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Diet-induced thermogenesis and substrate oxidation are not different between lean and obese women after two different isocaloric meals, one rich in protein and one rich in fat

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Abstract

Reduction in diet-induced thermogenesis (DIT) may promote weight gain and maintenance. Data on differences in DIT and macronutrient oxidation between lean and obese subjects are conflicting. In this study, we sought for differences in DIT and macronutrient oxidation between lean and obese women after consumption of 2 different isocaloric meals, one rich in protein and one rich in fat. Fifteen lean and 15 obese women were studied on 2 occasions, 1 week apart. In one visit, they consumed a protein-rich meal; in the other visit, a fat-rich meal. The 2 meals were isocaloric (~2026 kJ each), of equal volume, and given in random order. Resting energy expenditure and macronutrient oxidation rates were measured and calculated in the fasting state and every 1 hour for 3 hours after meal consumption. Diet-induced thermogenesis was not significantly different between lean and obese subjects after consumption of either the protein-rich ($P = .59$) or the fat-rich meal ($P = .68$). Diet-induced thermogenesis was significantly higher (by almost 3-fold) after consumption of the protein-rich meal in comparison with the fat-rich meal in both study groups. In addition, no significant differences in macronutrient oxidation rates were found between lean and obese women after the test meals. The results indicate that DIT is higher after protein intake than after fat intake in both lean and obese participants; however, DIT and macronutrient oxidation rate are not different between lean and obese subjects after consumption of either a protein-rich or a fat-rich meal. Over the long term, a low DIT after regular or frequent fat intake may contribute to the development and maintenance of obesity.

1. Introduction

Obesity is the most common nutritional disorder worldwide [1]. Reduction in physical activity and increase in energy intake, primarily from fat-rich, energy-dense foods, are the 2 main causes of the development of obesity [1]. Energy balance is achieved by oxidation of the 3 macronutrients: protein, carbohydrate (CHO), and fat. In humans, CHO and protein balance is efficiently self-regulated, as their oxidation is promoted by CHO and protein intake [2,3]. On the contrary, fat oxidation is stimulated less by fat intake [4]. The absence of metabolic pathways other than fat storage to balance a fraction of fat intake contributes to fat gain [4]. In addition, satiation is lower with fat intake in comparison with protein and/or CHO intake [5,6]. There is also evidence for the existence of a hierarchy in macronutrient oxidation rate in the postprandial state with the sequence protein > CHO > fat [7,8].

Diet-induced thermogenesis (DIT) is the increase in energy expenditure above resting associated with digestion, absorption, and storage of food. Diet-induced thermogenesis is the major form of thermogenesis in humans, accounting for 5% to 15% of the total daily energy expenditure [9]. The thermic effect of separate nutrients is highest for protein (20%-30%), followed by CHO (5%-10%) and fat (0%-3%) [10]. A reduced DIT may contribute to the development or/and maintenance of obesity. Studies, however, examining differences in DIT between lean and obese subjects have given conflicting results [11-17]. Some studies have shown reduced DIT in obesity [11-15], whereas others have shown no difference between lean and obese subjects [16,17]. In

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addition, data on differences in nutrient oxidation after protein-rich or fat-rich meals are limited.

The aim, therefore, of the present crossover study was to investigate for potential differences in DIT and substrate oxidation rate between lean and obese women after consumption of 2 different isoenergetic meals, one rich in protein and one rich in fat.

2. Subjects and methods

2.1. Subjects

A total of 15 lean and 15 obese female healthy volunteers, strictly matched for age, were examined. Patients were eligible if they were nonsmokers, were aged 20 to 60 years, were on a free—but not hypocaloric—diet, and had no change of more than 2 kg in their body weight in the previous 6 months. During the days preceding the test, no attempt was made to influence the usual diet of the participants. The day before the test, they were instructed to avoid any intense physical activity. They were always examined at the supine position, in a quiet room with stable temperature (22°C-24°C). After application and adjustment of the measured devices, a period of 30 minutes was left for acclimatization. As plasma estrogen levels may affect energy expenditure [18], all women at the reproductive age were examined at the first half of their cycle. Pre- and postmenopausal women were included in the study groups in almost equal numbers (lean group: premenopausal, n = 11; postmenopausal, n = 4; obese group: premenopausal, n = 12; postmenopausal, n = 3; $\chi^2 = 0.18$; $P = .67$). None of the postmenopausal women was on hormonal replacement therapy. After explanation of the purpose and the procedures of the protocol, all subjects provided written informed consent to participate in the study, which was approved by the ethics committee of our hospital. The demographics and clinical characteristics of the study subjects are shown in Table 1.

Table 1
Clinical and demographic characteristics of the study subjects

	Lean	Obese	*P*
n	15	15	
Age (y)	39.2 ± 14.6	39.5 ± 14.5	.95
BMI (kg/m^2)	22.9 ± 2.1	37.2 ± 4.3	<.0001
Waist (cm)	79.7 ± 13.8	108.2 ± 18.8	<.0001
WHR	0.77 ± 0.09	0.84 ± 0.05	.01
Weight (kg)	62.8 ± 10.2	100.9 ± 21.8	<.001
% Body fat	32.2 ± 6.9	50.2 ± 11.5	<.0001
Fat mass (kg)	20.6 ± 6.4	52.3 ± 12.4	<.001
FFM (kg)	42.2 ± 6.1	48.6 ± 8.4	.02
Heart rate (beats/min)	68.1 ± 8.2	68.6 ± 10.9	.97
MAP (mm Hg)	80.1 ± 9.8	92.3 ± 12.9	.005
Insulin (μU/mL)	11.5 ± 7.2	17.9 ± 10.8	.03
HOMA-IR[a]	2.2 (1.6-3.6)	4.3 (2.8-6.5)	.04

Data are shown as mean ± SD. Mean arterial blood pressure, heart rate, insulin, and HOMA-IR are the average values across the 2 testing days. MAP indicates mean arterial blood pressure.

[a] Median value (interquartile range).

2.2. Methods

In the morning, after a 12- to 14-hour fast, each subject attended the metabolic unit of our department. Anthropometric measurements took place after voiding at around 8:00 AM. Weight and height were measured using standard techniques. Percentage of body fat was measured using a bioimpendance analyzer (Tanita TBF-215 body composition analyzer; Tanita, Brooklyn, NY). Body mass index (BMI) and waist-to-hip ratio (WHR) were measured and calculated. Afterward, an intravenous catheter was inserted in a superficial forearm vein and kept patent by a slow infusion of saline solution 0.9% for blood sampling.

We designed a crossover study. Each subject received the standard test meals, one rich in protein (total energy content of 2026 kJ, consisting of 18.3 g CHO, 0.16 g fat, and 102.4 g protein) in the form of nonfat yogurt, skimmed milk, and egg whites and one rich in fat (total energy content of 481.4 kcal, consisting of 39.4 g fat, 19.9 g CHO, and 11.8 g protein) in the form of walnuts, toast bread, and 500 mL of water. The 2 meals were of equal volume. The meals were given in a random order and with an interval of about 7 days in between.

Respiratory gas exchange measurements were performed by an open-circuit ventilated hood system (Deltatrack monitor; Datex, Helsinki, Finland) for 30 minutes in the fasting condition and for 30 minutes every 1 hour for a total of 3 hours after meals. Fasting resting energy expenditure (REE) and respiratory quotient (RQ) were calculated from the oxygen consumption and the carbon dioxide production [19].

The thermic effect of the test meals (DIT) was calculated as the difference of the postprandial minus the fasting REE [17]. Macronutrient oxidation was calculated from VO_2 and VCO_2 using the following formulas: Fox (in grams per minute) = 1.67 VO_2 (in liters per minute) − 1.67 VCO_2 (in liters per minute) − 0.307 Pox; and Gox (in grams per minute) = 4.55 VCO_2 (in liters per minute) − 3.21 VO_2 (in liters per minute) − 0.459 Pox, where Fox is fat oxidation, Gox is glucose oxidation, and Pox is protein oxidation. Protein oxidation was estimated as follows: Pox (in grams per minute) = [REE (in kJ per minute) 0.15]/16.74 kJ [20]. We assumed that protein oxidation covered 15% of REE in both lean and obese subjects [21].

Plasma lipids (total cholesterol, high-density lipoprotein [HDL] cholesterol, triglycerides) were measured enzymatically on a Technicon analyzer RA-XT (Dublin, Ireland). Low-density lipoprotein (LDL) cholesterol levels were estimated using the equation of Friedewald et al [22]. Serum glucose was measured by colorimetric method GOD-POD (Zafiropoulos, Athens, Greece). Plasma insulin (Biosure, Brussels, Belgium; coefficient of variation = 3.3% ± 1.2%) was determined by radioimmunoassay. Insulin resistance was estimated using the homeostatic model assessment equation (homeostasis model assessment of insulin resistance [HOMA-IR]) [23].

2.3. Statistical analysis

Statistical analysis was performed using the SPSS program (version 10.0; SPSS, Chicago, IL). Analysis of variance (ANOVA) for repeated measurements was performed to test the timing effect of the studied parameters in the 2 phases of the study. The same analysis was used to examine for differences along the experiment in REE and DIT after adjustment for the obesity status (lean/obese) and the menopausal status (premenopausal/postmenopausal) (between-subjects effects) and for the time as well as the test meal (protein-rich/fat-rich meal) (within-subjects factors). The Greenhouse-Geisser adjustment was used when the sphericity assumptions were not fulfilled. Postprandial responses of the serial measurements of the study (DIT, macronutrient oxidation rate, and RQ) were summarized, calculating the areas under the respective 3-hour curve (AUCs) using the trapezoid rule [24]. The AUCs adjusted for baseline values (incremental or nonincremental AUC, Δ-AUC) were calculated by subtracting the values in the fasting state from each postprandial value before area calculation. A paired Student *t* test or the Wilcoxon test was used to compare the differences of the measured parameters between the 2 meals in each group, whereas a 2-sample *t* test or the Mann-Whitney test was used to compare differences between lean and obese women. In addition, 1-way analysis of covariance was used to assess differences in DIT and macronutrient oxidation rate, adjusting for the effect of menopausal status and fat-free mass (FFM). *P* values less than .05 (2-sided) were considered statistically significant.

3. Results

3.1. Baseline data

At baseline, BMI, waist, WHR, percentage of body fat, mean arterial blood pressure, plasma insulin levels, and HOMA-IR index were all significantly higher in the obese in comparison with the lean women. Heart rate was not different between the 2 groups. Resting energy expenditure was significantly higher in the obese women ($P < .0001$), whereas RQ ($P = .008$) was higher in the lean women (Tables 1-3).

Table 2
Fasting and postprandial profiles of the measured parameters after the fat-rich meal

	Fasting	1st h	2nd h	3rd h	P^a	P^b
REE (kJ/24 h)						
Lean	4805.1 ± 552.1	5189.4 ± 542.5	5208.6 ± 660.1	5080.1 ± 553.4	<.001	
Obese	6242.6 ± 846.8	6558.2 ± 945.2	6572.0 ± 938.1	6679.6 ± 839.7	<.001	.12
DIT (kJ/h)						
Lean	–	141.1 ± 112.7	149.8 ± 128.8	125.2 ± 93.4	.24	
Obese	–	105.1 ± 111.2	109.7 ± 143.6	145.6 ± 117.8	.40	.25
RQ						
Lean	0.89 ± 0.04	0.87 ± 0.05	0.85 ± 0.04	0.85 ± 0.04	<.001	
Obese	0.86 ± 0.05	0.85 ± 0.04	0.83 ± 0.02	0.83 ± 0.02	<.001	.81
Glucose (mg/mL)						
Lean	95.1 ± 14.8	103.0 ± 14.2	96.28 ± 8.1	100.4 ± 21.3	.68	
Obese	110.6 ± 33.9	111.6 ± 21.6	110.3 ± 38.2	97.6 ± 31.3	.27	.47
Insulin (μU/mL)						
Lean	10.6 ± 4.8	28.1 ± 25.7	14.5 ± 10.9	10.3 ± 5.6	<.001	
Obese	17.2 ± 10.2	36.9 ± 20.2	21.7 ± 11.5	17.5 ± 7.8	<.001	.87
Triglycerides (mg/mL)						
Lean	79.8 ± 30.7	80.0 ± 31.1	96.1 ± 35.7	106.1 ± 40.6	.01	
Obese	129.5 ± 55.0	132.1 ± 53.4	159.4 ± 64.7	152.9 ± 83.9	.04	.33
Total cholesterol (mg/mL)						
Lean	186.06 ± 41.4	182.3 ± 44.8	181.7 ± 44.9	184.2 ± 46.1	.29	
Obese	194.7 ± 35.5	191.2 ± 36.1	194.4 ± 38.7	177.5 ± 59.1	.39	.72
HDL cholesterol (mg/mL)						
Lean	49.7 ± 12.5	49.6 ± 12.2	49.8 ± 12.2	49.9 ± 12.5	.40	
Obese	35.9 ± 16.1	38.2 ± 12.5	40.9 ± 6.3	41.0 ± 6.4	.20	.47
LDL cholesterol (mg/mL)						
Lean	113.9 ± 47.6	116.9 ± 40.9	114.1 ± 39.3	107.4 ± 47.6	.46	
Obese	127.8 ± 29.9	123.5 ± 29.4	114.6 ± 42.7	108.1 ± 39.1	.25	.32
Heart rate (beats/min)						
Lean	69.2 ± 10.3	66.1 ± 6.5	64.3 ± 5.6	63.8 ± 5.1	.06	
Obese	67.4 ± 10.7	65.9 ± 8.5	66.0 ± 8.9	66.7 ± 9.2	.67	.23
MAP (mm Hg)						
Lean	81.1 ± 9.7	80.4 ± 8.0	81.3 ± 6.4	85.0 ± 7.8	.18	
Obese	93.5 ± 12.8	91.7 ± 13.2	95.3 ± 13.2	94.6 ± 12.6	.42	.68

Data are mean ± SD.

[a] *P* indicates the result of ANOVA for repeated measurements within each group (*P* value for the effect of time).

[b] *P* indicates the result of ANOVA for repeated measurements between the 2 groups (lean and obese) (time × group interaction).

Table 3
Fasting and postprandial profiles of the measured parameters after the protein-rich meal

	Fasting	1st h	2nd h	3rd h	P^a	P^b
REE (kJ/24 h)						
Lean	4630.6 ± 704.9	5725.2 ± 768.9	5768.7 ± 695.3	5654.4 ± 636.7	<.001	
Obese	6291.9 ± 1059.8	7353.4 ± 857.3	7306.2 ± 963.6	7514.7 ± 838.1	<.001	.81
DIT (kJ/h)						
Lean	–	372.7 ± 128.4	384.7 ± 110.5	356.8 ± 129.0	.47	
Obese	–	354.2 ± 130.5	346.7 ± 191.7	348.8 ± 171.3	.97	.70
RQ						
Lean	0.88 ± 0.03	0.87 ± 0.02	0.87 ± 0.03	0.87 ± 0.03	.67	
Obese	0.86 ± 0.05	0.85 ± 0.03	0.85 ± 0.03	0.85 ± 0.03	.53	.50
Glucose (mg/mL)						
Lean	97.1 ± 15.7	91.7 ± 16.9	95.7 ± 12.3	98.2 ± 10.0	.06	
Obese	98.2 ± 34.9	107.6 ± 29.1	112.9 ± 38.0	111.8 ± 42.8	.41	.84
Insulin (μU/mL)						
Lean	12.3 ± 9.5	44.9 ± 27.4	31.3 ± 28.1	17.8 ± 12.6	<.001	
Obese	18.6 ±11.5	78.7 ± 45.6	47.8 ± 16.4	32.3 ± 16.8	<.001	.07
Triglycerides (mg/mL)						
Lean	70.2 ± 25.8	59.1 ± 30.4	69.1 ± 21.9	76.6 ± 24.6	.02	
Obese	102.4 ± 56.6	124.1 ± 53.3	128.8 ± 56.7	140.1 ± 62.6	.03	.07
Total cholesterol (mg/mL)						
Lean	186.8 ± 44.4	173.1 ± 44.9	179.6 ± 41.5	177.4 ± 39.9	.10	
Obese	177.4 ± 60.6	190.3 ± 40.3	183.3 ± 44.7	182.6 ± 44.2	.65	.21
HDL cholesterol (mg/mL)						
Lean	50.6 ± 12.5	50.6 ± 12.4	50.4 ± 12.6	50.5 ± 12.3	.66	
Obese	35.9 ± 16.1	38.2 ± 12.5	40.9 ± 6.3	41.0 ± 6.4	.20	.25
LDL cholesterol (mg/mL)						
Lean	122.5 ± 40.4	110.3 ± 44.2	108.8 ± 46.6	112.4 ± 36.1	.18	
Obese	108.1 ± 52.7	119.3 ± 48.9	116.6 ± 34.8	113.5 ± 33.5	.63	.57
Heart rate (beats/min)						
Lean	67.0 ± 5.9	67.6 ± 5.5	68.0 ± 4.2	66.7 ± 3.9	.60	
Obese	67.4 ± 10.7	65.9 ± 8.5	66.0 ± 8.9	66.7 ± 9.2	.67	.54
MAP (mm Hg)						
Lean	79.1 ± 9.6	82.3 ± 7.4	81.4 ± 7.6	83.0 ± 5.1	.30	
Obese	91.0 ± 12.9	89.6 ± 12.1	92.5 ± 12.9	91.9 ± 12.0	.50	.24

Data are mean ± SD.
[a] P indicates the result of ANOVA for repeated measurements within each group (P value for the effect of time).
[b] P indicates the result of ANOVA for repeated measurements between the 2 groups (lean and obese) (time × group interaction).

3.2. Postprandial comparisons between lean and obese subjects

The REE increased significantly after test meals in both lean and obese subjects (P value for the effect of time < .001). The increase was not significantly different after consumption of the protein-rich meal between lean and obese or between premenopausal and postmenopausal subjects (ANOVA for repeated measurements: time × obesity status interaction, $P = .81$; time × menopausal status interaction, $P = .89$; time × obesity status × menopausal status interaction, $P = .95$) (Tables 2 and 3). The same was valid after consumption of the fat-rich meal (time × obesity status interaction, $P = .12$; time × menopausal status interaction, $P = .54$; time × obesity status × menopausal status interaction, $P = .60$). The same analysis showed that REE increase was significantly higher after the protein-rich meal than after the fat-rich meal in both lean and obese subjects (time × meal interaction, $P < .001$) (Tables 2 and 3).

The DIT after the test meals did not change significantly in either lean or obese subjects (P value for the effect of time > .05). The values of DIT were not significantly different after consumption of the protein-rich meal between lean and obese or between premenopausal and postmenopausal subjects (time × obesity status interaction, $P = .70$; time × menopausal status interaction, $P = .71$; time × obesity status × menopausal status interaction, $P = .88$) (Tables 2 and 3). The same was valid after consumption of the fat-rich meal (time × obesity status interaction, $P = .25$; time × menopausal status interaction, $P = .35$; time × obesity status × menopausal status interaction, $P = .44$) (Tables 2 and 3). In addition, DIT in the postprandial state, expressed as Δ-AUC, was not different between lean and obese subjects after the protein-rich meal (294.7 ± 71.3 vs 260.2 ± 118.7 kJ/h, $P = .59$). The same was valid for the fat-rich meal (87.5 ± 50.3 vs 88.2 ± 82.7 kJ/h, $P = .68$). These differences between the lean and obese participants did not change after adjustment for FFM for either the protein-rich or the fat-rich meal ($P = .12$ or $P = .59$, respectively) and menopausal status ($P = .70$ and $P = .78$, respectively). The increase of DIT was significantly higher after the protein-rich meal in comparison

with the fat-rich meal in both lean (mean difference [Δ] = 205.6 ± 78.9 kJ/h, $P < .001$) and obese individuals (Δ = 169.8 ± 114.3 kJ/h, $P < .001$) (Fig. 1).

Changes in total oxidation rate of the 3 macronutrients in the postprandial period, expressed as Δ-AUC, were not significantly different between lean and obese women after consumption of the protein-rich meal (118.2 ± 11.4 vs 87.1 ± 21.6 mg/min, $P = .20$). The same was valid for the fat-rich meal (−2.5 ± 14.6 vs −18.9 ± 18.7 mg/min, $P = .49$, respectively). These nonsignificant differences did not change after adjustment for the FFM or the menopausal status. In the lean group, total oxidation rate of the 3 macronutrients was significantly higher after the protein-rich meal than after the fat-rich meal (Δ = 122.6 ± 23.4 mg/min, $P = .002$). The same was valid for the obese group (Δ = 108.4 ± 26.3 mg/min, $P = .006$).

Total protein oxidation rate during the study period, expressed as Δ-AUC, was not different between lean and obese subjects after the protein-rich meal (27.9 ± 2.1 vs 26.1 ± 3.3 mg/min, respectively, $P = .63$). The same was valid for the fat-rich meal (9.9 ± 2.0 vs 8.7 ± 2.1 mg/min, $P = .68$). These nonsignificant differences did not change after adjustment for FFM or menopausal status. Protein oxidation rate was significantly higher after the protein-rich meal in comparison with the fat-rich meal in both lean (Δ = 17.8 ± 3.5 mg/min, $P < .001$) and obese subjects (Δ = 17.1 ± 53.2 mg/min, $P < .001$) (Fig. 2).

Total glucose oxidation rate during the study period, expressed as Δ-AUC, was not different between lean and obese individuals after the protein-rich meal (110.9 ± 21.6 vs 47.5 ± 43.3 mg/min, $P = .51$). These nonsignificant differences did not change after adjustment for FFM or menopausal status. Glucose oxidation declined after the fat-rich meal in both lean and obese participants (both Ps < .001). Total glucose oxidation rate during the study period, expressed as Δ-AUC, was not significantly different between lean and obese subjects (−57.5 ± 26.1 vs −96.1 ± 39.0 mg/min, $P = .62$). These nonsignificant differences did not change after adjustment for FFM or menopausal status. Glucose oxidation was significantly lower after the fat-rich meal in comparison with the protein-rich meal in both lean (Δ = −174.3 ± 38.9 mg/min, $P = .001$) and obese subjects (Δ = −149.1 ± 48.2 mg/min, $P = .009$) (Fig. 2).

Fat oxidation rate during the study period, expressed as Δ-AUC, was not different between lean and obese individuals after the protein-rich meal (20.7 ± 6.9 vs 41.4 ± 16.7 mg/min, $P = .58$). These nonsignificant differences did not change after adjustment for FFM or menopausal status. Fat oxidation increased in both groups after the fat-rich meal ($P < .01$); but again, no significant difference was found between the lean and obese individuals (45.6 ± 8.0 vs 57.4 ± 14.9 mg/min, $P = .49$). This difference did not change after adjustment for FFM or menopausal status. There was a trend for the fat oxidation rate to be higher in the lean women after the fat-rich meal in comparison with the protein-rich meal (Δ = 27.6 ± 11.8 mg/min, $P = .06$). In the obese women, no significant difference was found in fat oxidation rate after the protein-rich meal (Δ = 18.4 ± 16.3 mg/min, $P = .28$) (Fig. 2).

The RQ values declined significantly after the fat-rich meal in both lean and obese subjects ($P < .0001$); however, they did not change significantly after the protein-rich meal in either study group (Tables 2 and 3). The overall decrease of the RQ after the test meals, expressed as Δ-AUC, was higher after the fat-rich meal in comparison with the protein-rich meal in both lean (Δ = −0.15 ± 0.16 mg/min, $P = .003$) and obese subjects (Δ = −0.09 ± 0.15 mg/min, $P = .03$).

3.3. Other associations

Protein oxidation rate after the protein-rich meal showed a trend for a positive association with fat oxidation rate ($r = 0.52$, $P = .056$) in the lean but not in the obese subjects ($r = 0.18$, $P = .56$). No significant relationship was found between the protein oxidation rate and the glucose oxidation rate in both groups. In addition, fat oxidation rate after the fat-rich meal showed a significant negative association with glucose oxidation rate in both lean ($r = −0.91$, $P < .001$) and obese women ($r = −0.94$, $P < .001$); no such significant association was found with protein oxidation rate in either group.

Plasma glucose, total cholesterol, LDL cholesterol, HDL cholesterol, blood pressure, and heart rate did not change significantly from baseline values after consumption of either the fat-rich or the protein-rich meal in both groups; however, plasma total triglyceride and insulin concentrations increased significantly after the test meals in both lean and obese subjects (Tables 2 and 3). In addition, there was a trend ($P = .07$) for a higher increase in plasma insulin and triglyceride concentrations in the

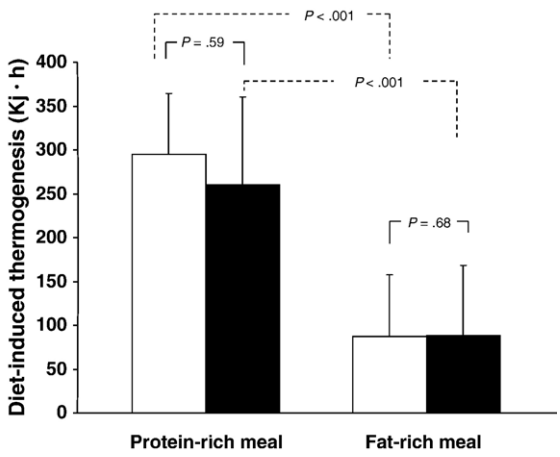

Fig. 1. Data are mean ± SD of incremental AUC throughout the experiment of DIT in lean (white bars) and obese (black bars) women after the test meals.

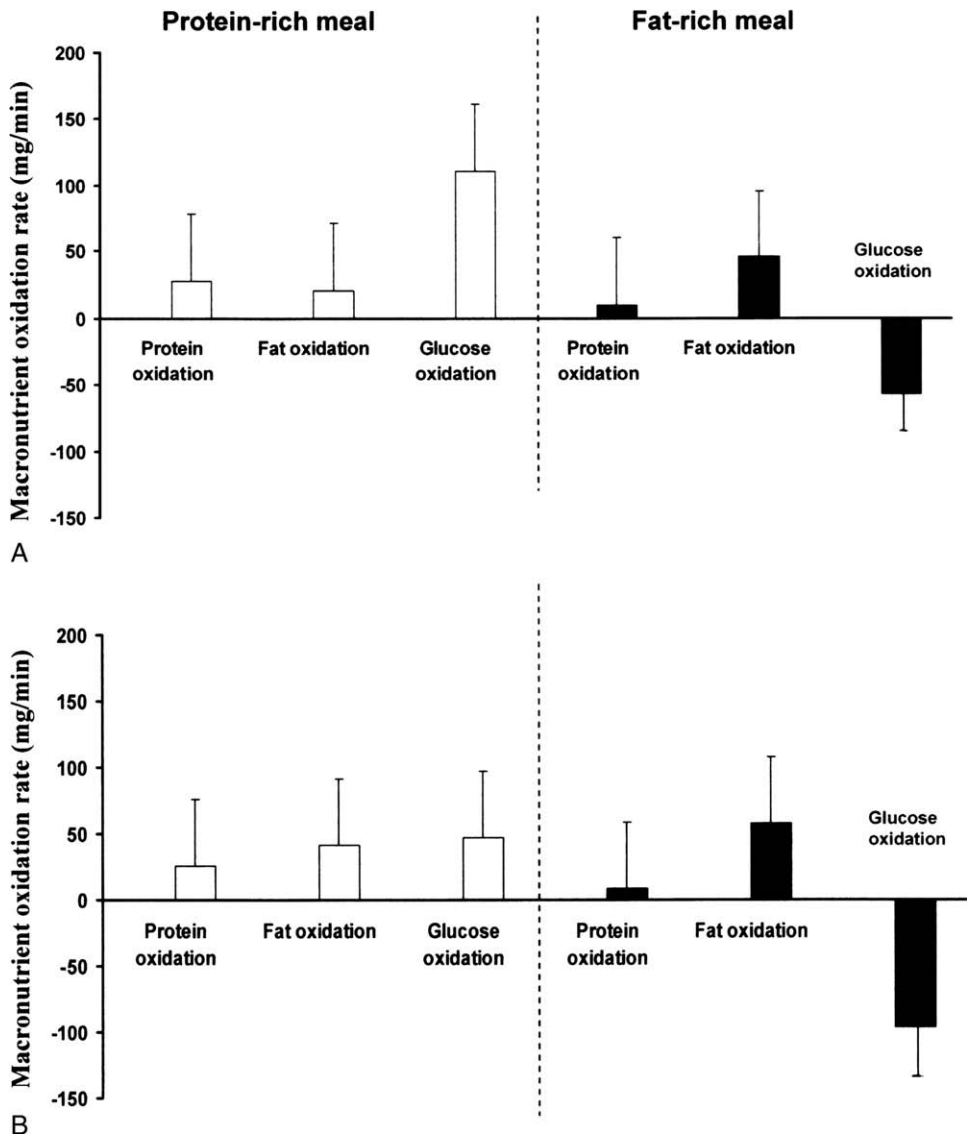

Fig. 2. Data are mean ± SD. Macronutrient oxidation rate in lean (A) and obese (B) women after consumption of the protein-rich meal (on the left side of the dashed line, white bars) and the fat-rich meal (on the right side of the dashed line, black bars). Bars represent the incremental AUC of macronutrient oxidation rate throughout the experiment.

obese in comparison with the lean participants after the protein-rich meal (Table 3).

4. Discussion

Different types of meals do invoke different thermogenic responses [17]. In the present study, protein intake was associated with an almost 3-fold higher DIT in comparison with fat intake. The higher thermogenic response after protein intake has been described previously, and it is due to the fact that assimilation of protein is an energetically costly process [17].

The present study showed that the DIT was not different between lean and obese participants after consumption of either a fat-rich or a protein-rich isoenergetic test meal. Whether obesity is associated with a lower DIT remains controversial [25,26]. Many previous studies demonstrated that DIT is not different between lean and obese subjects after consumption of mixed [16], fat-rich [21], protein-rich [27], or CHO-rich meals [28]. An interesting study demonstrated that, in any given subject, the thermic effect of the food increases linearly with energy intake, but it is independent of body fatness [16]. In addition, findings of the studies performed in postobese subjects suggest that the diminished DIT in the obese might be a secondary phenomenon rather than the primary pathogenic factor in human obesity because it is reversed by weight reduction [14,15]. Moreover, an older comprehensive review reported that there was no clear-cut evidence for a reduced thermic effect of the food in obesity [29]. On the other hand, an impressive number of studies have reported decreased DIT in

obese subjects after mixed meals [11-15,27] or fat-rich meals [27]. To the same direction are the findings of a recent multicenter study performed in the central and north Europe, which showed that DIT is impaired in obese subject after a fat-rich meal [30]. A factor contributing to the divergent results is the different macronutrient composition of the test meals and mostly related to the equivalent energy load in the test meals given to subjects of different body weights [17,25,26]. An additional difficulty relies on the fact that the thermic effect of the food is the most difficult and the least reproducible component of daily energy expenditure because it is affected—beyond the meal size and composition, the palatability of the food, the time of the meal, the consumption frequency, the duration of measurements, and the position of the participants—by other factors that are difficult to control, including subject's genetic background, age, physical fitness, and sensitivity to insulin [17].

In agreement with previous reports, we have shown that obese subjects had lower RQ values in the postabsorptive state, which can be attributed to higher fat oxidation in these individuals [31-33]. Increased fat oxidation rate is considered to be a compensatory, although slow, process; an increase in fat mass may increase fat oxidation, reestablishing fat balance and opposing further weight gain [31].

The present study has shown that the postprandial oxidation rates of CHO, fat, and protein in the immediate response to eating were independent of leanness or obesity after both test meals. Data on differences in macronutrient oxidation after consumption of pure protein or fat meals in lean and obese individuals are limited. Most of the studies did not find differences in macronutrient oxidation between lean and obese subjects after mixed meals [34], after meals rich in either fat or CHO [35], and after a CHO or a protein load [36]. To the same directions are the findings after controlled overfeeding with different types of CHO and fat [37]. Similarly, a recent study showed no differences in macronutrient oxidation between lean and obese children after mixed meals of different fat content [21]. In addition, protein oxidation rate in both the fasting state and during a euglycemic clamp, with plasma insulin concentrations similar to those observed in the postprandial state, was similar in obese and lean subjects [38]. Other studies however have shown a decreased ability for protein oxidation and an altered rate for fat oxidation in obese subjects after consumption of mixed meals [39]. Differences in the intervention and examination period in these studies may explain the divergent results and do not allow for a direct comparison among these studies.

It is well established that the organism needs a few days to adjust amino acid oxidation to protein intake. When changing from situations of low to high protein intake or vice versa, a few days are required before nitrogen balance is again achieved [40]. This probably explains why a high protein intake in our short-term experiment resulted in a relatively small increase (by ~20%) in protein oxidation rate.

An inverse relationship between CHO intake and fat oxidation has been previously described [41]. We also found a negative relationship between fat and glucose oxidation after the fat meal, and this was equally true in lean and obese subjects. Increased fat oxidation inhibits glucose utilization via glycolytic and aerobic pathways. The sites of inhibition of glucose utilization are the steps catalyzed by phosphofructokinase, the rate-limiting enzyme in glucolysis, and pyruvate dehydrogenase, the enzyme required for the formation of acetyl coenzyme A from pyruvate. This relationship is known as the *glucose–fatty acid cycle* and proposes that an elevation in fat oxidation interferes with glucose oxidation [41].

Expectedly, increased fat oxidation after the fat-rich meal resulted in a reduction in RQ postprandially, whereas protein intake did not result in any significant change in RQ throughout the study. However, no significant differences in the respiratory exchange ratio between lean and obese subjects after either the protein-rich or the fat-rich meal were found.

Our study is not without limitations. First, the 3-hour measurement period used in the present study was chosen to avoid sleeping or fidgeting of the participants; a more prolonged postmeal period for the measurement of DIT and substrate oxidation may be required to increase the validity of the results. Second, genetic factors such as variations in the β3-adrenoceptor have been shown to alter DIT [42]. Third, variations in intestinal fatty acid binding proteins contribute to variations in fat absorption and oxidation and thus might also contribute to variations in DIT [43]. In addition, the design of our study is not longitudinal; and therefore, a cause and effect relationship between DIT or oxidation of macronutrients and development of obesity cannot be established.

This study has shown that DIT was ~270 kJ (65 kcal) over the 3-hour period after the protein-rich meal, which is equal to 13.4% of the energy consumed, and ~90 kJ (21 kcal) after the fat-rich meal, which is equal to 4.3% of the energy consumed. A difference of 180 kJ (43 kcal) does not make sense. It can be, however, translated into an important caloric deficit in the long term, given that a positive energy balance of this degree can result in a weight gain of ~2 kg in 1 year.

In conclusion, the present study has shown that DIT is not different between lean and obese subjects after consumption of either a protein-rich or a fat-rich standard test meal. In addition, it was shown that DIT is higher (by almost 3-fold) after consumption of a protein-rich meal in comparison with a fat-rich meal. Moreover, we showed that the macronutrient oxidation rate after the test meals was not different between lean and obese subjects. Over the long term, a low DIT after regular or frequent fat intake may contribute to the development and maintenance of obesity.

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References

[1] WHO. Division of Noncommunicable Diseases/World Health Organization/Programme of Nutrition Family and Reproductive Health. Obesity—preventing and managing the global epidemic. Report of a WHO consultation on obesity. Geneva: World Health Organization; 1998 (WHO/NUT/NCD/98.1).

[2] Acheson KJ, Flatt JP, Jequier E. Glycogen synthesis versus lipogenesis after a 500 gram carbohydrate meal in man. Metabolism 1982;31: 1234-40.

[3] Flatt JP, Ravussin E, Acheson KJ, et al. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J Clin Invest 1985;76:1019-24.

[4] Schutz Y, Flatt JP, Jequier E. Failure of dietary fat intake to promote fat oxidation: a factor favoring the development of obesity. Am J Clin Nutr 1989;50:307-14.

[5] Lawton CL, Burley VJ, Wales JK, et al. Dietary fat and appetite control in obese subjects: weak effects on satiation and satiety. Int J Obes Relat Metab Disord 1993;17:409-16.

[6] Rolls BJ, Kim-Harris S, Fischman MW, et al. Satiety after preloads with different amounts of fat and carbohydrate: implications for obesity. Am J Clin Nutr 1994;60:476-87.

[7] Jebb SA, Prentice AM. Physiological regulation of macronutrient balance. In: Björntorp P, editor. International textbook of obesity. Chichester, UK: John Wiley & Sons, Ltd; 2001. p. 125-35.

[8] Raben A, Agerholm-Larsen L, Flint A, et al. 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. Am J Clin Nutr 2003;77:91-100.

[9] Schutz Y, Bessard T, Jequier E. Diet-induced thermogenesis measured over a whole day in obese and nonobese women. Am J Clin Nutr 1984; 40:545-52.

[10] Flatt JP. The biochemistry of energy expenditure. In: Bray GA, editor. Recent advances in obesity research. London: Newman; 1978. p. 211-28.

[11] Segal KR, Guttin B, Nyman AM, et al. Thermic effect of food at rest, during exercise, and after exercise in lean and obese men of similar body weight. J Clin Invest 1985;76:1107-12.

[12] Segal KR, Edano A, Blando L, et al. Comparison of thermic effects of constant and relative caloric loads in lean and obese men. Am J Clin Nutr 1990;51:14-21.

[13] Segal KR, Edano A, Tomas MB. Thermic effect of a meal over 3 and 6 hours in lean and obese men. Metabolism 1990;39:985-92.

[14] Thorne A, Hallberg D, Wahren J. Meal-induced thermogenesis in obese patients before and after weight reduction. Clin Physiol 1989;9: 481-98.

[15] Thorne A, Naslund I, Wahren J. Meal-induced thermogenesis in previously obese patients. Clin Physiol 1990;10:99-109.

[16] D'Alessio DA, Kavle EC, Mozzoli MA, et al. Thermic effect of food in lean and obese men. J Clin Invest 1988;81:1781-9.

[17] Ravussin E, Swinburn BA. Energy expenditure and obesity. Diabetes Rev 1996;4:403-22.

[18] Lissner L, Stevens J, Levitsky DA, et al. Variation in energy intake during the menstrual cycle: implications for food-intake research. Am J Clin Nutr 1988;48:956-62.

[19] Wier JDV. New methods of calculating metabolic rate with special reference to protein metabolism. J Phisiol 1949;109:1-9.

[20] Jequier E, Acheson K, Schutz Y. Assessment of energy expenditure and fuel utilization in man. Annu Rev Nutr 1987;7:187-208.

[21] Maffeis C, Schutz Y, Grezzani A, et al. Meal-induced thermogenesis and obesity: is a fat meal a risk factor for fat gain in children? J Clin Endocrinol Metab 2001;86:214-9.

[22] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentifuge. Clin Chem 1972;18:499-502.

[23] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and b-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28: 412-9.

[24] Matthews JN, Altman DG, Gampbell MJ, et al. Analysis of serial measurements in medical research. BMJ 1990;300:230-5.

[25] Granada GP, Brandom LJ. The thermic effect of food and obesity: discrepant results and methodological variations. Nutr Rev 2002;60: 223-33.

[26] Prentice A. Are defects in energy expenditure involved in the causation of obesity? Obes Rev 2007;8(Suppl 1):89-91.

[27] Swaminathan R, King RF, Holmfield J, Siwek RA, Baker M, Wales JK. Thermic effect if feeding carbohydrate, fat, protein, and mixed meal in lean and obese subjects. Am J Clin Nutr 1985;42: 177-81.

[28] Marques-Lopes I, Forga L, Martinez A. Thermogenesis induced by a high-carbohydrate meal in fasted lean and overweight young men: insulin, body fat, and sympathetic nervous system involvement. Nutrition 2003;19:25-9.

[29] Ravussin E, Swinburn B. Energy metabolism. In: Stunkard AJ, Wadden TA, editors. Obesity: theory and therapy. 2nd ed. New York: Raven; 1993. p. 163-89.

[30] Blaak EE, Hul G, Verdich C, et al. Impaired fat-induced thermogenesis in obese subjects: the NUGENOB study. Obesity 2007;15:653-63.

[31] Gazzaniga JM, Burns TL. Relationship between diet composition and body fatness, with adjustment for resting energy expenditure and physical activity, in preadolescent children. Am J Clin Nutr 1993;58: 21-8.

[32] Tentolouris N, Tsigos C, Perea D, et al. Differential effects of high-fat and high-carbohydrate isoenergetic meals on cardiac autonomic nervous system activity in lean and obese women. Metabolsim 2003; 52:1426-32.

[33] Maffeis C, Armellini F, Tato L, et al. Fat oxidation and adiposity in prepubertal children: exogenous versus endogenous fat utilization. J Clin Endocrinol Metab 1999;84:654-8.

[34] Owen OE, Mozzoli MA, Smalley KJ, et al. Oxidative and nonoxidative macronutrient disposal in lean and obese men after mixed meals. Am J Clin Nutr 1992;55:630-6.

[35] Thomas CD, Peters JC, Reed GW, et al. Nutrient balance and energy expenditure during ad libitum feeding of high-fat and high-carbohydrate diets in humans. Am J Clin Nutr 1992;55:934-42.

[36] Labayen I, Diez N, Parra D, et al. Basal and postprandial substrate oxidation rates in obese women receiving two test meals with different protein content. Clin Nutr 2004;3:571-8.

[37] McDevitt RM, Poppitt SD, Murgatroyd PR, et al. Macronutrient disposal during controlled overfeeding with glucose, fructose, sucrose, or fat in lean and obese women. Am J Clin Nutr 2000;72:369-77.

[38] Solini A, Bonora E, Bonadonna R, et al. Protein metabolism in human obesity: relationship with glucose and lipid metabolism and with visceral adipose tissue. J Clin Endocrinol Metab 1997;82:2552-8.

[39] Bruce AC, McNurlan MA, McHardy KC, et al. Nutrient oxidation patterns and protein metabolism in lean and obese subjects. Int J Obes Relat Metab Disord 1990;14:631-46.

[40] Flatt JP. Substrate utilization and obesity. Diabetes Reviews 1996;4: 433-49.

[41] Jebb SA, Prentice AM, Goldberg GR, et al. Changes in macronutrient balance during over- and underfeeding assessed by 12-d continuous whole-body calorimetry. Am J Clin Nutr 1996;64:259-66.

[42] Walston J, Andersen RE, Seibert M, et al. Arg64 beta3-adrenoceptor variant and the components of energy expenditure. Obes Res 2003;11: 509-11.

[43] Kim CH, Yun SK, Byun DW, et al. Codon 54 polymorphism of the fatty acid binding protein 2 gene is associated with increased fat oxidation and hyperinsulinemia, but not with intestinal fatty acid absorption in Korean men. Metabolism 2001;50:473-6.