

Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men

Citation for published version (APA):

Larsen, T. M., Toubro, S., van Baak, M. A., Gottesdiener, K. M., Larson, P., Saris, W. H. M., & Astrup, A. V. (2002). Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. *American Journal of Clinical Nutrition*, 76(4), 780-788. <https://doi.org/10.1093/ajcn/76.4.780>

Document status and date:

Published: 01/01/2002

DOI:

[10.1093/ajcn/76.4.780](https://doi.org/10.1093/ajcn/76.4.780)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 02 Jun. 2023

Effect of a 28-d treatment with L-796568, a novel β_3 -adrenergic receptor agonist, on energy expenditure and body composition in obese men¹⁻³

Thomas M Larsen, Søren Toubro, Marleen A van Baak, Keith M Gottesdiener, Patrick Larson, Wim HM Saris, and Arne Astrup

ABSTRACT

Background: Stimulation of energy expenditure (EE) with selective thermogenic β -adrenergic agonists may be a promising approach for treating obesity.

Objective: We analyzed the effects of the highly selective human β_3 -adrenergic agonist L-796568 on 24-h EE, substrate oxidation, and body composition in obese, weight-stable men.

Design: In this 2-center, double-blind, randomized, parallel-group study, we measured 24-h EE before and after 28 d of treatment with L-796568 (375 mg/d) or placebo during weight maintenance (ie, without dietary intervention) in nondiabetic, nonsmoking men aged 25–49 y with body mass index (in kg/m²) of 28–35 ($n = 10$ subjects per treatment group).

Results: The mean change in 24-h EE from before to after treatment did not differ significantly between groups (92 ± 586 and 86 ± 512 kJ/24 h for the L-796568 and placebo groups, respectively). The change in 24-h nonprotein respiratory quotient from before to after treatment did not differ significantly between groups (0.009 ± 0.021 and 0.009 ± 0.029 , respectively). No changes in glucose tolerance were observed, but triacylglycerol concentrations decreased significantly with L-796568 treatment compared with placebo (-0.76 ± 0.76 and 0.42 ± 0.31 mmol/L, respectively; $P < 0.002$). Overall, treatment-related changes in body composition were not observed, but higher plasma L-796568 concentrations in the L-796568 group were associated with greater decreases in fat mass ($r = -0.69$, $P < 0.03$).

Conclusions: Treatment with L-796568 for 28 d had no major lipolytic or thermogenic effect but it lowered triacylglycerol concentrations. This lack of chronic effect on energy balance is likely explained by insufficient recruitment of β_3 -responsive tissues in humans, down-regulation of the β_3 -adrenergic receptor-mediated effects with chronic dosing, or both. *Am J Clin Nutr* 2002;76:780–8.

KEY WORDS L-796568, β_3 -adrenergic receptor, β_3 -adrenergic receptor agonist, β_3 agonist, selectivity, energy expenditure, lipolysis, respiratory quotient, indirect calorimetry, triacylglycerol, obesity, obese men

INTRODUCTION

Several sympathomimetic agents are currently being used to treat human obesity. These agents have potential anorectic and thermogenic effects. However, the earlier use of nonselective β -adrenergic compounds was associated with adverse reactions

such as tachycardia and tremor, which were attributable to β_1 and β_2 stimulation, respectively. The characterization of the β_3 -adrenergic receptor, mainly expressed in white adipose tissue (WAT) and brown adipose tissue (BAT) (1, 2), evoked hope for a new potential target in the pharmacologic treatment of obesity and diabetes.

A marked thermogenic response to selective β_3 -adrenergic receptor agonists was found in rodents (3, 4). When administered over a period of weeks, these agonists induce weight loss and have antidiabetic effects (3, 5). Furthermore, in addition to their thermogenic effects, β_3 agonists may promote fat loss by stimulating fatty acid mobilization and, directly or indirectly, fat oxidation. Studies of isolated human fat cells show lipolytic activity not involving the β_1 and β_2 receptors (6, 7), and in vivo studies in humans show lipolytic activity of the partial β_3 -adrenergic receptor agonist CGP12177. This effect was not inhibited by the combined β_1 and β_2 antagonist propranolol (8), and it was suggested that the β_3 -adrenergic receptor may be responsible for a substantial proportion of the lipolytic response to norepinephrine in humans (9).

Among the β_3 agonists tested in human clinical trials to date, only the compound CL 316 243 has been recognized as weakly potent, but selective, toward the human β_3 -adrenergic receptor (10). In an 8-wk study in lean male subjects, this partial β_3 -adrenergic receptor agonist significantly increased insulin-stimulated glucose disposal, fasting plasma fatty acid concentrations, and 24-h fat oxidation without any side effects mediated by β_1 or β_2 ; however, no effects on energy expenditure or body weight were observed.

L-796568, a novel β_3 -adrenergic receptor agonist for the human β_3 -adrenergic receptor, shows potency as a selective human β_3 agonist when tested in cyclic AMP assays on Chinese hamster

¹ From the Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Copenhagen (TML, ST, and AA); the Nutrition and Toxicology Research Institute (NUTRIM), Department of Human Biology, Maastricht University, Maastricht, Netherlands (MAVB and WHMS); and Merck & Co, Rahway, NJ (KMG and PL).

² Supported by Merck & Co Inc, Whitehouse Station, NJ.

³ Address reprint requests to TM Larsen, Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg, Copenhagen, Denmark. E-mail: tml@kvl.dk.

Received January 1, 2001.

Accepted for publication October 24, 2001.

ovary cells transfected with the human β_3 -adrenergic receptor, and the compound has an $EC_{50} = 3.6$ nmol/L (94% activation compared with isoprenaline) with >600-fold selectivity over the human β_1 and β_2 receptors (11). A related β_3 agonist that has similar potency, L-755507, elicited a dose-dependent rise in metabolic rate and lipolysis in rhesus monkeys; there was an $\approx 30\%$ increase in metabolic rate after intravenous bolus administration. Chronic treatment with L-755507 was also accompanied by significant increases in BAT and uncoupling protein 1 expression (12). Human studies with L-796568 showed dose-dependent increases in plasma glycerol and fatty acid concentrations (K Gottesdiener and Merck & Co, unpublished observations, 1999). More importantly, in obese subjects who had fasted overnight, an $\approx 8\%$ acute thermogenic effect of a single 1000-mg dose of L-796568 was found (13). The purpose of the present study was to test whether this acute effect was maintained after 28 d of chronic treatment with a dosing regimen (375 mg L-796568 once daily with food) that was shown in a separate study to achieve plasma concentrations of L-796568 similar to or higher than those achieved with a single 1000-mg dose of L-796568 administered in the fasted state.

SUBJECTS AND METHODS

Study design

This double-blind, randomized, placebo-controlled, parallel-group study was performed at 2 centers, Copenhagen and Maastricht. A total of 20 male subjects were randomly assigned to receive either 375 mg L-796568 or placebo, administered once daily with breakfast. In each treatment group (placebo or L-796568) there were 10 subjects (5 at each study site).

Energy expenditure (EE) and substrate oxidation rates were determined with indirect calorimetry in respiration chambers on a fixed physical-activity protocol. L-796568 was administered at 0915 in the respiration chamber after a meal. The study consisted of a baseline 24-h measurement (0915 to 0915) on day 0 (the day before treatment began) and a second measurement obtained with an identical protocol on day 27. At the baseline visit, the measurement was continued for 6 h after each subject ingested his first dose of study medication to evaluate a possible acute effect of the compound. To investigate a possible drug effect during particular periods of the day, the 24-h measurement period (0915–0915) was divided into the following specific intervals: sleep EE (0300–0600), basal metabolic rate (0730–0830), daytime EE (0915–1315), exercise EE (1430–1445), and postdinner EE (1915–2215).

Subjects

All subjects underwent a full physical examination with an electrocardiogram, medical history, and routine hematology, biochemistry, and urine screening tests. All were found to be in good health (except for their obesity), without previous or current endocrine, cardiovascular, or hepatic diseases. The subjects reported no acute illnesses within the 2 wk immediately preceding the start of the trial. None of the subjects were dieting or took any medication regularly. They were all nonsmokers, aged 25–49 y, with a body mass index (BMI; in kg/m^2) between 28 and 35 and stable weight (± 4 kg) for ≥ 3 mo before the study.

All subjects were carefully instructed to maintain their usual dietary habits and body weight throughout the study. During the 28-d treatment period, the subjects consumed breakfasts with a

consistent composition each morning (1620 kJ, 30% of energy from fat) to improve drug absorption (≈ 3 -fold increase in area under the curve when administered with food). They also avoided excessive or strenuous physical activity and consumed no more than 2 units (24 g) of alcohol and 4 cups of caffeinated beverages/d. Alcohol intake was prohibited for 48 h before the respiration chamber sessions. No other medication was allowed, and subjects were instructed to report any additional medication used during this period. The study was approved by the Ethics Committee of Frederiksberg and Copenhagen (Copenhagen site) and the Medical Ethics Committee of Maastricht University (Maastricht site). The subjects gave their written, informed consent according to the Helsinki Declaration.

Anthropometry

Body weight was measured in light clothing on a decimal scale on days 0, 7, 14, and 28 (in Copenhagen: Lindeltronic 8000, Lindells Inc, Kristianstad, Sweden; in Maastricht: Sauter model D-7440, Sauter Inc, Ebingen, Germany). Body composition was estimated with dual-energy X-ray absorptiometry within 7 d before treatment (prestudy) and again within 2 d after the last treatment day (poststudy) (both sites: Lunar DPX-IQ; General Electric, Madison, WI) and with the bioelectrical impedance method (Copenhagen: Animeter; HTS-Engineering Inc, Odense, Denmark and Maastricht: Hydra ECF/ICF model 4200; Xitron Technologies, San Diego). Heart rate and blood pressure were measured with an automatic device (both sites: Omron Automatic HEM-705CP; Omron Electronics GmbH, Hamburg, Germany).

Indirect calorimetry

EE and substrate oxidation were measured in open-circuit respiration chambers. At the Copenhagen site, the floor area and volume were 6.5 m^2 and 14.7 m^3 , respectively (14), whereas at the Maastricht site, the floor area and volume were 7.0 m^2 and 14.0 m^3 , respectively (15). The room temperature was maintained at 24 °C during the daytime and 18 °C at night. The oxygen and carbon dioxide exchange was determined by taking measurements of the oxygen fraction (Copenhagen: Magnus 4G; Hartman and Braun, Frankfurt, Germany and Maastricht: Magnus 6G, Hartman and Braun, in combination with OA184A; Servomex, Crowborough, United Kingdom) and carbon dioxide fraction (both sites: Uras 3G; Hartman and Braun) at the inlet and outlet of the chamber and from the flow of the outgoing air.

Urine was collected for a 24-h period on days 0 and 27 and also during the 6-h period after the first dose. Urine samples were used to estimate protein oxidation by assessing nitrogen excretion. Protein oxidation was calculated on the basis of the assumption that 6.25 g protein is combusted per gram of nitrogen excreted. Subsequently, nonprotein oxygen and carbon dioxide exchanges were calculated on the basis of the assumption that 0.952 L O_2 is consumed and 0.795 L CO_2 is produced per gram of protein oxidized. Carbohydrate and fat oxidation were then calculated by using the constants published by Elia and Livesey (16).

Spontaneous physical activity in the respiration chambers was assessed with 2 microwave radar instruments (Sisor Mini-Radar; Static Input System SA, Lausanne, Switzerland) at the Copenhagen site and with an analogue ultrasound system (Artech Advisor DU160; Artech BV, Roermond, Netherlands) at the Maastricht site. The spontaneous physical activity scores were used to

redefine the sleeping period if spontaneous physical activity exceeded 2% during the prescribed period.

During the subjects' stays in the respiration chamber, the activity protocols (ie, fixed meal times and sessions of physical activity) were similar at the 2 sites. Two 15-min sessions of bicycling at a work output of 75 W were included in the protocol at 1430 and 1630. To ensure adherence to the protocol, the subjects were monitored constantly by a laboratory technician during the daytime. To accustom the subjects to the respiration chamber environment, they spent the night (Copenhagen) or at least some hours (Maastricht) in the chamber before the experiments.

Diet

To achieve energy balance (defined as energy intake – EE, ± 0.5 MJ) during the stay in the respiration chamber, the appropriate dietary energy content (kJ/24 h) was individually calculated by using the subjects' fat-free mass (FFM) in the following equation: $136.1 \text{ kJ/kg} \times \text{FFM in kg} + 2154 \text{ kJ}$ (17). FFM was calculated with an algorithm determined on the basis of impedance measurements in men (18). The dietary composition was similar at the 2 study sites, providing an average of 50% of energy from carbohydrates (range: 48.6–53.6%), 35% from fat (32.2–35.6%), and 15% from protein (13.4–16.9%). The diet had a calculated respiratory quotient (RQ; derived from the dietary macronutrient composition) of ≈ 0.881 , depending on the actual energy intake. Dietary energy content and composition were calculated by using BECEL dietary assessment software (Unilever, Rotterdam, Netherlands) and the Dutch Food Composition Table at the Maastricht site. At the Copenhagen site, we used DANKOST dietary assessment software, version 2.0 (Danish Catering Center Denmark). Both software programs use the Atwater factors for metabolizable energy. Any food not consumed by the subjects was reweighed and subtracted to calculate actual energy and macronutrient intakes. A maximum of 2 cups tea or coffee was allowed during the respiratory chamber measurements, and intake during the baseline measurement was replicated during the second measurement.

Blood and urine sample collection

While the subjects were in a fasted state and resting, blood samples were obtained from a forearm vein; this was done before subjects entered the chamber on day 0 and after they left the chamber on day 28. The blood was analyzed for norepinephrine, epinephrine, fatty acids, triacylglycerol, glycerol, lactate, ketones (β -hydroxybutyrate), total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and leptin. In addition, fasting triacylglycerol, fatty acids, glycerol, and total cholesterol were measured on day 14. We also obtained blood samples to be analyzed for L-796568 concentration on day 0 and before the dose of L-796568 on days 21, 25, and 28; subjects were fasting when the blood was drawn.

A 24-h urine sample was collected for measurement of nitrogen and catecholamines from 0900 on day 0 to 0900 on day 1 and again from 0900 on day 27 to 0900 on day 28. In addition, urine was collected on day 1 from 0900 to 1500.

Blood and urine analyses

For the analyses of plasma glycerol and fatty acid concentrations, 2 mL blood was drawn into a tube containing EDTA, kept on ice, and centrifuged within 1 h at $1200 \times g$ for 15 min at 4°C ; the plasma was stored at -70°C until analyzed. The analysis was done with an automated spectrophotometric procedure (19). For

the analyses of serum leptin, 4 mL blood was drawn into a tube, kept on ice, and centrifuged within 1 h at $1200 \times g$ for 15 min at 20°C . The serum was stored at -70°C until analyzed with the DSL-23100 leptin immunoradiometric assay kit (Diagnostic Systems Laboratories, Webster, TX).

For the analyses of plasma catecholamines, 6 mL blood was drawn and immediately transferred into a fresh solution of reduced glutathione; this was centrifuged at $806 \times g$ for 10 min at 4°C . The plasma was immediately frozen in liquid nitrogen and stored at -70°C until analyzed by using HPLC with electrochemical detection (20). For the analyses of plasma L-796568, 5 mL blood was drawn into a tube containing heparin, placed on ice, and centrifuged at $1670 \times g$ for 15 min at 4°C . The plasma was stored at -20°C until analyzed by using solid-liquid extraction, electrospray liquid chromatography–tandem mass spectrophotometry (LC-MS/MS) method (Merck & Co, West Point, PA).

For the analyses of serum or plasma lipids, 4 mL blood was drawn into a tube, centrifuged at $2000 \times g$ for 10 min at 4°C , and kept at -18°C until analyzed. At the Copenhagen site, we measured serum triacylglycerols with an enzymatic method (21) and serum HDL cholesterol with an enzymatic method (22); serum LDL cholesterol was calculated (23). At the Maastricht site, serum triacylglycerols and serum total cholesterol were measured with an enzymatic method (24), plasma HDL cholesterol was measured with an enzymatic method (25), and plasma LDL cholesterol was calculated (23).

For the analyses of β -hydroxybutyrate and lactate, at the Copenhagen site 5 mL blood was drawn into a tube containing EDTA and mixed, 4 mL ice-cold perchloric acid was added, and the solution was kept at -18°C until analyzed. β -hydroxybutyrate concentration was analyzed with a colorimetric method (Boehringer Mannheim, Mannheim, Germany), and blood lactate was measured with enzymatic determination (26). At the Maastricht site, serum β -hydroxybutyrate was analyzed with an enzymatic method (27) automated on a Cobas Fara centrifugal analyzer (Roche, Basel, Switzerland). Plasma lactate was analyzed via enzymatic determination (YSI model 2300 glucose and lactate analyzer; Yellow Springs Instruments Inc, Yellow Springs, OH).

Subjects underwent a 120-min oral-glucose-tolerance test (OGTT) with 75 g glucose on 2 occasions: within the 7 d before the study and on day 28 (the end of the study). Both OGTTs were performed in the fasting state, but on day 28 the glucose was consumed with 375 mg L-796568. For the analyses of glucose and insulin concentrations, at the Copenhagen site 4 mL blood was drawn into a gel tube, allowed to coagulate, and centrifuged at $2000 \times g$ for 10 min at 4°C . The serum was kept at 18°C until analyzed. Serum insulin was measured with a radioimmunoassay kit from Pharmacia AS, Copenhagen and serum glucose was measured with enzymatic glucose oxidase (21). At the Maastricht site, serum insulin was analyzed with an Autodelphia fluoroimmunoassay kit from Perkin-Elmer Wallac Inc, Oy, Finland, and plasma glucose was measured with an enzymatic method (28). For the analyses of plasma glucagon, at the Copenhagen site 5 mL blood was drawn into a tube containing EDTA, stored at 4°C for < 30 min, and centrifuged at $2000 \times g$ for 10 min at 4°C . The plasma layer was transferred into a glass tube holding 1.500 kallikrein-inactivating units (KIE) of Trasylol (Bayer Pharma, Lyngby, Denmark). The sample was stored at 25°C until analyzed with a radioimmunoassay kit from Diagnostic Products Corp, Los Angeles. At the Maastricht site, serum glucagon was analyzed with a radioimmunoassay kit (glucagon double antibody; DPC Biermann GmbH, Bad Nauheim, Germany).

TABLE 1

Anthropometric and cardiovascular measures at baseline and energy expenditure (EE) and nonprotein respiratory quotient (RQ_{np}) during the baseline stay in the respiration chamber¹

	Placebo group (n = 10)	L-796568 group (n = 10)
Age (y)	35 ± 8	37 ± 6
Height (cm)	184 ± 8	181 ± 5
Body weight (kg)	102.7 ± 12.7	103.5 ± 7.9
BMI (kg/m ²)	30.3 ± 2.2	31.5 ± 2.1
Fat-free mass (kg) ²	74.9 ± 10.7	70.9 ± 4.6
Fat mass (kg) ²	28.7 ± 5.2	31.7 ± 5.0
Systolic BP (mm Hg)	128 ± 7	126 ± 6
Diastolic BP (mm Hg)	76 ± 9	81 ± 7
Heart rate (beats/min)	63 ± 6	63 ± 11
24-h EE (kJ/min)	8.13 ± 0.93	8.10 ± 0.45
24-h RQ_{np}	0.846 ± 0.021	0.858 ± 0.020
Sleeping EE (kJ/min)	6.03 ± 0.93	5.92 ± 0.33
Basal metabolic rate (kJ/min)	7.48 ± 1.14	7.05 ± 0.73
Daytime RQ_{np}	0.806 ± 0.028	0.838 ± 0.035 ³

¹ $\bar{x} \pm SD$. BP, blood pressure.

²Determined within the 7 d before the baseline stay in the respiration chamber.

³Significantly different from the placebo group, $P = 0.04$.

For the analyses of urinary nitrogen, urine was collected in bottles containing 10 mL sulfuric acid (2 mol/L); 8 mL was transferred into scintillation vials and stored at -20°C before analysis via oxidative decomposition with heat conductance detection (Heraeus CHN-O-RAPID; Heraeus GmbH, Hanau, Germany). For the analyses of urinary norepinephrine and epinephrine, 3 mL acidified urine was stored at -70°C before analysis via HPLC with electrochemical detection (20).

Appetite profiles

Subjects were asked to complete appetite questionnaires during both stays in the respiration chamber (29). The questionnaires include questions related to hunger and satiety, both of which were rated on a 100-mm visual analogue scale. Subjects recorded their responses before lunch (while fasting), every hour for the 3 h after lunch, before dinner, and every hour for the 3 h after dinner (a total of 8 times).

Statistical analyses

The effect of treatment with L-796568 on 24-h EE was the primary endpoint of this study. Within each treatment group, changes over time relative to baseline were tested by using a paired t test. Mean ($\pm SD$) changes over time (eg, from day 0 to day 27) were calculated and group differences were tested by using a two-sample unpaired t test. If the group differences were significant or nearly significant ($P < 0.10$) at baseline, group differences from day 0 to day 27 were recalculated with a general linear model univariate analysis with baseline values added as cofactors. Changes in OGTT response were tested with a general linear model on the basis of a repeated-measures analysis of variance model, and significant group-by-time interaction effects were subsequently analyzed with an unpaired t test. Correlations were determined by using simple correlation analysis. Because body weight and FFM were stable throughout the treatment periods, these factors were not included in the analyses. Analyses were performed with SPSS 10.0 for Windows (SPSS Inc, Chicago). $P < 0.05$ was regarded as statistically significant.

TABLE 2

Blood and urine variables at baseline¹

	Placebo group (n = 10)	L-796568 group (n = 10)
Blood		
Glucose (mmol/L)	5.35 ± 0.67	5.46 ± 0.52
Insulin (pmol/L)	64 ± 28	87 ± 56
Glucagon (pmol/L)	19 ± 5	26 ± 13
Fatty acids ($\mu\text{mol/L}$)	324 ± 93	415 ± 122 ²
Glycerol ($\mu\text{mol/L}$)	45 ± 12	58 ± 21
Triacylglycerols (mmol/L)	1.23 ± 0.64	2.03 ± 0.89 ³
Lactate ($\mu\text{mol/L}$)	720 ± 210	913 ± 180 ³
Ketones ($\mu\text{mol/L}$)	87.7 ± 79.5	115.7 ± 73.6
Leptin (ng/mL)	11 ± 5	14 ± 10
Norepinephrine (nmol/L)	2.45 ± 1.40	2.00 ± 0.82
Epinephrine (pmol/L)	156 ± 80	147 ± 84
Total cholesterol (mmol/L)	4.9 ± 0.8	5.7 ± 1.2 ²
HDL cholesterol (mmol/L)	1.1 ± 0.3	1.0 ± 0.2
LDL cholesterol (mmol/L)	3.2 ± 0.8	3.6 ± 1.0
Urine		
Norepinephrine ($\mu\text{g}/24\text{ h}$)	47.4 ± 13.3	56.5 ± 39.2
Epinephrine ($\mu\text{g}/24\text{ h}$)	6.77 ± 2.44	6.94 ± 3.25

¹ $\bar{x} \pm SD$.

²Tendency toward group difference, $P < 0.10$.

³Significantly different from placebo group, $P < 0.05$.

RESULTS

All subjects completed the study in accordance with our protocol. The 2 groups were well matched with regard to anthropometric and indirect calorimetry measures at baseline (Table 1). There were no significant differences at baseline between the groups in biochemical variables, except for triacylglycerol and lactate, which were significantly higher ($P < 0.05$) in subjects allocated to the L-796568 treatment. There was also a tendency ($P < 0.10$) for higher total cholesterol and fatty acid concentrations in the L-796568 group (Table 2).

In both groups, there were no significant changes in body weight, body composition, heart rate, or blood pressure from day 0 to day 28. The magnitude of the changes in these variables from day 0 to day 28 did not differ significantly between groups (Table 3).

TABLE 3

Changes from day 0 (baseline) to day 28 (day 28 value minus day 0 value) for body weight, body composition, and cardiovascular measures¹

	Placebo group (n = 10)	L-796568 group (n = 10)
Body weight (kg)	-0.2 ± 1.2	-0.5 ± 1.6
Fat-free mass (kg) ²	-1.7 ± 2.8	0.4 ± 2.0
Fat mass (kg) ²	1.0 ± 2.6	0.2 ± 1.5
Fat mass:fat-free mass ²	0.02 ± 0.05	-0.00 ± 0.03
Systolic BP (mm Hg)	5 ± 8	3 ± 11
Diastolic BP (mm Hg)	3 ± 5	-2 ± 6
Heart rate (beats/min)	2 ± 5	-3 ± 13

¹ $\bar{x} \pm SD$. BP, blood pressure. There were no significant differences between the 2 groups in the magnitude of the change from day 0 to day 28.

²Determined within the 7 d before the baseline stay in the respiration chamber.

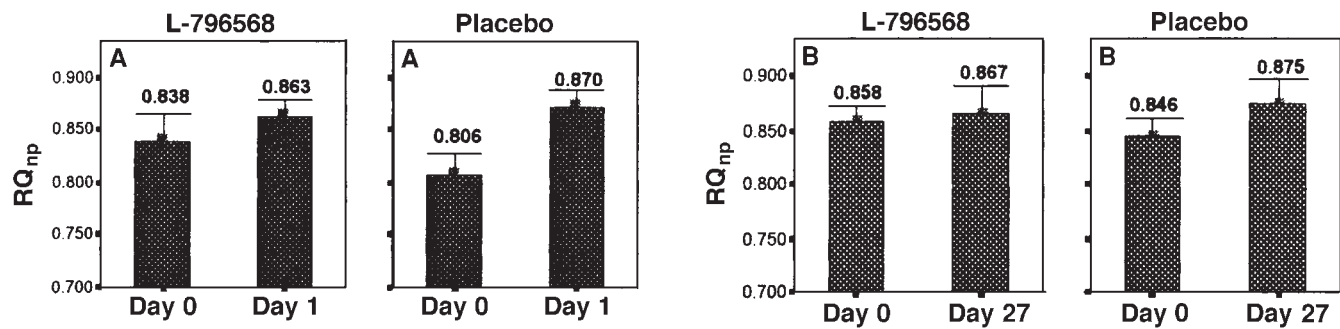


FIGURE 1. Acute effect (A): change in daytime nonprotein respiratory quotient (RQ_{np}) between day 0 (baseline) and day 1 (measured from 0 to 4 h postdose). There was a significant between-group difference without correction for baseline values ($P < 0.02$); however, when baseline data were included as a cofactor the difference was not significant ($P = 0.21$). Chronic effect (B): change in 24-h RQ_{np} between day 0 and day 27 ($P = 0.09$). $n = 10$ per group.

When we compared day 0 with day 1 to test for an acute effect, we observed no between-group differences in daytime EE (0.37 ± 0.47 and 0.41 ± 0.58 kJ/min for the placebo and L-796568 groups, respectively), but there was a significantly smaller increase ($P < 0.02$) in daytime nonprotein respiratory quotient (RQ_{np}) in the L-796568 group (0.025 ± 0.041) than in the placebo group (0.064 ± 0.021) (Figure 1). However, the difference was not significant ($P = 0.21$) when the higher baseline RQ_{np} values for the L-796568 group were included as a cofactor in the analysis. The same tendency was observed for RQ_{np} during exercise, although the difference was not significant (0.000 ± 0.040 and -0.039 ± 0.055 for the placebo and L-796568 groups, respectively).

For all the time intervals (ie, 24 h, sleep, daytime, postdinner, during exercise, and basal metabolic rate), changes in EE and RQ_{np} from day 0 to day 27 were not significantly different between the 2 groups (with or without adjustment for changes in FFM and energy balance), except for a significantly smaller

increase in daytime RQ_{np} in the L-796568 group (unadjusted for energy balance; 0.044 ± 0.031 and 0.001 ± 0.040 in the placebo and L-796568 groups, respectively; $P < 0.02$) (Table 4). However, this significant difference in daytime RQ_{np} for the chronic response was no longer significant ($P = 0.15$) when the higher baseline RQ_{np} values for the L-796568 group were included as a cofactor in the analysis.

Compliance was assured by observation of daily dosing and by measurement of the mean predose plasma L-796568 concentrations on days 21, 25, and 28 (3-d mean \pm SD = 77.2 ± 30.3 nmol/L); these values were as predicted on the basis of previous studies. Even though there was no overall difference between groups in the body composition variables, there was a significant inverse correlation between the change in fat mass (poststudy minus prestudy) and the average predose plasma L-796568 concentration in the L-796568 group ($r = -0.69$, $P < 0.03$), ie, higher L-796568 concentrations were associated with greater decreases in fat mass (Figure 2). The decreases in fat mass were small,

TABLE 4

Changes from day 0 (baseline) to day 27 (day 27 value minus day 0 value) for energy expenditure (EE) and nonprotein respiratory quotient (RQ_{np})¹

	Placebo group ($n = 10$)	L-796568 group ($n = 10$)
24-h EE (kJ/min)	0.06 ± 0.36	0.06 ± 0.41
Sleeping EE (kJ/min)	0.01 ± 0.44	0.15 ± 0.32
Basal metabolic rate (kJ/min)	-0.14 ± 0.60	0.14 ± 0.62
Daytime EE (kJ/min)	0.17 ± 0.40	0.03 ± 0.44
Postdinner EE (kJ/min)	0.10 ± 0.48	0.16 ± 0.86
Exercise EE (kJ/min) ²	-3.49 ± 5.64	-0.52 ± 1.60
24-h RQ_{np}	0.029 ± 0.021	0.009 ± 0.029
Sleeping RQ_{np}	0.046 ± 0.041	0.041 ± 0.037
Basal RQ_{np}	0.067 ± 0.039	0.044 ± 0.050
Daytime RQ_{np} ³	0.044 ± 0.031	0.001 ± 0.040
Postdinner RQ_{np}	0.025 ± 0.031	0.029 ± 0.063
Exercise RQ_{np}	-0.027 ± 0.077	-0.045 ± 0.056

¹ $\bar{x} \pm$ SD.

²Exercise value was measured during a 15-min session of cycling (1430–1445).

³Without the cofactor, $P < 0.02$ for the difference between the 2 groups in the magnitude of change from day 0 to day 27. With the baseline value as a cofactor, $P = 0.15$.

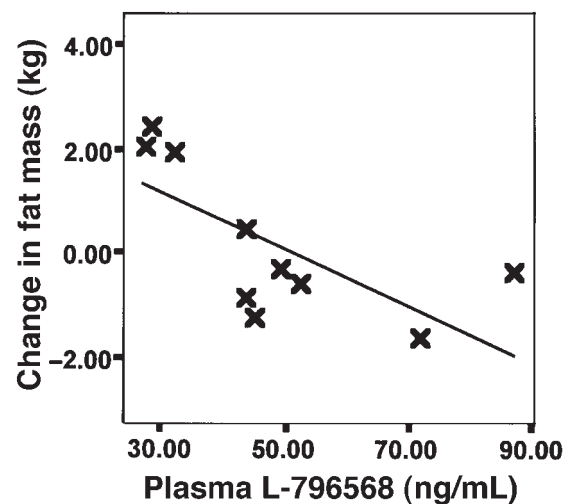


FIGURE 2. Correlation between mean predose plasma L-796568 concentration on days 21, 25, and 28 and change in fat mass from pretreatment to posttreatment ($r = -0.69$, $P < 0.03$); $n = 10$. Each \times represents the mean L-796568 concentration for 1 subject on the 3 measurement days.

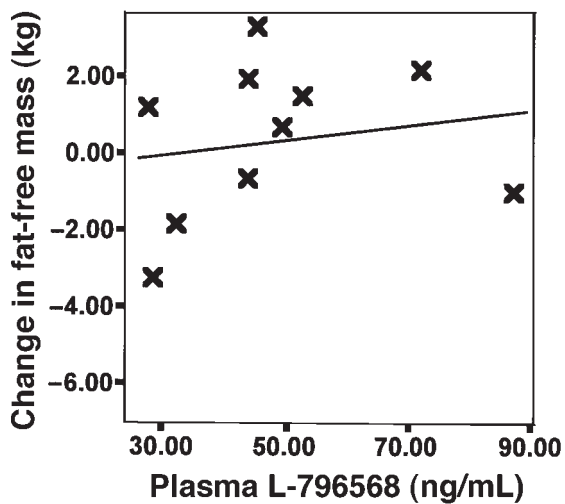


FIGURE 3. Correlation between mean predose plasma L-796568 concentration on days 21, 25, and 28 and change in fat-free mass from pretreatment to posttreatment ($r = 0.21$, $P = 0.56$; $n = 10$. Each \times represents the mean L-796568 concentration for 1 subject on the 3 measurement days.

however. No significant correlations were found between plasma L-796568 concentration and changes in FFM (**Figure 3**) or other endpoints, including changes in 24-h EE ($r = -0.08$, $P = 0.98$), 24-h RQ_{np} ($r = 0.56$, $P = 0.09$), and the ratio of fat mass to FFM ($r = -0.52$, $P = 0.13$).

The results of the OGTTs showed no significant differences between the treatment groups in the changes in the incremental areas under the curve for plasma glucose, insulin, or glucagon responses. However, a time \times day \times group interaction effect was found ($P < 0.02$) for the glucose response, and subsequent unpaired t test analysis at each time point showed a significantly higher glucose concentration at the 120-min time point in the L-796568 group than in the placebo group on day 28 ($P < 0.03$) (**Figure 4**).

On day 14, fasting triacylglycerol, total cholesterol, and fatty acid concentrations were significantly decreased in the L-796568 group compared with the placebo group, but only the decrease in total cholesterol was significant when baseline values were included as a covariate ($P < 0.005$) (data not shown). There were no significant differences in the changes in glycerol between the groups.

For triacylglycerol and total cholesterol, there were significant decreases in the L-796568 group compared with the placebo group from baseline (day 0) to day 28 (**Table 5**). When the higher baseline values of the L-796568 group were taken into account, only the change in triacylglycerol remained significantly different between the groups (-0.76 ± 0.76 and 0.42 ± 0.31 mmol/L for the L-796568 and placebo groups, respectively; $P < 0.002$).

We found no significant differences between groups for blood concentrations of LDL cholesterol, HDL cholesterol, ketones, lactate, leptin, norepinephrine, or epinephrine, or for urinary nitrogen or catecholamine excretion. The 2 groups did

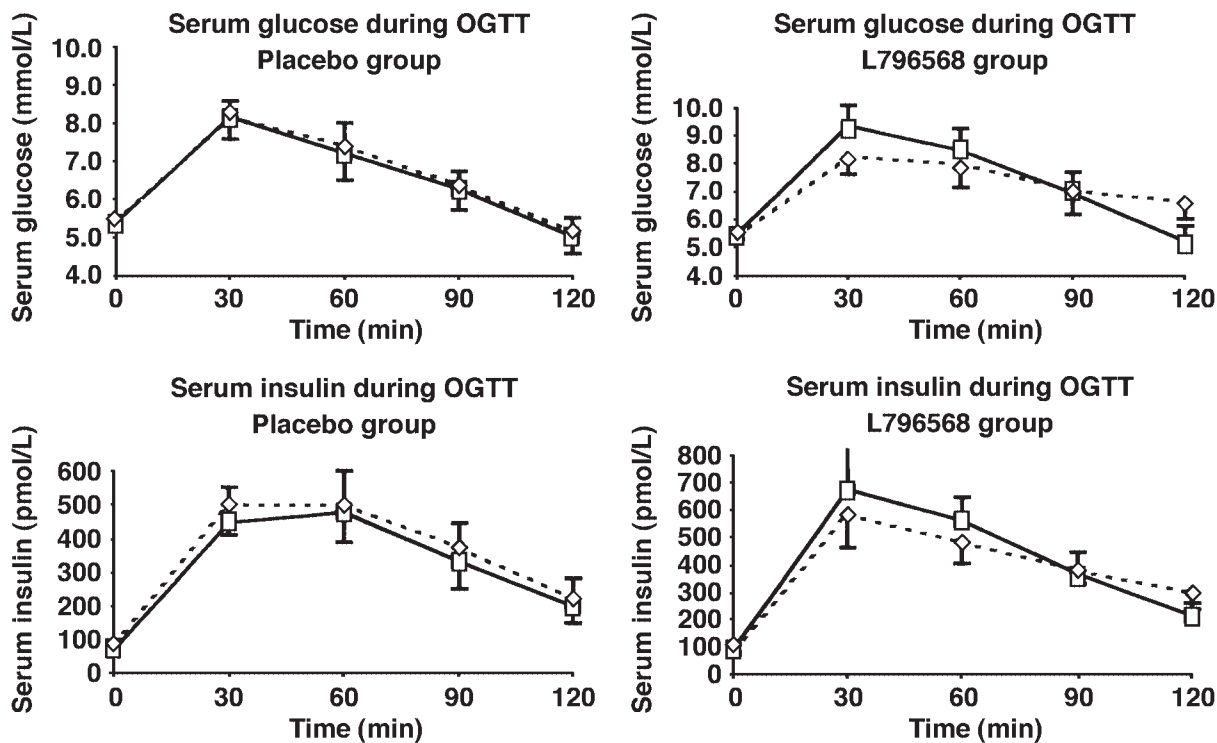


FIGURE 4. Glucose and insulin responses during oral-glucose-tolerance tests (OGTTs) performed pretreatment (\square) and on day 28 (\blacklozenge); $n = 10$ per group. No significant changes were found in the incremental area under the curve, but there was a significant time \times day \times group interaction effect ($P < 0.02$; general linear model for repeated-measures ANOVA) for glucose, with higher serum glucose at 120 min in the L-796568 group than in the placebo group on day 28 ($P < 0.03$).

TABLE 5
Changes in blood lipids from day 0 (baseline) to days 14 and 28

	Placebo group (n = 10)	L-796568 group (n = 10)	P ¹	
			No cofactor	With cofactor
Fatty acids (μmol/L)				
Day 14 – day 0	14 ± 31 ²	-11 ± 11	<0.03	0.07
Day 28 – day 0	-17 ± 128	-8 ± 215	0.91	—
Glycerol (μmol/L)				
Day 14 – day 0	21 ± 50	5 ± 19	0.34	—
Day 28 – day 0	-4 ± 15	-6 ± 19	0.81	—
Triacylglycerols (mmol/L)				
Day 14 – day 0	0.19 ± 0.55	-0.84 ± 0.88	<0.01	0.13
Day 28 – day 0	0.42 ± 0.31	-0.76 ± 0.76	<0.001	<0.002
Total cholesterol (mmol/L)				
Day 14 – day 0	0.2 ± 0.5	-0.8 ± 0.6	<0.002	<0.005
Day 28 – day 0	0.1 ± 0.7	-0.7 ± 0.6	<0.02	0.07

¹P values are for differences between groups (unpaired *t* test); the cofactor was the baseline value.

² $\bar{x} \pm$ SD.

not differ significantly with regard to hunger or satiety ratings at lunch or dinner.

Nine subjects in the L-796568 group and 2 in the placebo group reported gastrointestinal side effects, primarily diarrhea, during the treatment period. No subjects reported tremor (data not shown).

DISCUSSION

Previous pharmacologic studies have used a variety of approaches to try to elucidate the thermogenic potential of β_3 receptors in humans, but these studies have reported conflicting results. One approach has been to study EE with nonspecific β agonists in the presence of β_1 and β_2 antagonists to isolate any effects caused by β_3 agonist stimulation. In one study, 43% of the thermogenic effect of ephedrine (which potentiates the synaptic release of noradrenaline) remained after pretreatment with the β_1 and β_2 antagonist nadolol (30). Other studies showed that isoprenaline and BRL35135 (nonspecific β_3 agonists) have thermogenic effects that are not completely abolished by nadolol (31, 32). However, these findings were not confirmed in other studies that used a similar approach (33, 34). A second approach has been to investigate the effect of putative β_3 agonists. Of all the previous studies on this topic, the one that seems the most informative evaluated the effect of the β_3 agonist CL316 243, at a dose of 1500 mg/d, on EE and RQ (10). CL316 243 is a partial but selective human β_3 agonist (60% activity compared with isoprenaline; >1500-fold selectivity over β_1 and β_2) (35), but is \approx 1000-fold less potent ($EC_{50} = 3700$ nmol/L) than is L-796568 (K Gottesdiener and Merck & Co, unpublished observations, 1999). Plasma concentrations in the study were low compared with its EC_{50} value, and CL316 243 failed to increase EE after 8 wk of chronic administration.

In the present study of obese subjects, 375 mg of the human β_3 -adrenergic receptor agonist L-796568, taken once daily, had no effect on 24-h EE or on EE during any other time interval, neither acutely nor after 28 d of administration. These results were surprising, because an acute 8% increase in EE was observed after administration of a single 1000-mg dose of L-796568 to obese subjects who had fasted overnight (13). The lack of acute effect on EE on the first treatment day in this study was less surprising, because the present study had a different design in which subjects

were not resting and were served a large breakfast during the measurements; these factors are both likely to increase variability.

One explanation for the lack of chronic effect of L-796568 on EE could be that the plasma concentrations were too low. Overall, we think this is unlikely for the following reasons: 1) L-796568 is a very potent ($EC_{50} \approx 3.6$ nmol/L) and full agonist of the human β_3 -adrenergic receptor and average plasma concentrations were ≈ 77 nmol/L, or >20-fold higher than EC_{50} values, throughout the day; 2) lower plasma concentrations were associated with significant increases in EE in the rhesus monkey (K Gottesdiener and Merck & Co, unpublished observations, 1999); and 3) in a separate study, this dosing regimen (375 mg with food) resulted in peak plasma concentrations of ≈ 175 nmol/L, which is well above the peak plasma concentrations (≈ 100 nmol/L) achieved in the acute study that led to an 8% increase in EE (13).

An alternative explanation for the lack of chronic effect on EE might be that the agonist-induced BAT proliferative capability of β_3 receptor-responsive tissues in humans was too low during chronic stimulation. BAT, the primary tissue that responds to β_3 stimulation in rodents and possibly in rhesus monkeys, is only present in very limited amounts in adult humans and has questionable functional capacity. Thus, an acute β_3 -stimulated increase in EE in humans is less likely to be dependent on BAT and might be mediated directly by WAT and perhaps indirectly by the liver and muscles. This is consistent with studies in transgenic mice that showed that the major part of acute β_3 -agonist-induced thermogenesis and lipolysis is mediated by WAT and not by BAT (36). Compared with β_1 and β_2 receptors, β_3 receptors have quite low expression in human WAT (37), yet seem capable of producing an acute increase in EE. In addition, BAT might be up-regulated after chronic β_3 receptor stimulation. One study showed that 28 d of treatment with the β_3 adrenergic receptor agonist L-755507 was sufficient to induce significant BAT proliferation and uncoupling protein 1 up-regulation in rhesus monkeys (12). If humans are similar to rhesus monkeys in this respect, this 28-d study might have led to BAT proliferation, uncoupling protein 1 up-regulation, and increases in EE that exceeded the acute effects.

Another likely explanation for the lack of chronic effect on EE is down-regulation of β_3 receptors, even though previous *in vivo* studies in rodents showed no signs of β_3 -adrenergic receptor

agonist-induced down-regulation (38, 39). If down-regulation is the cause of the lack of chronic β_3 -adrenergic receptor-mediated thermogenesis, this suggests that a different β_3 -adrenergic receptor-desensitizing mechanism occurs in humans. However, we also cannot rule out the possibility that some type of negative feedback occurs, possibly via inhibition of lipolysis with indirect effects on EE. Studies in humans showed increased thermogenesis during intravenous infusions of lipid plus heparin (ie, increased fatty acids) (40), suggesting that mobilization of fatty acids may be the major determinant of β_3 -agonist-induced thermogenesis. However, an attenuated response of β_3 agonist-mediated increases in plasma fatty acid concentrations was observed after 9 d of treatment with 375 mg L-796568 in healthy volunteers; the acute increases measured on the first treatment day were completely absent after 9 d of treatment (K Gottesdiener and Merck & Co, unpublished observations, 1999).

Interestingly, however, we did not find any β_3 agonist-induced changes in catecholamine concentrations or excretion, suggesting that there was no major influence on lipolysis via changes in endogenous catecholamine metabolism. The lack of effect of the agonist under conditions when food intake is not altered does not exclude the possibility that the drug may show thermogenic efficacy in preventing the fall in EE under conditions of hypocaloric dieting, ie, when sympathetic activity is low and there is a tendency for adrenergic receptors to be up-regulated.


In the present weight-maintenance study, FFM and fat mass did not differ significantly between groups. However, the observed changes in body fat mass in the L-796568 group were inversely correlated with plasma L-796568 concentrations measured at the end of the study period, suggesting sustained increased fat oxidation. After 28 d of treatment, there was no sustained increase in the mobilization of fatty acids, as assessed by measuring fasting concentrations of fatty acids and glycerol.

When baseline RQ_{np} values were included as a cofactor in the analysis, there was no significant effect of L-796568 on substrate partitioning represented by RQ or nonprotein RQ, but regarding acute induced thermogenesis, any acute effect on daytime RQ_{np} may be masked by interference induced by diet and physical activity. However, among all the measuring periods, daytime RQ_{np} shows the largest decrease and thus indicates an acute stimulatory effect of a drug on fat oxidation, which may be dependent on fatty acid mobilization. On the contrary, we did not find a significant chronic effect on substrate partitioning, as indicated by the lack of effect on 24-h RQ_{np} (Figure 1).

Treatment with L-796568 reduced plasma triacylglycerol and total cholesterol concentrations to a greater extent than did placebo after 14 d. In L-796568 studies conducted in dogs and monkeys, decreased triacylglycerol concentrations were also found (K Gottesdiener and Merck & Co, unpublished observations, 1999), and chronically decreased triacylglycerol concentrations were observed previously in selective β_3 agonist studies in obese mice (41). β_3 agonists were shown to improve glucose tolerance in humans (10, 42), but in this study, we found no significant effect of L-796568 on glucose tolerance. The plasma glucose profile over time in the L-796568 group shows lower and more sustained concentrations, which may have been caused by decreased gastric emptying resulting from the known effects of β_3 agents on gastric motility (43–45).

There was no evidence in this study that L-796568 had effects on β_1 and β_2 receptors, as indicated by the absence of tachycardia, tremor, and increased blood pressure and the lack of effects on

plasma potassium. Significantly more subjects in the L-796568 group had gastrointestinal side effects such as diarrhea, an effect which was previously found in dogs (K Gottesdiener and Merck & Co, unpublished observations, 1999) but not in human studies of CL316243 (C Weyer, personal communication, 2001). In animal models, β_3 agonists had anorectic effects, but L-796568 did not seem to have any effect on appetite parameters assessed during the subjects' stays in the respiration chamber.

In conclusion, no chronic thermogenic effect of L-796568 was found in obese, nondiabetic young men. There was also no evidence of stimulation of fat oxidation by L-796568, although changes in fat mass correlated negatively with drug concentrations, suggesting some potential activity at the β_3 receptor. In addition, triacylglycerol concentrations decreased substantially in the L-796568 group. In contrast with most studies of nonspecific β_3 agonists, there was no evidence of β_1 or β_2 stimulation to confound these results. The lack of chronic effects most likely resulted from down-regulation of β_3 effects (indirectly or directly via down-regulation of receptors) or a lack of recruitment of BAT in humans, or both. 

At the Copenhagen site, we thank the kitchen staff, Inge Timmermann, Kirsten Bryde Rasmussen, Martin Kreutzer, John Lind, and Benjamin Buemann and at the Maastricht site, we thank Gabby Hul, Paul Schoffelen, Joan Senden, and Jos Stegen.

REFERENCES

1. Krief A, Lönnqvist F, Raimbault S, et al. Tissue distribution of β_3 -adrenergic receptor mRNA in man. *J Clin Invest* 1993;91:344–9.
2. Berkowitz DE, Nardone NA, Smiley RM, et al. Distribution of β_3 -adrenergic receptor mRNA in human tissues. *Eur J Pharmacol* 1995;289:223–8.
3. Yoshida T, Sakane N, Wakabayashi Y, Umekawa T, Kondo M. Anti-obesity and anti-diabetic effects of CL316,243, a highly specific β_3 -adrenoceptor agonist, in yellow KK mice. *Life Sci* 1994;54:491–8.
4. Himms-Hagen J, Cui J, Danforth E, et al. Effect of CL-316,243, a thermogenic β_3 -agonist, on energy balance and brown and white adipose tissues in rats. *Am J Physiol* 1994;266:R1371–82.
5. Arbeny CM, Meyers DS, Hillyer DE, Bergquist KE. Metabolic alterations associated with the antidiabetic effect of β_3 -adrenergic receptor agonists in obese mice. *Am J Physiol* 1995;268:E678–84.
6. Hoffstedt J, Lönnqvist F, Shimizu M, Blaak E, Arner P. Effects of several putative β_3 -adrenoceptor agonists on lipolysis in human omental adipocytes. *Int J Obes Relat Metab Disord* 1996;20:428–34.
7. Galitzky J, Langin D, Verwaerde P, Montastruc J-L, Lafontan M, Berlan M. Lipolytic effects of conventional β_3 -adrenoceptor agonists and of CGP 12,177 in rat and human fat cells: preliminary pharmacological evidence for a putative β_4 -adrenoceptor. *Br J Pharmacol* 1997;122:1244–50.
8. Enocksson S, Shimizu M, Lönnqvist F, Nordenström J, Arner P. Demonstration of an in vivo functional β_3 -adrenoceptor in man. *J Clin Invest* 1995;95:2239–45.
9. Zaagsma J, Nahorski SR. Is the adipocyte beta-adrenoceptor a prototype for the recently cloned atypical 'beta-3 adrenoceptor'? *Trends Pharmacol Sci* 1990;11:3–7.
10. Weyer C, Tataranni PA, Snitker S, Danforth E, Ravussin E. Increase in insulin action and fat oxidation following treatment with CL 316,243, a highly selective beta₃-adrenoceptor agonist in humans. *Diabetes* 1998;47:1555–61.
11. Mathvink RJ, Tolman JS, Chitty D, et al. Discovery of a potent, orally

- bioavailable β_3 adrenergic receptor agonist, (R)-N-[4-[2-[[2-hydroxy-2-(3-pyridinyl)ethyl]amino]ethyl]phenyl]-4-[4-(4-(trifluoromethyl)phenyl)thiazol-2-yl]benzenesulfonamide. *J Med Chem* 2000;43:3832–6.
12. Fisher MH, Amend AM, Bach TJ, et al. A selective human β_3 adrenergic receptor agonist increases metabolic rate in rhesus monkeys. *J Clin Invest* 1998;101:2387–93.
 13. van Baak MA, Hul GB, Toubro S, et al. Acute effect of L-796568, a novel β_3 -adrenergic receptor agonist, on energy expenditure in obese men. *Clin Pharmacol Ther* 2002;71:272–9.
 14. Astrup A, Thorbek G, Lind J, Isaksson B. Prediction of 24-h energy expenditure and its components from physical characteristics and body composition in normal-weight humans. *Am J Clin Nutr* 1990;52:777–83.
 15. Schoffelen PFM, Westerterp K, Saris W, Ten Hoor F. A dual-respiration chamber system with automatic calibration. *J Appl Physiol* 1997;83:2064–72.
 16. Elia M, Livesey G. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. *World Rev Nutr Diet* 1992;70:68–131.
 17. Klausen B, Toubro S, Astrup A. Age and sex effects on energy expenditure. *Am J Clin Nutr* 1997;65:895–907.
 18. Heitmann BL. Prediction of body water and fat in adult Danes from measurements of electrical impedance. A validation study. *Int J Obes* 1990;14:789–802.
 19. Li R, Keymulen B, Gerlo E. Determination of glycerol in plasma by an automated enzymatic spectrophotometric procedure. *Clin Chem Lab Med* 2001;39:20–4.
 20. Smedes F, Kraak JC, Poppe H. Simple and fast solvent extraction system for selective and quantitative isolation of adrenaline, noradrenaline and dopamine from plasma and urine. *J Chromatogr* 1982;231:25–39.
 21. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. *Ann Clin Biochem* 1969;6:24–7.
 22. Sugiuchi H, Uji Y, Okabe H, et al. Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulphated alpha-cyclodextrin. *Clin Chem* 1995;41:717–23.
 23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasmas, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
 24. Tietz NW, ed. *Clinical guide to laboratory tests*. 2nd ed. Philadelphia: WB Saunders, 1990.
 25. Tietz NW, ed. *Fundamentals of clinical chemistry*. 3rd ed. Philadelphia: WB Saunders, 1987.
 26. Lundholm L, Lundholm EM, Vamos N. Lactic acid assay with L(+) lactic acid dehydrogenase from rabbit muscle. *Acta Physiol Scand* 1963;58:243–9.
 27. Elia M, Folmer P, Schlatman A, Goren A, Austen S. Carbohydrate, fat and protein metabolism in muscle and in the whole body after mixed meal ingestion. *Metabolism* 1988;37:542–51.
 28. Burtis CA, Ashwood ER, eds. *Tietz textbook of clinical chemistry*. 2nd ed. Philadelphia: WB Saunders, 1994.
 29. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord* 1999;23:1–11.
 30. Liu Y-L, Toubro S, Astrup A, Stock MJ. Contribution of β_3 -adrenoceptor activation to epinephrine-induced thermogenesis in humans. *Int J Obes Relat Metab Disord* 1995;19:678–85.
 31. Wheeldon NM, McDevitt DG, Lipworth BJ. Do beta 3-adrenoceptors mediate metabolic responses to isoprenaline. *Q J Med* 1993;86:595–600.
 32. Wheeldon NM, Mcdevitt DG, Mcfarlane LC, Lipworth BJ. Beta-adrenoceptor subtypes mediating the metabolic effects of BRL 35135 in man. *Clin Sci (Colch)* 1994;86:331–7.
 33. Blaak EE, van Baak MA, Kempen KPG, Saris WHM. Role of α - and β -adrenoceptors in sympathetically mediated thermogenesis. *Am J Physiol* 1993;264:E11–7.
 34. Schiffelers SL, Blaak EE, Saris WH, van Baak MA. In vivo beta3-adrenergic stimulation of human thermogenesis and lipid use. *Clin Pharmacol Ther* 2000;67:558–66.
 35. Dolan JA, Muenkel HA, Burns MG, et al. Beta-3 adrenoceptor selectivity of the dioxolane dicarboxylate phenethanolamines. *J Pharmacol Exp Ther* 1994;269:1000–6.
 36. Grujic D, Susulic VS, Harper M-E, et al. β_3 -adrenergic receptors on white and brown adipocytes mediate β_3 -selective agonist-induced effects on energy expenditure, insulin secretion and food intake. *J Biol Chem* 1997;272:17686–93.
 37. Revelli J-P, Muzzin P, Paoloni A, Moinat M, Giacobino J-P. Expression of the β_3 -adrenergic receptor in human white adipose tissue. *J Mol Endocrinol* 1993;10:193–7.
 38. Thomas RF, Holt BD, Schwinn OA, Liggett SB. Long-term agonist exposure induces up-regulation of β_3 -adrenergic receptor expression via multiple cAMP response elements. *Proc Natl Acad Sci U S A* 1992;89:4490–4.
 39. Yoshida T, Umekawa T, Sakane Nkogure A, Kondo M. Long-term exposure of a β_3 -adrenoceptor agonist induces up-regulation of β_3 -, but not β_1 -adrenoceptor in adipose tissue in OLEFT rats with obesity and diabetes. *Int J Obes Relat Metab Disord* 1998;22:S187 (abstr).
 40. Schiffelers SLH, Saris WHM, van Baak MA. The effect of an increased NEFA concentration on thermogenesis and substrate oxidation in obese and lean men. *Int J Obes Relat Metab Disord* 2001;25:33–8.
 41. Yoshitomi H, Yamazaki K, Abe S, Tanaka I. Differential regulation of mouse uncoupling proteins among brown adipose tissue, white adipose tissue, and skeletal muscle in chronic β_3 adrenergic receptor agonist treatment. *Biochem Biophys Res Commun* 1998;253:85–91.
 42. Mitchell TH, Ellis RMD, Smith SA, Robb G, Cawthorne MA. Effects of BRL 35135, a β -adrenoceptor agonist with novel selectivity in obese subjects. *Int J Obes* 1989;13:757–66.
 43. Fletcher DS, Candelore MR, Grujic D, et al. Beta-3 adrenergic receptor agonists cause an increase in gastrointestinal transit time in wild-type mice, but not in mice lacking the beta-3 adrenergic receptor. *J Pharmacol Exp Ther* 1998;287:720–4.
 44. De Ponti F, Modini C, Gibelli G, Crema F, Frigo G. Atypical β -adrenoceptors mediating relaxation in the human colon: functional evidence for β_3 - rather than β_4 -adrenoceptors. *Pharmacol Res* 1999;39:345–8.
 45. Manara L, Croci T, Aureggi G, et al. Functional assessment of β adrenoceptor subtypes in human colonic circular and longitudinal (taenia coli) smooth muscle. *Gut* 2000;47:337–42.