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β_1- and β_2-Adrenoceptor-Mediated Thermogenesis and Lipid Utilization in Obese and Lean Men*

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ABSTRACT

The aim of this study was to elucidate the roles of the β_1- and the β_2-adrenoceptors in thermogenesis and lipid utilization in obesity. The β_1-adrenoceptor study was performed in 9 obese and 10 lean men and consisted of 4 30-min periods during which subjects received consecutive infusions of 0, 3, 6, and 9 μg/kg fat-free mass (FFM)·min dobutamine. Energy expenditure, lipid oxidation, and plasma nonesterified fatty acids and glycerol concentrations increased similarly in both groups during β_1-adrenergic stimulation. The β_2-adrenoceptor study was performed in 10 obese and 11 lean men and involved 3 45-min periods during which 0, 50, and 100 ng/kg FFM·min salbu-

tamol were given in combination 1.2 μg/kg FFM·min atenolol (bolus, 50 μg/kg FFM). During β_2-adrenergic stimulation, the increases in energy expenditure and plasma nonesterified fatty acids and glycerol concentrations were reduced in the obese group. Furthermore, lipid oxidation significantly increased in the normal weight group, but remained similar in the overweight group. In conclusion, these data suggest that β_1-adrenoceptor-mediated metabolic processes are similar in both groups, but β_2-adrenoceptor-mediated increases in thermogenesis and lipid utilization are impaired in the obese. (*J Clin Endocrinol Metab* **86:** 2191–2199, 2001)

THE SYMPATHETIC nervous system plays an important role in the regulation of thermogenesis and lipid utilization. Studies in which the endogenous catecholamines norepinephrine (1, 2) and epinephrine (3–5) (both nonselective α- and β-adrenoceptor agonists) are infused show significant increases in energy expenditure, lipid oxidation, and lipolysis. The roles of the individual adrenoceptor subtypes in thermogenesis have also been studied. α-Adrenergic stimulation does not affect whole body thermogenesis (3, 6), whereas nonselective β-adrenergic stimulation with isoprenaline significantly increases energy expenditure and lipid utilization (7). During only β_1-adrenergic stimulation with dobutamine (8, 9) or only β_2-adrenergic stimulation with salbutamol (6) or terbutaline (10), energy expenditure, lipid oxidation, and lipolysis increase as well. In rats, β_3-adrenergic stimulation leads to significant increases in energy expenditure and lipid utilization (11, 12). However, the rat β_3-adrenoceptor differs pharmacologically from the human β_3-adrenoceptor (13, 14), and consequently, the specific β_3-adrenoceptor agonists used in rats are only weak agonists in humans. Until now, no highly selective β_3-adrenoceptor agonist or antagonist has been available for administration in humans.

Obese subjects may show an impaired response of thermogenesis during norepinephrine infusion (15, 16), but responses similar to those in lean subjects are also frequently

found during norepinephrine (17, 18), epinephrine (5, 19), and isoprenaline (7) infusion. Others only found an impaired thermogenic response when very obese men were compared with very lean men (20) or only during overfeeding (18). More evident are the differences in lipid utilization between obese and lean subjects. During epinephrine (5, 19) or isoprenaline (7) infusion, the increase in lipid oxidation is reduced in overweight men. Furthermore, their increases in plasma nonesterified fatty acids (NEFA) and glycerol concentrations are impaired during epinephrine (5, 21) or isoprenaline (7) infusion. Only Katzeff *et al.* (17) reported an opposite finding, *e.g.* that the increases in plasma glycerol and NEFA concentrations in response to norepinephrine infusion were proportional to the total fat mass of each individual and therefore were greater in the obese. Until now, it has been unclear which β-adrenoceptor subtype is responsible for the impaired responses of thermogenesis and lipid utilization.

The aim of the present studies was to elucidate the roles of β_1- and β_2-adrenoceptors in thermogenesis, lipid oxidation, and lipolysis in obese and lean men.

Subjects and Methods

Subjects

Fourteen obese and 15 lean male volunteers participated in these studies. Six obese and 6 lean men participated in both studies within a time frame of 9 ± 2 months. The physical characteristics of the subjects, grouped per study, are summarized in Table 1. All subjects were in good health as assessed by medical history and physical examination and were weight stable for at least 6 months. Furthermore, both obese and lean subjects spent no more than 2 h a week in organized sports activities. The study protocols were reviewed and approved by the ethics committee of Maastricht University, and all subjects gave informed consent before participating in the tests.

TABLE 1. Physical characteristics of subjects participating in the β_1- and β_2-adrenoceptor studies

	β_1-Adrenoceptor study		β_2-Adrenoceptor study	
	Obese (n = 9)	Lean (n = 10)	Obese (n = 10)	Lean (n = 11)
BW (kg)	103.1 (85.6–119.1)	73.3 (57.0–82.2)[a]	102.3 (88.6–120.7)	73.7 (55.2–94.6)[a]
Ht (m)	1.78 (1.72–1.84)	1.76 (1.63–1.84)	1.76 (1.62–1.84)	1.77 (1.63–1.90)
BMI (kg/m^2)	32.4 (29.1–36.0)	23.7 (20.4–26.6)[a]	33.2 (29.5–37.1)	23.4 (19.1–26.4)[a]
Body fat (%)	31.4 (24.0–36.1)	22.2 (14.0–31.0)[b]	31.8 (22.0–43.9)	22.1 (13.2–26.4)[a]
Fat-free mass (kg)	70.4 (60.7–77.8)	57.1 (39.4–68.5)[b]	69.5 (61.7–77.9)	57.3 (38.1–71.4)[b]
Age (yr)	46 (39–51)	41 (34–50)[c]	47 (40–50)	43 (34–50)

Values are means (range).
[a] $P < 0.001$, obese *vs.* lean, by unpaired t test.
[b] $P < 0.01$, obese *vs.* lean, by unpaired t test.
[c] $P < 0.05$, obese *vs.* lean, by unpaired t test.

Experimental design

Subjects were studied in the morning after an overnight fast. They came to the laboratory by car or bus to minimize the amount of physical activity before the test. On arrival, a cannula was inserted into a forearm vein of each arm. One cannula was used for the infusion of drugs, and one cannula was used for the sampling of blood. Next, ventilated hood measurements were started with the subject in supine position and continued for the remainder of the experiment. At the end of each study period, a blood sample was taken. Room temperature was kept at 21–23 C.

The β_1-adrenoceptor study consisted of four study periods. After a 30-min baseline measurement, subjects received consecutive infusions of 3, 6, and 9 μg/kg fat-free mass (FFM)·min dobutamine (Dobax, Byk, Zwanenburg, The Netherlands), each dose for 30 min.

The β_2-adrenoceptor study consisted of three study periods. At the start of the experiment, subjects received a priming dose of 50 μg/kg FFM atenolol (β_1-adrenoceptor antagonist, Tenormin, Zeneca Pharmaceuticals, Ridderkerk, The Netherlands) in 5 min, after which a continuous infusion of 1.2 μg/kg FFM·min atenolol was started for the remainder of the experiment. After a 45 min baseline measurement, subjects additionally received consecutive infusions of 50 and 100 ng/kg FFM·min salbutamol (Ventolin, GlaxoWellcome, Zeist, The Netherlands), each infusion for 45 min.

Clinical methods

Body density was determined by hydrostatic weighing with simultaneous lung volume measurement (Volugraph 2000, Mijnhardt, Bunnik, The Netherlands). Body composition was calculated according to the equation of Siri (22).

Whole body energy expenditure and respiratory exchange ratio (RER) were measured by indirect calorimetry, using an open-circuit ventilated hood system. In the β_1-adrenoceptor study, a homemade system was used (23). The volume of air drawn through the hood was measured by a dry gas meter (Schlumberger, Dordrecht, The Netherlands), and the composition of the in-flowing and out-flowing air was analyzed by a paramagnetic O_2 analyzer (Servomex, Crowborough, UK) and an infrared CO_2 analyzer (Hartmann and Braun, Frankfurt, Germany). In the β_2-adrenoceptor study, energy expenditure and RER were measured by an Oxycon (Mijnhardt, Bunnik, The Netherlands). The airflow rate and the O_2 and CO_2 concentrations of the in- and outflowing air were used to compute O_2 consumption and CO_2 production on-line through an automatic acquisition system connected to a personal computer. The coefficient of variation for O_2 consumption was 2.4% for the homemade system and 2.5% for the Oxycon; the coefficient of variation for CO_2 production was 3.1% for the homemade system and 2.0% for the Oxycon. Energy expenditure was calculated according to the formula proposed by Weir (24). Energy expenditure and RER values were averaged over the last 10 min of each 30-min (β_1) or 45-min (β_2) period during which steady state occurred. During the β_2-adrenoceptor study, subjects collected their urine for nitrogen determination over a 12-h period before arriving at the laboratory. Nitrogen excretion was used to estimate protein oxidation at baseline and was assumed to be constant during the remainder of the test. For subjects who only participated in the β_1-adrenoceptor study, the mean nitrogen excretion rate for the corresponding group in the β_2-adrenoceptor study was used.

After correction for protein oxidation, carbohydrate and lipid oxidation rates were calculated from O_2 consumption and CO_2 production as described by Ferrannini (25).

Heart rate was monitored continuously by conventional electrocardiography, and the mean value over the last 10 min of each measuring period was used for further analysis. Blood pressure was measured by an automated blood pressure device (Tonoprint, Speidel & Keller, Jungingen, Germany) during the last 10 min of each 30-min (β_1) or 45-min (β_2) interval. The means of four measurements per interval were used for further analysis.

Analytical methods

Blood samples for the determination of NEFA, glycerol, glucose, lactate, and insulin were preserved in sodium ethylenediamine tetraacetate; samples for potassium were preserved in heparin; and those for dobutamine, salbutamol, norepinephrine, and epinephrine were preserved in heparin plus glutathione (1.5%, wt/vol). Blood samples were immediately centrifuged for 10 min at 800 $\times$ g at 4 C. Plasma was transferred into microtest tubes, rapidly frozen in liquid nitrogen, and stored at -70 C until further analysis.

The plasma NEFA concentration was measured with a NEFA C kit (99475409, WAKO, Neuss, Germany), the plasma glycerol concentration was measured with a glycerol kit (148270, Roche, Mannheim, Germany), the plasma glucose concentration was measured with a glucose kit (Unimate 5, 0736724, Roche, Basel, Switzerland), and the plasma lactate concentration was measured by the method of Gutmann and Wahlefeld (26), all on a Cobas-Fara centrifugal analyzer (Roche, Basel, Switzerland). The plasma insulin concentration was determined with a double antibody RIA (Insulin RIA 100, Pharmacia, Uppsala, Sweden), and the plasma potassium concentration was determined by an ion-selective electrode (Salm & Kipp, Breukelen, The Netherlands). Plasma dobutamine, norepinephrine, and epinephrine levels were determined by high performance liquid chromatography according to the method described by Alberts *et al.* (27). Plasma salbutamol concentrations were measured by an in-house method (Analytico Medinet, Breda, The Netherlands) Salbutamol was first extracted from its matrix by means of a solid phase extraction procedure. After derivatization with BSTFA (trimethylsilyl-trifluoroacetamide), its concentration was determined using a capillary gas chromatography-mass spectrometry method. Quantification was performed by monitoring the ion fragments at 456 m/z for salbutamol and 459 m/z for the internal standard D$_3$-salbutamol and calculation of peak height ratio analyte/internal standard amounts. The limit of quantification in plasma was 1.0 nmol/L, based on a 1-mL sample volume. The calibration range was between 1 and 40 nmol/L. Standard samples with known concentrations were included in each run for quality control.

Data analysis

All data are presented as the mean $\pm$ SEM. Data for energy expenditure were adjusted for FFM for group comparison (28).

The effect of β_1- or β_2-adrenergic stimulation between groups was analyzed with two-way repeated measurements ANOVA. *Post-hoc* testing was performed with Student's unpaired t test. $P < 0.05$ was regarded as statistically significant.

Results

β_1-Adrenoceptor study

Baseline energy expenditure was significantly higher in obese compared with lean men (5.49 ± 0.21 *vs.* 4.61 ± 0.18 kJ/min; P < 0.01), but after adjustment for FFM, it was comparable between groups (obese *vs.* lean, 5.19 ± 0.25 *vs.* 5.15 ± 0.14 kJ/min adjusted for FFM; P = NS). During β_1-adrenergic stimulation, energy expenditure increased significantly (Fig. 1). RER was similar at baseline between obese and lean men (0.799 ± 0.013 *vs.* 0.797 ± 0.011; P = NS). RER significantly decreased during β_1-adrenergic stimulation. Lipid and carbohydrate oxidations were comparable in obese and lean subjects at baseline [lipid oxidation, 76 ± 7 *vs.* 66 ± 6 mg/min (P = NS); carbohydrate oxidation, 93 ± 19 *vs.* 77 ± 14 mg/min (P = NS)]. Lipid oxidation significantly increased and carbohydrate oxidation significantly decreased during β_1-adrenergic stimulation. The changes in energy expenditure, RER, lipid oxidation, and carbohydrate oxidation were similar in obese and lean men (Fig. 1).

At baseline, plasma NEFA and glycerol levels were similar in obese and lean men [NEFA, 542 ± 60 *vs.* 409 ± 46 μmol/L (P = NS); glycerol, 77.7 ± 8.2 *vs.* 62.6 ± 8.6 μmol/L (P = NS)]. Both groups showed similar dose-related increases in plasma NEFA and glycerol levels (Fig. 2). Baseline glucose and insulin levels were significantly higher in the obese compared with the lean group (glucose, P < 0.05; insulin, P < 0.01; Table 2). Plasma glucose levels significantly decreased and plasma insulin levels significantly increased during β_1-adrenergic stimulation with dobutamine. The changes in these parameters compared with baseline were not significantly different between groups. At baseline, plasma lactate and potassium concentrations were similar in obese and lean men. During β_1-adrenergic stimulation, plasma lactate levels remained similar, whereas plasma potassium levels showed some variation, but no dose-dependent changes in either group (Table 2).

Plasma dobutamine levels significantly increased to similar concentrations in obese and lean men (Fig. 3). Baseline norepinephrine and epinephrine levels were comparable between overweight and normal weight subjects (norepinephrine, 1.59 ± 0.35 *vs.* 1.47 ± 0.27 nmol/L; epinephrine, 0.19 ± 0.04 *vs.* 0.22 ± 0.03 nmol/L; both P = NS) and were significantly decreased in both groups during β_1-adrenergic stimulation (Fig. 3).

Baseline values for heart rate and systolic blood pressure were not significantly different between groups (Table 3), but diastolic blood pressure was significantly higher in obese men (P < 0.01). Heart rate and systolic blood pressure significantly increased, and diastolic blood pressure significantly decreased in both groups during β_1-adrenergic stimulation with dobutamine. The changes in heart rate and in systolic and diastolic blood pressure were comparable in both groups (Table 3).

β_2-Adrenoceptor study

Baseline energy expenditure was similar in obese and lean men (5.13 ± 0.16 *vs.* 4.97 ± 0.09 kJ/min adjusted for FFM; P = NS). During β_2-adrenergic stimulation, adjusted energy expenditure significantly increased. However, the increase in energy expenditure was significantly lower in the obese com-
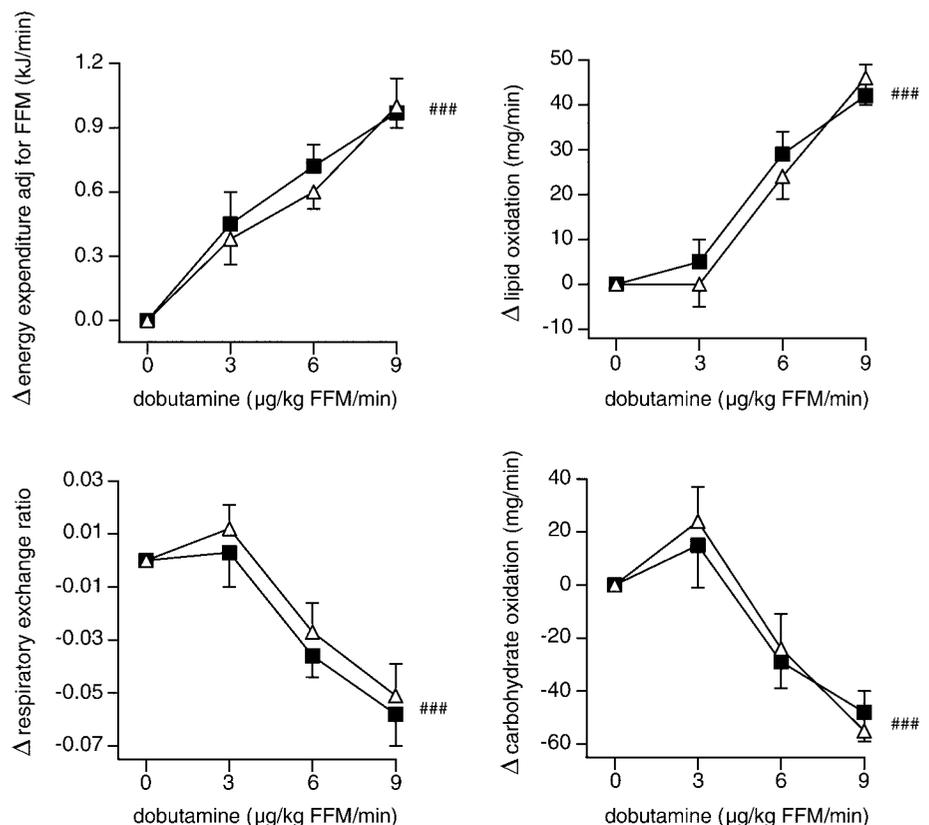

FIG. 1. Changes in energy expenditure adjusted for FFM, RER, and lipid and carbohydrate oxidation during β_1-adrenergic stimulation with dobutamine in 9 obese ($\triangle$) and 10 lean ($\blacksquare$) men. Values are the mean ± SEM. ###, P < 0.001, by ANOVA for treatment.

FIG. 2. Changes in plasma NEFA and glycerol concentrations during β_1-adrenergic stimulation with dobutamine in 9 obese ($\triangle$) and 10 lean ($\blacksquare$) men (*left panels*) and during β_2-adrenergic stimulation with salbutamol in combination with atenolol in 10 obese ($\triangle$) and 11 lean ($\blacksquare$) men (*right panels*). Values are the mean $\pm$ SEM. ###, $P <$ 0.001 (by ANOVA for treatment). *, $P <$ 0.05; **, $P <$ 0.01; ***, $P <$ 0.001 (by unpaired t test, obese *vs.* lean).
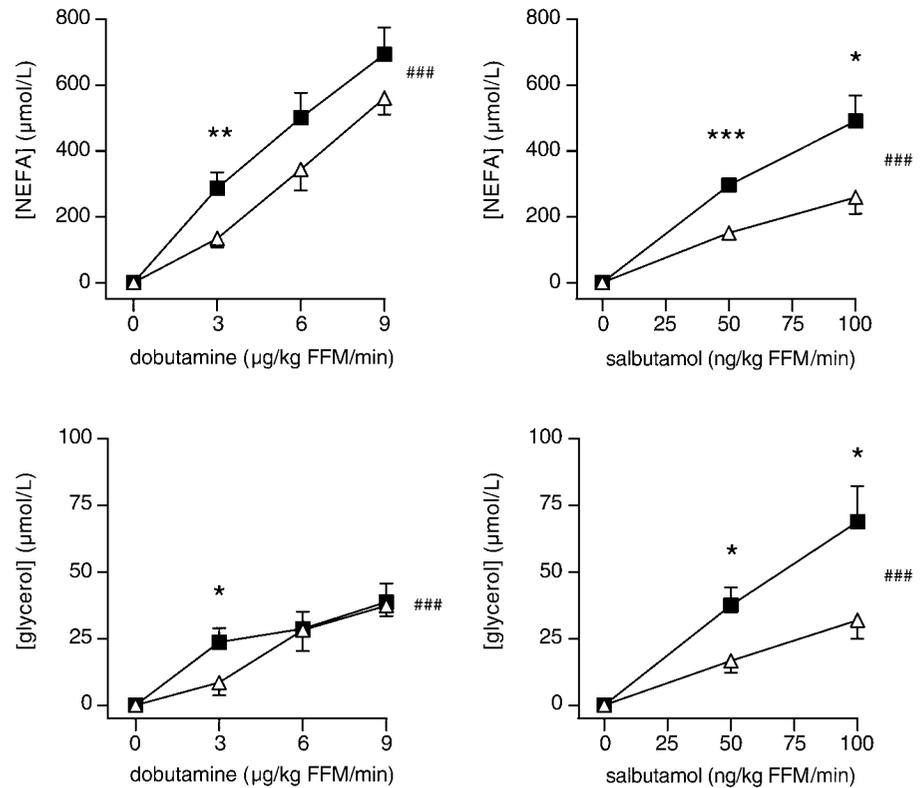
TABLE 2. Plasma concentrations of glucose, insulin, lactate, and potassium during β_1-adrenergic stimulation with dobutamine in obese and lean men

Parameter	Group	Dobutamine (μg/kg FFM·min)				ANOVA		
		0	3	6	9	Group	Treatment	Group $\times$ treatment
Glucose (mmol/L)	Obese	5.56 ± 0.16	5.31 ± 0.21	5.16 ± 0.18	5.01 ± 0.18	<0.05	<0.001	NS
	Lean	5.04 ± 0.15^a	4.86 ± 0.15	4.74 ± 0.12	4.67 ± 0.11			
Insulin (mU/L)	Obese	14.0 ± 2.7	19.4 ± 4.7	21.0 ± 4.5	22.7 ± 5.4	<0.05	<0.001	NS
	Lean	6.1 ± 0.6^b	7.8 ± 1.0^a	9.3 ± 1.2^a	10.5 ± 1.2^a			
Lactate (mmol/L)	Obese	1.28 ± 0.16	1.28 ± 0.17	1.27 ± 0.17	1.13 ± 0.10	<0.01	NS	NS
	Lean	0.93 ± 0.14	0.83 ± 0.07^a	0.81 ± 0.06^a	0.74 ± 0.03^b			
Potassium (mmol/L)	Obese	4.22 ± 0.11	4.26 ± 0.09	4.17 ± 0.09	4.16 ± 0.08	NS	<0.05	NS
	Lean	4.07 ± 0.08	4.16 ± 0.09	4.18 ± 0.08	4.02 ± 0.07			

Values are the mean $\pm$ SEM. Obese, n = 9; lean, n = 10.
[a] $P < 0.05$, obese *vs.* lean, by unpaired t test.
[b] $P < 0.01$, obese *vs.* lean, by unpaired t test.

pared with the lean group (ANOVA for energy expenditure $\times$ group, $P < 0.05$; Fig. 4). At baseline, RER was similar in obese and lean men (0.838 ± 0.011 *vs.* 0.825 ± 0.008; $P =$ NS). RER significantly decreased during β_2-adrenergic stimulation, but the decrease was significantly larger in the lean group (ANOVA for RER $\times$ group, $P < 0.05$; Fig. 4). Baseline lipid and carbohydrate oxidation were similar in obese and lean subjects [lipid oxidation, 55 ± 6 *vs.* 42 ± 3 mg/min ($P =$ NS); carbohydrate oxidation, 143 ± 17 *vs.* 116 ± 9 mg/min ($P =$ NS)]. Lipid oxidation significantly increased during β_2-adrenergic stimulation, but this increase was significantly higher in the lean group (ANOVA for lipid oxidation $\times$ group, $P = 0.05$). Carbohydrate oxidation rates significantly decreased (ANOVA for treatment, $P < 0.05$) during β_2-

adrenergic stimulation, but did not differ significantly between groups (Fig. 4).

At baseline, plasma NEFA levels were similar in obese and lean men (443 ± 21 *vs.* 395 ± 34 μmol/; $P =$ NS; Fig. 2). Baseline glycerol levels were significantly higher in the overweight compared with the normal weight group (76.5 ± 4.3 *vs.* 61.7 ± 4.8 μmol/L; $P < 0.05$). During β_2-adrenergic stimulation with salbutamol, plasma NEFA and glycerol levels increased significantly more in the lean compared with the obese group (ANOVA for group $\times$ treatment: NEFA, $P < 0.01$; glycerol, $P < 0.05$; Fig. 2). Plasma glucose and insulin levels were significantly higher in the obese group at baseline (Table 4). Plasma glucose levels remained similar in the overweight group, but increased significantly in the normal
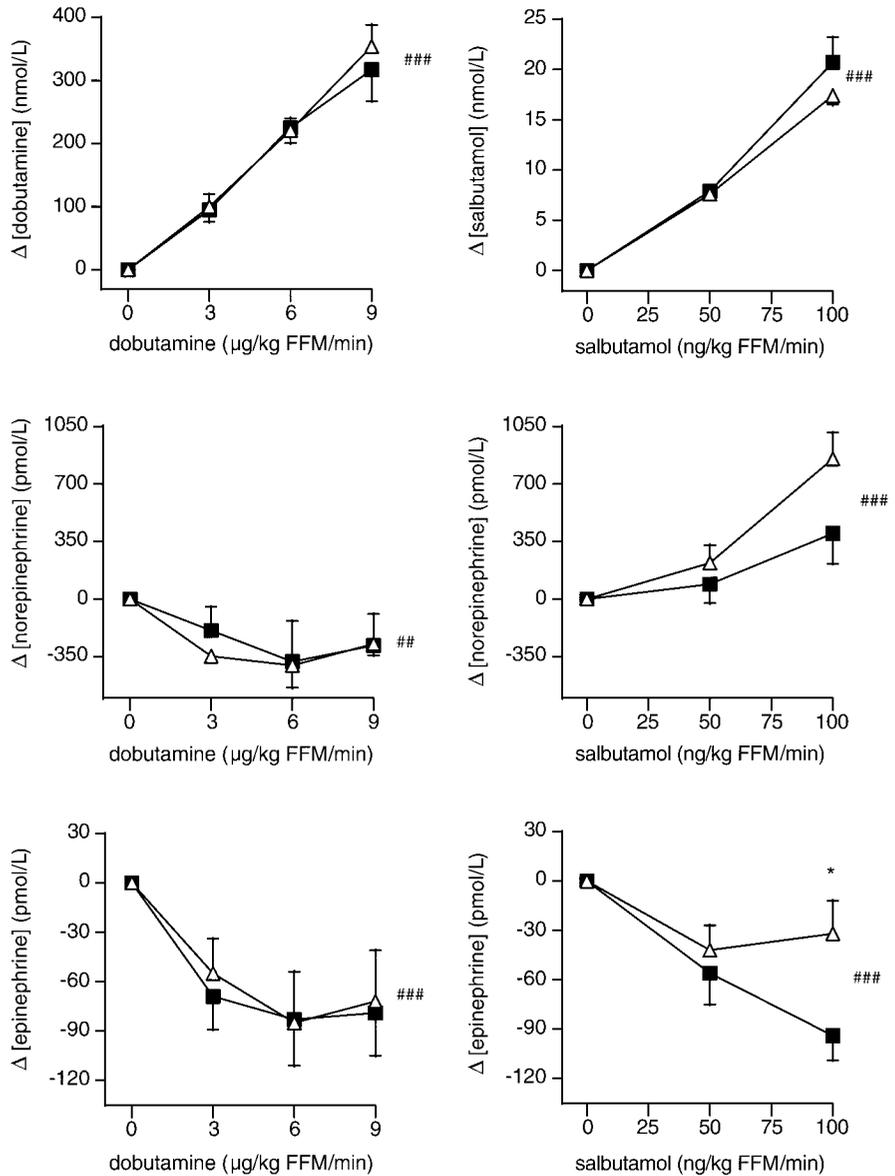
FIG. 3. Changes in plasma dobutamine (*left panel*) or salbutamol (*right panel*) and plasma norepinephrine and epinephrine concentrations during β_1-adrenergic stimulation with dobutamine in 9 obese ($\triangle$) and 10 lean ($\blacksquare$) men (*left panels*) and during β_2-adrenergic stimulation with salbutamol in combination with atenolol in 10 obese ($\triangle$) and 11 lean ($\blacksquare$) men (*right panels*). Values are the mean $\pm$ SEM. ##, $P < 0.01$, ###, $P < 0.001$ (by ANOVA for treatment). *, $P < 0.05$; **, $P < 0.01$ (by unpaired t test, obese *vs.* lean).

TABLE 3. Heart rate and systolic and diastolic blood pressures during β_1-adrenergic stimulation with dobutamine in obese and lean men

Parameter	Group	Dobutamine (μg/kg FFM·min)				ANOVA		
		0	3	6	9	Group	Treatment	Group $\times$ treatment
Heart rate (beats/min)	Obese	66 ± 3	65 ± 4	68 ± 4	76 ± 5	NS	<0.001	NS
	Lean	59 ± 2	59 ± 2	64 ± 2	77 ± 5			
Systolic blood pressure (mm Hg)	Obese	126 ± 4	140 ± 6	150 ± 7	157 ± 6	NS	<0.001	NS
	Lean	122 ± 4	142 ± 5	153 ± 4	160 ± 4			
Diastolic blood pressure (mm Hg)	Obese	95 ± 2	89 ± 3	82 ± 3	80 ± 3	NS	<0.001	NS
	Lean	84 ± 3^a	80 ± 3	76 ± 4	75 ± 5			

Values are the mean $\pm$ SEM. Obese, n = 9; lean, n = 10.
[a] $P < 0.05$, obese *vs.* lean, by unpaired t test.

weight group during β_2-adrenergic stimulation. Plasma insulin levels increased significantly more in the obese compared with the lean group during salbutamol infusion. Baseline lactate and potassium concentrations were similar in both groups. Lactate levels significantly increased, and po-

tassium levels significantly decreased during β_2-adrenergic stimulation, but remained comparable between groups (Table 5).

Plasma salbutamol concentrations increased to a similar level in obese and lean men during both infusion periods

FIG. 4. Changes in energy expenditure adjusted for FFM, RER, and lipid and carbohydrate oxidation during β_2-adrenergic stimulation with salbutamol in combination with atenolol in 10 obese ($\triangle$) and 11 lean ($\blacksquare$) men. Values are the mean ± SEM. #, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$ (by ANOVA for treatment). *, $P < 0.05$ (by unpaired t test, obese *vs.* lean).
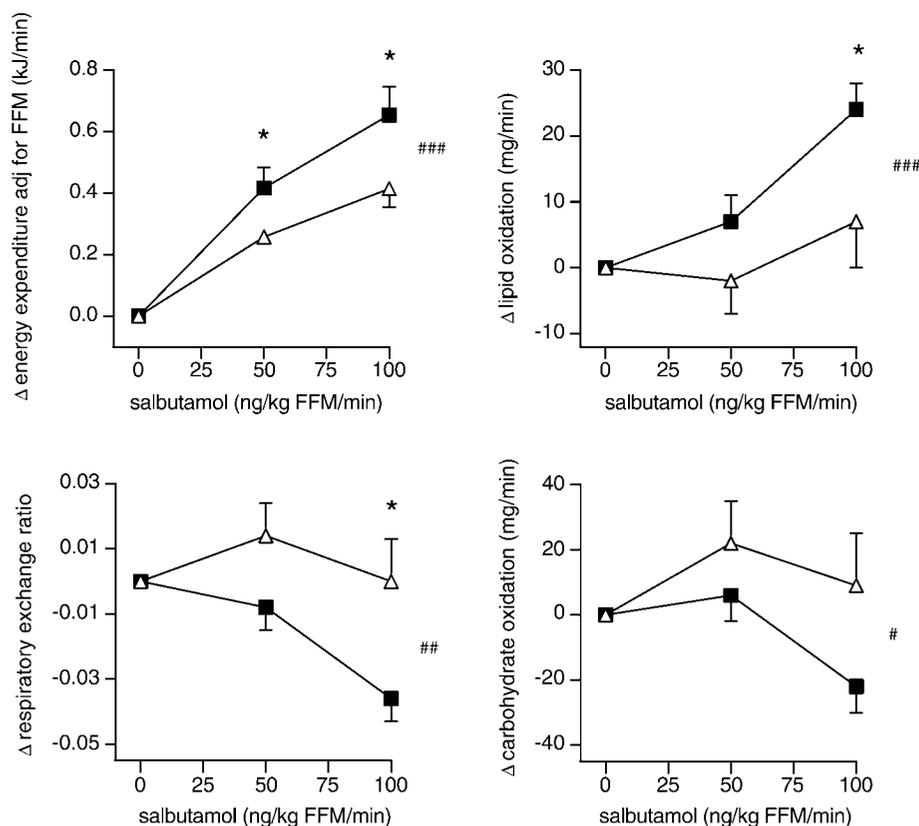
TABLE 4. Plasma concentrations of glucose, insulin, lactate, and potassium during β_2-adrenergic stimulation with salbutamol in obese and lean men

Parameter	Group	Salbutamol (ng/kg FFM·min)			ANOVA		
		0	50	100	Group	Treatment	Group × treatment
Glucose (mmol/L)	Obese	5.64 ± 0.18	5.56 ± 0.17	5.67 ± 0.14	<0.01	<0.01	<0.05
	Lean	4.89 ± 0.12[a]	4.96 ± 0.15[b]	5.17 ± 0.15[b]			
Insulin (mU/L)	Obese	11.8 ± 2.4	17.2 ± 4.0	17.9 ± 3.3	<0.05	<0.001	<0.05
	Lean	5.0 ± 0.4[a]	6.8 ± 0.8[b]	8.5 ± 1.1[b]			
Lactate (mmol/L)	Obese	1.06 ± 0.11	1.12 ± 0.11	1.27 ± 0.10	NS	<0.001	NS
	Lean	0.86 ± 0.13	0.89 ± 0.08	1.03 ± 0.07			
Potassium (mmol/L)	Obese	4.32 ± 0.01	4.32 ± 0.07	4.05 ± 0.07	NS	<0.001	NS
	Lean	4.21 ± 0.07	4.29 ± 0.08	4.03 ± 0.08			

Values are the mean ± SEM. Obese, n = 10; lean, n = 11.
[a] $P < 0.05$, obese *vs.* lean, by unpaired t test.
[b] $P < 0.01$, obese *vs.* lean, by unpaired t test.

(Fig. 3). Baseline norepinephrine levels were comparable between overweight and normal weight subjects (2.57 ± 0.16 *vs.* 2.36 ± 0.16 nmol/L; P = NS), but baseline epinephrine levels were significantly higher in the lean group (0.13 ± 0.02 *vs.* 0.22 ± 0.03 nmol/L; $P < 0.01$). Norepinephrine levels increased similarly in both groups during β_2-adrenergic stimulation. Epinephrine levels decreased significantly in both groups during β_2-adrenergic stimulation, but the decrease was significantly higher in the lean group (Fig. 3).

Baseline values for heart rate and systolic and diastolic blood pressure were not significantly different between obese and lean men (Table 5). Heart rate significantly increased, systolic blood pressure remained similar, and diastolic blood pressure significantly decreased in both groups

during β_2-adrenergic stimulation with salbutamol. The changes in heart rate and systolic and diastolic blood pressure were similar in obese and lean men (Table 5).

Discussion

The aim of the present studies was to examine the roles of the β_1- and the β_2-adrenoceptor in thermogenesis and lipid utilization in obese and lean men. During β_1-adrenergic stimulation with dobutamine, no differences were found in the changes in energy expenditure and lipid utilization between groups. During β_2-adrenergic stimulation with salbutamol, obese subjects had a reduced increase in energy expenditure; a reduced decrease in RER, suggesting a blunted increase in

TABLE 5. Heart rate and systolic and diastolic blood pressures during β_2-adrenergic stimulation with salbutamol in obese and lean men

Parameter	Group	Salbutamol (ng/kg FFM·min)			ANOVA		
		0	50	100	Group	Treatment	Group × treatment
Heart rate (beats/min)	Obese	58 ± 2	65 ± 2	74 ± 3	NS	<0.001	NS
	Lean	53 ± 2	59 ± 2	67 ± 3			
Systolic blood pressure (mm Hg)	Obese	121 ± 4	119 ± 4	122 ± 5	NS	NS	NS
	Lean	113 ± 2	117 ± 3	118 ± 2			
Diastolic blood pressure (mm Hg)	Obese	90 ± 4	85 ± 4	83 ± 4	NS	<0.001	NS
	Lean	82 ± 3	80 ± 3	75 ± 2			

Values are the mean ± SEM. Obese, n = 10; lean, n = 11.

lipid oxidation; and a reduced increase in plasma NEFA and glycerol concentrations, suggesting a reduced lipolytic response. Even when comparing similar increases in thermogenesis in the lean subjects between studies, the accompanying changes in energy expenditure, RER, and plasma NEFA and glycerol concentrations in the obese were blunted during β_2-adrenergic stimulation. This is in line with other studies (5, 7, 19) that found similar impaired responses during sympathetic activation in the obese.

The interpretation of the data from our study highly depends on the selectivity of the β-adrenoceptor agonists used. An earlier study from our group (9) showed that dobutamine induced β_1-adrenoceptor-specific changes in thermogenesis and lipid utilization in dosages of 10 μg/kg BW·min or less. The maximum dose we used was 9 μg/kg FFM·min, which is comparable with 7.5 μg/kg BW·min and thus lies within the range of β_1-adrenoceptor specificity. Our earlier study (9) also showed that the β_2-adrenoceptor agonist salbutamol in a concentration of 85 ng/kg BW·min (or 100 ng/kg FFM·min) also induced β_1-adrenoceptor-specific changes in lipid utilization. Addition of the β_1-adrenoceptor antagonist atenolol prevented simultaneous β_1-adrenergic stimulation, but did not affect β_2-adrenoceptor-specific changes. Therefore, in the current study salbutamol was given in combination with atenolol to investigate β_2-adrenoceptor specific changes in thermogenesis and lipid utilization.

Our study suggests that it is the β_2-adrenoceptor that is responsible for the impaired responses in thermogenesis, lipid oxidation, and lipolysis in the obese *in vivo*. In *in vitro* studies, similar results are found in relation to lipolysis. Glycerol release from sc abdominal fat cells from normal weight and overweight women was similar after incubation with dobutamine, but after incubation with isoprenaline or terbutaline, glycerol release was reduced in fat cells from the obese. This appeared to be due to a significant reduction in cell surface density of β_2-adrenoceptors, although messenger ribonucleic acid (mRNA) levels were similar in both groups (29). In another study, lean subjects with low isoprenaline sensitivity, as measured by *in vitro* sc abdominal fat cell lipolysis, appeared to have lower β_2-adrenoceptor number and mRNA level compared with lean subjects with high isoprenaline sensitivity, whereas β_1-adrenoceptor number and mRNA levels were similar in both groups (30). Both studies suggest that the β_2-adrenoceptor is responsible for the reduced β-adrenoceptor-mediated increase in lipolysis, which is in line with our findings.

Further evidence for a role of the β_2-adrenoceptor in the etiology of obesity is provided by two recently found polymorphisms in the β_2-adrenoceptor that are associated with obesity. The Arg[16]Gly polymorphism in the β_2-adrenoceptor is associated with obesity in Japanese women (31). In a group of Swedish women, this mutation is not associated with obesity, but fat cells from women homozygous for Arg[16] showed a 5-fold lower agonist sensitivity for β_2-adrenoceptors than women heterozygous or homozygous for Gly[16] (32). The Gln[27]Glu polymorphism is associated with obesity in Japanese males and females (31, 33). Swedish women homozygous for Glu[27] had an average fat mass excess of 20 kg and approximately 50% larger fat cells than women homozygous for Gln[27]. However, no significant association with changes in β_2-adrenoceptor function was observed, as assessed by *in vitro* fat cell lipolysis experiments (32). Obesity in Swedish males tends to be negatively associated with the Gln[27]Glu polymorphism (34). As we did not determine β_2-adrenoceptor polymorphisms, it is unknown whether the impaired responses in thermogenesis and lipid utilization found in our obese group are associated with one or both of the above-mentioned polymorphisms. Until now, no associations between polymorphisms in the β_1-adrenoceptor and obesity have been reported.

The reduced increases in thermogenesis and lipid oxidation during β_2-adrenergic stimulation in the obese might also be explained by the reduced increase in NEFA in blood. The amount of NEFA presented to skeletal muscle was therefore reduced, which may have resulted in a smaller increase in lipid oxidation and thermogenesis. As shown in Fig. 5, there was a clear relationship between the increases in plasma NEFA concentration and the increases in energy expenditure and lipid oxidation during β_1- and β_2-adrenergic stimulation. Furthermore, we recently reported that for a certain increase in plasma NEFA concentration produced by lipid heparin infusion, similar increases in thermogenesis and lipid oxidation are found in obese and lean men (35). These data suggest that not only β_2-adrenergic stimulation but also NEFA availability might be related to the blunted responses in thermogenesis and lipid oxidation. Other studies reported not only impaired responses in adipose tissue metabolism, but also in skeletal muscle metabolism, where thermogenesis and lipid oxidation are presumed to be predominantly localized (36). Blaak *et al.* (7) showed that although plasma NEFA levels increased significantly during nonselective β-adrenergic stimulation, no net uptake of NEFA in skeletal muscle occurred in the obese. Moreover, others found that obese women have a decreased capacity to oxidize substrates

FIG. 5. Relationship between mean changes in plasma NEFA concentration and mean changes in energy expenditure and lipid oxidation per infusion period during β_1-adrenergic stimulation with dobutamine ($\triangle$) or β_2-adrenergic stimulation with salbutamol in combination with atenolol ($\bigcirc$) in obese ($\bigcirc$ and $\triangle$) and lean ($\bullet$ and $\blacktriangle$) men.
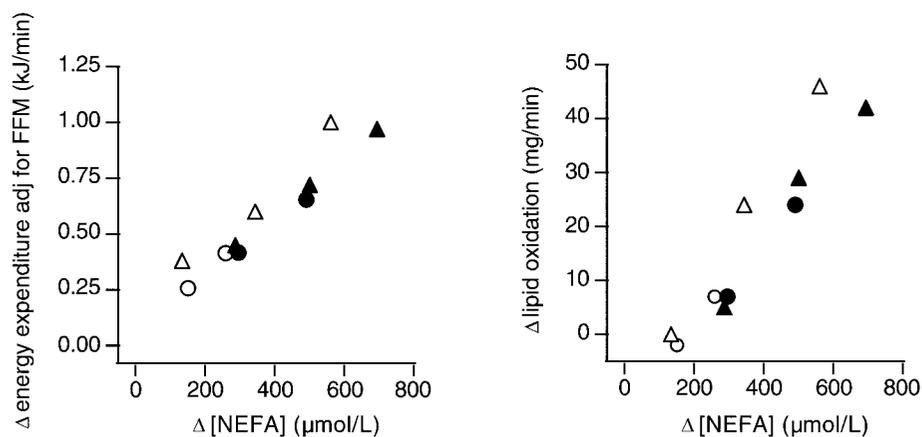
and have increased glycolytic and anaerobic capacities, as measured by the activities of several key enzymes in skeletal muscle biopsies (37, 38). This suggests that lipid oxidation and thermogenesis are impaired in the obese independently from NEFA availability. Our β_1-adrenoceptor study and the study using lipid heparin infusion (35) show that similar increases in thermogenesis and lipid oxidation occur in obese and lean men for a certain increase in plasma NEFA concentration, and therefore do not support these findings.

The reduced increase in plasma NEFA and glycerol concentrations and the consequent reduced increases in lipid oxidation and thermogenesis during β_2-adrenergic stimulation might be explained not only by a defect in the β_2-adrenoceptor or the pathways it mediates, as stated above, but also by the slightly higher increase in insulin concentration in the obese, because insulin inhibits lipolysis. As the obese group was insulin resistant according to their high baseline insulin levels, the impact of this higher increase is difficult to interpret. Comparing the β_1- with the β_2-adrenoceptor study, the increases in plasma insulin level were almost identical between studies (obese *vs.* lean, β_1 study, 8.7 ± 3.5 *vs.* 4.4 ± 0.8 mU/L; β_2 study, 6.0 ± 1.0 *vs.* 3.5 ± 0.8 mU/L), whereas the increases in NEFA and glycerol levels, lipid oxidation, and thermogenesis were only impaired in the β_2-adrenoceptor study. Furthermore in *in vitro* lipolysis tests, β_2-adrenoceptor-mediated lipolysis was reduced in fat cells from the obese, although no insulin was present in the incubation medium (29, 30). These data suggest that the impaired increases in plasma NEFA and glycerol concentration were not due to the slightly higher increase in plasma insulin concentration in the obese during β_2-adrenergic stimulation. However, as changes in insulin concentration and not in insulin action were measured, repeating the experiment during a hyperinsulinemic clamp can only provide direct evidence for a role of insulin in the blunted responses in the obese. Two other studies investigated the role of insulin in epinephrine-induced thermogenesis. One study showed that epinephrine induced energy expenditure independently from insulin concentrations (39), whereas the other study found an inhibitory effect of insulin (40).

Aging is also known to reduce the sensitivity for catecholamines and thus for β-adrenoceptor agonists (41, 42). In our β_2-adrenoceptor study, obese and lean subjects were of similar age, but in the β_1-adrenoceptor study, the obese

group was slightly, but significantly, older than the lean one. However, as our groups differed by only 5 yr in age, whereas subjects in studies on the effect of aging commonly differ by more than 30 yr of age, we believe that the difference in catecholamine sensitivity between our subjects was only minor and therefore did not influence the interpretation of our data.

The impaired responses to β_2-adrenergic stimulation may be caused by differences in norepinephrine kinetics. Studies with tritiated norepinephrine have shown that norepinephrine appearance rates are similar (43, 44) or higher (45, 46) and norepinephrine clearance rates are similar (17, 45, 46) or lower (18) in subjects with a greater fat mass. This suggests that basal sympathetic nervous system activity may be chronically increased in the obese. As a consequence, β-adrenoceptors may become desensitized and/or down-regulated, resulting in reduced sympathetic nervous system responses during additional β-adrenergic stimulation, as shown in this and other studies (5, 7, 15, 16, 19–21). With regard to our study, it is unclear why this desensitization and/or down-regulation would only affect the β_2-adrenoceptor and not the β_1-adrenoceptor.

The question remains of whether the impaired responses during β_2-adrenergic stimulation are a cause or a consequence of obesity. Blaak *et al.* (47) showed that β-adrenoceptor-mediated thermogenesis tended to increase after weight loss. This suggests that the impaired sympathetic nervous system response is a consequence of the obese state. On the other hand, Astrup *et al.* (48) showed that glucose-induced increases in energy expenditure and norepinephrine levels improved in obese subjects after 30-kg weight loss, but were still lower than those in control subjects. Furthermore, Blaak *et al.* (47) showed that β-adrenoceptor-mediated increases in arterial NEFA concentration and muscle NEFA uptake remained impaired after weight reduction. This suggests that a defective sympathetic nervous system may be a primary factor leading to the development of obesity rather than a secondary factor resulting from the obese state.

In conclusion, our studies suggest that β_1-adrenoceptor-mediated thermogenesis and lipid utilization are similar in obese and lean men, but β_2-adrenoceptor-mediated increases in energy expenditure, lipid oxidation, and lipolysis are impaired in the obese.

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