1.1 $\pm$ 0.3	1.7 $\pm$ 0.4	0.07
Prospective consumption (cm)	-1.4 $\pm$ 0.4	-1.8 $\pm$ 0.5	-1.2 $\pm$ 0.3	-1.6 $\pm$ 0.3	0.08
Fullness (cm)					0.04
Women	2.8 $\pm$ 0.5	3.1 $\pm$ 0.8	2.2 $\pm$ 0.4	2.6 $\pm$ 0.7	
Men	1.3 $\pm$ 0.5	1.8 $\pm$ 0.6	1.2 $\pm$ 0.4	1.4 $\pm$ 0.4	
Desire for					
Some meat or fish (cm) <sup>2</sup>	0.2 $\pm$ 0.5	0.0 $\pm$ 0.5	0.2 $\pm$ 0.4	0.0 $\pm$ 0.2	0.94
Something salty (cm) <sup>2</sup>	-0.4 $\pm$ 0.4	-0.1 $\pm$ 0.4	-0.1 $\pm$ 0.2	-0.3 $\pm$ 0.5	0.30
Something sweet (cm) <sup>2</sup>	0.1 $\pm$ 0.5	1.0 $\pm$ 0.4	0.5 $\pm$ 0.4	0.7 $\pm$ 0.5	0.29
Something fatty (cm) <sup>2</sup>					0.02
Women	1.4 $\pm$ 0.8	2.0 $\pm$ 0.5	1.5 $\pm$ 0.7	1.5 $\pm$ 0.7	
Men	0.6 $\pm$ 0.6	0.5 $\pm$ 0.5	0.4 $\pm$ 0.6	0.3 $\pm$ 0.4	

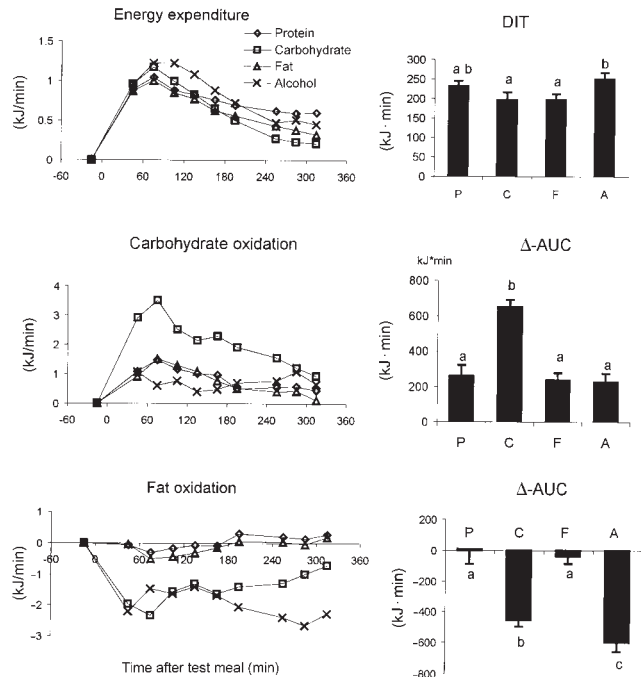
<sup>1</sup> $\bar{x} \pm$  SEM of 5-h postprandial values from fasting ratings;  $n = 19$ . There were no significant meal or meal  $\times$  sex effects.

<sup>2</sup>A high value means no desire.

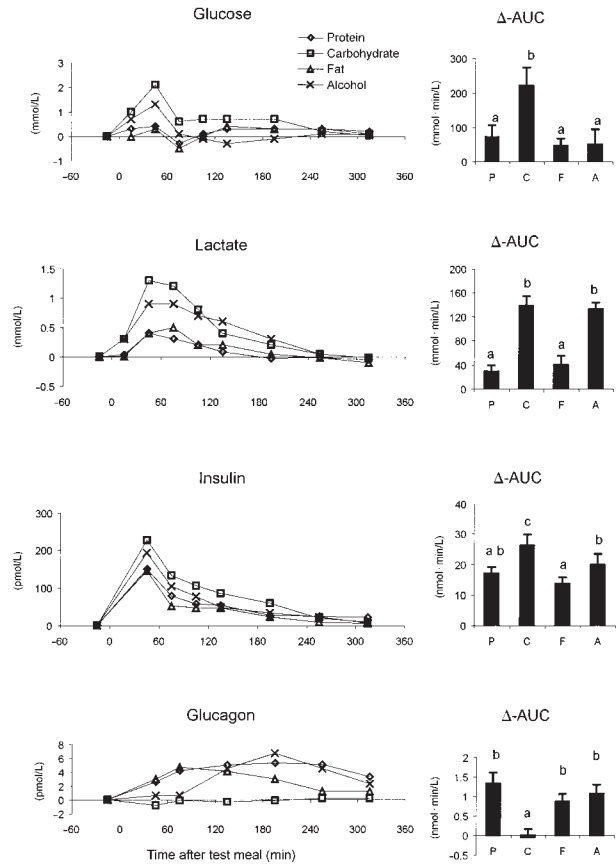


**FIGURE 2.** Mean ( $\pm$ SEM) ad libitum energy intake of normal-weight men ( $\blacksquare$ ;  $n = 10$ ) and women ( $\square$ ;  $n = 9$ ) at lunch 5 h after test meals rich in protein, carbohydrate, fat, or alcohol. By ANOVA, there was a significant meal  $\times$  sex effect after adjustment for the breakfast meal evaluations ( $P < 0.05$ ). \*\*\*\*Significantly different from men (post hoc tests with Tukey adjustment): \*\* $P < 0.001$ , \*\*\* $P < 0.0001$ .

patterns and a late peak after the alcohol meal (meal  $\times$  time effect:  $P < 0.0001$ ; Figure 4). A significant difference in the  $\Delta$ -AUCs was found, with high responses after the protein, fat, and alcohol meals and a negative  $\Delta$ -AUC after the carbohydrate meal (meal effect:



**FIGURE 3.** Energy expenditure and substrate oxidation in 19 normal-weight subjects after intake of test meals rich in protein (P), carbohydrate (C), fat (F), or alcohol (A). Bars are mean ( $\pm$ SEM). DIT, diet-induced thermogenesis;  $\Delta$ -AUC, change in area under the curve. Left panels: by ANOVA, there was a significant meal  $\times$  time effect for energy expenditure, carbohydrate oxidation, and fat oxidation ( $P < 0.0001$ ) and a significant time  $\times$  sex effect for carbohydrate oxidation ( $P < 0.01$ ) and fat oxidation ( $P < 0.05$ ). Right panels: bars with different letters are significantly different,  $P < 0.05$  (post hoc tests with Tukey's adjustment).



**FIGURE 4.** Plasma concentrations of glucose, lactate, insulin, and glucagon in 10 normal-weight subjects after intake of test meals rich in protein (P), carbohydrate (C), fat (F), or alcohol (A). Bars are mean ( $\pm$ SEM).  $\Delta$ -AUC, change in area under the curve. Left panels: by ANOVA, there was a significant meal  $\times$  time effect for glucose, lactate, glucagon (all  $P < 0.0001$ ), and insulin ( $P < 0.01$ ) and a significant time  $\times$  sex effect for insulin ( $P < 0.05$ ) and glucagon ( $P < 0.001$ ). Right panels: bars with different letters are significantly different,  $P < 0.05$  (post hoc tests with Tukey's adjustment).

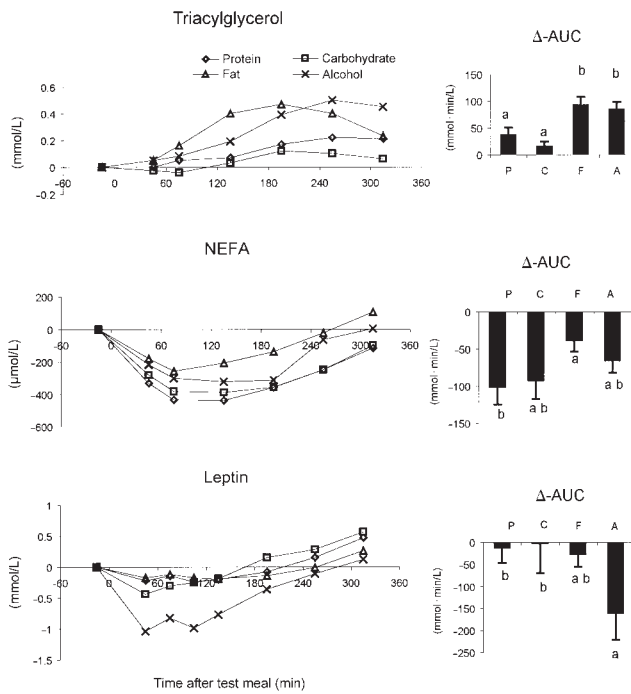
$P < 0.0001$ ). The mean glucagon  $\Delta$ -AUC was 5 times higher in the men than in the women (sex effect:  $P < 0.001$ ; data not shown).

### Triacylglycerol, nonesterified fatty acids, and leptin

For triacylglycerol, interactions between meal and time ( $P < 0.0001$ ) and time and sex ( $P < 0.05$ ) were observed (Figure 5). The fat and alcohol meals induced the highest responses, although the alcohol meal caused a slower postprandial increase. The  $\Delta$ -AUCs for triacylglycerol were also larger after the fat and alcohol meals than after the protein and carbohydrate meals (meal effect:  $P < 0.0001$ ; Figure 5).

In general, plasma nonesterified fatty acids decreased from fasting concentrations after the onset of all 4 meals, but more so and with a later return to fasting concentrations after the protein and carbohydrate meals (meal  $\times$  time effect:  $P < 0.05$ ; Figure 5). The  $\Delta$ -AUC was lower after the protein meal than after the fat meal ( $P < 0.05$ ).

For leptin, a significant interaction was observed between meal, time, and sex ( $P < 0.001$ ) and between time and meal ( $P < 0.0001$ ). A marked



**FIGURE 5.** Plasma concentrations of triacylglycerol, nonesterified fatty acids (NEFAs), and leptin in 5 men and 5 women after intake of test meals rich in protein (P), carbohydrate (C), fat (F), or alcohol (A). Bars are mean ( $\pm$ SEM).  $\Delta$ -AUC, change in area under the curve. Left panels: by ANOVA, there was a significant meal  $\times$  time effect for triacylglycerol, leptin (both  $P < 0.0001$ ), and NEFAs ( $P < 0.05$ ); a significant time  $\times$  sex effect for triacylglycerol ( $P < 0.05$ ); and a significant meal  $\times$  time  $\times$  sex effect for leptin ( $P < 0.001$ ). Right panels: bars with different letters are significantly different,  $P < 0.05$  (post hoc tests with Tukey's adjustment).

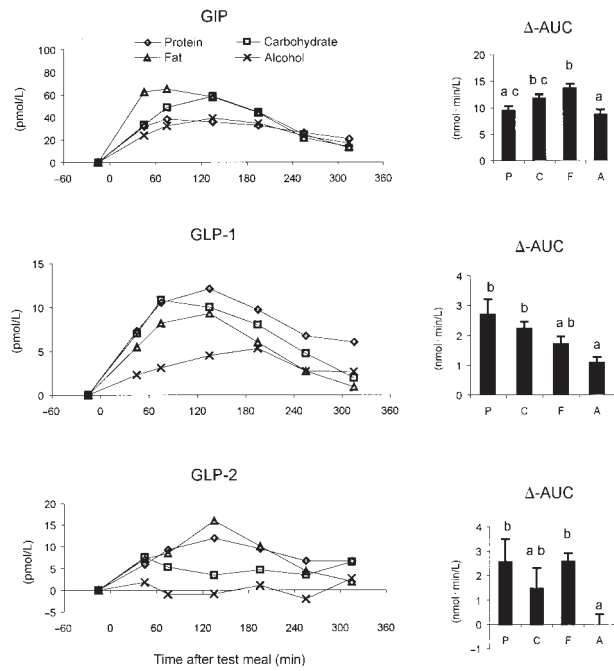
suppression was seen the first 4 h after the alcohol meal compared with a slight suppression the first 3 h after the other meals (Figure 5). The  $\Delta$ -AUC for leptin was significantly lower after the alcohol meal than after the protein and carbohydrate meals (meal effect:  $P < 0.05$ ).

### GIP, GLP-1, and GLP-2

The GIP response showed an interaction between meal and time ( $P < 0.0001$ ) and meal and sex ( $P < 0.05$ ; Figure 6). The fat meal produced the most rapid and steepest response curve, followed by the carbohydrate meal. The  $\Delta$ -AUCs were highest after the fat meal, followed by the carbohydrate meal (meal effect:  $P < 0.0001$ ; meal  $\times$  sex interaction:  $P < 0.05$ ). The women had a lower GIP than did the men after the protein meal ( $P < 0.05$ ; data not shown).

GLP-1 showed an interaction between meal and time ( $P < 0.0001$ ; Figure 6). The protein meal gave the highest response, whereas the alcohol meal induced both a lower and slower response compared with the other meals. The protein meal created the highest  $\Delta$ -AUC, followed by the carbohydrate meal, and the alcohol meal produced the lowest  $\Delta$ -AUC (meal effect:  $P < 0.01$ ).

GLP-2 was almost unchanged after the alcohol meal, but increased after the fat and protein meals (meal  $\times$  time effect:  $P < 0.001$ ; Figure 6). After the carbohydrate meal, GLP-2 increased less and seemed to reach a plateau 1 h after the meal. The  $\Delta$ -AUCs showed a difference between the meals ( $P < 0.05$ ), with the highest  $\Delta$ -AUCs after the protein and fat meals.



**FIGURE 6.** Plasma concentrations of glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1 (GLP-1), and glucagon-like peptide 2 (GLP-2) in 5 men and 5 women after intake of test meals rich in protein (P), carbohydrate (C), fat (F), or alcohol (A). Bars are mean ( $\pm$ SEM).  $\Delta$ -AUC, change in area under the curve. Left panels: by ANOVA, there was a significant meal  $\times$  time effect for GIP, GLP-1 (both  $P < 0.0001$ ), and GLP-2 ( $P < 0.001$ ) and a significant meal  $\times$  sex effect for GIP ( $P < 0.05$ ). Right panels: bars with different letters are significantly different,  $P < 0.05$  (post hoc tests with Tukey's adjustment).

## DISCUSSION

### Diet-induced thermogenesis

A clear finding in the present study was a 27% larger DIT after the alcohol meal than after the carbohydrate and fat meals. DIT was also larger after protein meal (by 17%), but this was not significantly different from the other meals. That DIT remained increased after the alcohol and protein meals at the end of the 5-h measurement period suggests that the effects of these meals might have been more pronounced had our measurement period been longer. The high DIT after the alcohol meal is presumably related to the reoxidation of cytoplasmic NADH not being tightly linked to ATP generation. Alcohol oxidation starts instantly after intake, and alcohol is eventually completely eliminated by oxidation (22, 33). Still, the results of previous studies are not concordant on the magnitude of DIT after alcohol consumption, and reported DIT values range between 9% and 28% (22, 34–36). Furthermore, a long-term study reported similar metabolizable energy after alcohol and carbohydrate (37), so discrepancies do exist.

Although DIT was 17% higher after the protein meal, it was not significantly increased compared with the carbohydrate and fat meals. The lack of a larger DIT after the protein meal, as was expected from previous suggestions (5) and short-term studies (38–40), may relate to the use of a more stringent post hoc test

(Tukey's) in the present than in the previous studies or to the use of more compound meals instead of single macronutrients. The lack of difference in DIT between the fat and carbohydrate meals was, however, similar to other studies in which the ratio of fat to carbohydrate was manipulated (41, 42). On the basis of the present 5-h results, our oxidation hierarchy can be summarized as follows: alcohol > protein = fat = carbohydrate.

### Appetite and ad libitum intake

We were unable to show significant differences in the subjective sensations of satiety, hunger, fullness, or prospective food consumption in our study. VAS scores could therefore not be used to show the satiety hierarchy of protein > carbohydrate > fat. Nor could we propose a position for alcohol in this hierarchy on the basis of the VAS scores. The lack of differences was somewhat surprising because higher satiety after protein than after carbohydrate and fat has been shown before (7, 8, 11, 43, 44), although not consistently (45–48). Our use of meals with similar energy densities could, however, be a major reason for the lack of differences in hunger and satiety ratings (4). Discrepancies in the previous study outcomes probably relate to different methods, eg, the use of fluid rather than solid meals, differences in energy loads (an amount > 1.5 MJ may be needed), preload versus test meal, different measurement periods, and differences in subject characteristics (such as sex, age, and body mass index).

It was interesting to note that during the first period after breakfast, the subjects had a lower desire for something sweet after the carbohydrate meal and a higher desire for something sweet after the protein meal. This phenomenon probably reflects sensory-specific satiety (45). A carbohydrate-rich diet may therefore decrease the urge for sweet snacks between meals.

We did not observe any significant differences in ad libitum energy intake after the 4 meals. On the basis of previous studies, we had expected to find a reduced energy intake after the protein meal (10, 43–45) compared with the carbohydrate and fat meals. This would also have been in line with the results of a 6-mo ad libitum study showing a greater reduction in body weight after a low-fat, high-protein diet than after a low-fat, high-carbohydrate diet (49) and with the findings of habitual protein intake being negatively correlated with energy intake (50). That protein did not suppress energy intake here is, however, in agreement with other short-term studies (7, 11, 46, 51).

In our study, the effect of the alcohol-rich meal on energy intake was not significantly different from the effects of the other meals. Thus, alcohol did not stimulate energy intake, as was been reported when alcohol was given as an aperitif (52) or with dinner (B Buemann, S Toubro, A Astrup, unpublished observations, 2002). Other studies did not, however, report any difference in energy intake after alcohol compared with carbohydrate or fat intake (53). When the alcohol is served may therefore play a role, ie, whether it is given before or with a meal, in addition to whether it is given in addition to or as a substitute for other macronutrients. On the basis of our ad libitum data, we also could not propose a satiety hierarchy for the 4 macronutrients.

We observed that energy intake was twice as high in the men as in the women. Because the thermogenic response was similar in the women and men, nonphysiologic mechanisms may have been involved in the women's ad libitum food intake. These women may have been steered by psychological mechanisms (dietary restraint and concerns about body weight) rather than physiologic needs for energy. This hypothesis is supported by the results of

previous studies (54–56) and may be especially relevant because our subjects ate under supervision.

### Substrates and hormones

As expected, carbohydrate oxidation was higher after the carbohydrate meal than after the other meals. This was also reflected in higher postprandial glucose, lactate, and insulin concentrations after this meal. The suppression of fat oxidation and nonesterified fatty acids and the minor effect on triacylglycerol and glucagon all agree with the stimulation of insulin after carbohydrate intake. Although both insulin and GLP-1 were increased after the carbohydrate meal, hunger and energy intake were not lower after this diet than after the other meals, as might have been expected (57, 58).

After the alcohol meal, net fat oxidation was greatly suppressed, even more than after the carbohydrate meal. Also, carbohydrate oxidation was relatively low after the alcohol meal considering the carbohydrate content in this meal (43% of energy). This was probably due to the fate of acetate, the major product of ethanol metabolism in the liver (59). Acetate is released to the circulation and primarily oxidized, probably with priority over other substrates. Furthermore, acetate released into plasma inhibits lipolysis (59). We also saw an increase in triacylglycerol, occurring later, but with as high a  $\Delta$ -AUC as after the fat meal, despite a wide difference in fat intake (24% compared with 65% of energy). This increase could have been due to a stimulation of hepatic lipogenesis, increasing the contribution of fatty acids to circulating VLDL-triacylglycerol (59). Leptin concentrations were also greatly suppressed after the alcohol meal in contrast with the other 3 meals. This observation has not been published before in the literature. In contrast, habitual alcohol intake was reported to be positively associated with circulating leptin in young, healthy men (60). Furthermore, major changes in leptin are normally not seen acutely after meal intake (61). The reason may be related to the above-mentioned effects of alcohol on fat metabolism, causing a suppression of leptin release from adipose tissue. No or only small effects of alcohol were seen on GIP, GLP-1, and GLP-2. This may relate to alcohol being largely absorbed through the stomach wall and not entering the small intestine.


After the fat meal we observed the expected increase in triacylglycerol, with a peak  $\approx$ 3 h postprandially, whereas fat oxidation was almost unchanged. Furthermore, we observed an increase in both GIP and GLP-2 after the fat meal. This may explain why insulin increased despite comparatively low carbohydrate (24% of energy) and protein (12% of energy) intakes with the fat meal. Fat intake has been shown to stimulate GIP before (62). The increased GIP may activate lipoprotein lipase and thereby increase chylomicron clearance from the circulation (63).

The carbohydrate oxidation rate was low and fat oxidation almost unchanged after the protein meal. However, the protein meal suppressed nonesterified fatty acids as much as did the carbohydrate meal and stimulated GLP-1 and GLP-2. GLP-1 has been proposed to have satiating properties (58), but the differences here were apparently not large enough to produce differences in appetite ratings or ad libitum energy intake.

### Conclusion

A higher thermogenic response was observed after a meal rich in alcohol than after meals rich in protein, fat, or carbohydrate with similar energy densities and dietary fiber contents. Despite meas-



urable differences in substrate oxidation, plasma substrates, and hormones, we observed no significant differences in subjective hunger and satiety sensations or in ad libitum energy intake. Our data from compound meals, therefore, do not support the existence of a satiety hierarchy resembling the oxidative hierarchy of the 4 macronutrients. The apparently positive effects of alcohol on energy expenditure should be considered together with the greatly suppressed fat oxidation and leptin concentrations and increased triacylglycerol concentrations after the alcohol meal. The response patterns in a 5-h period may also change if longer measurement periods are used 

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