Increase in Insulin Action and Fat Oxidation After Treatment With CL 316,243, a Highly Selective β_3-Adrenoceptor Agonist in Humans

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Stimulation of β_3-adrenoceptors by selective agonists improves insulin action and stimulates energy metabolism in various rodent models of obesity and type 2 diabetes. Whether selective β_3-adrenoceptor stimulation exerts metabolic actions in humans remains to be proven. The effects of a highly selective β_3-adrenoceptor agonist on insulin action, energy metabolism, and body composition were assessed in 14 healthy young lean male volunteers (age 22.5 ± 3.3 years, 15 ± 5% body fat [mean ± SD]) randomly assigned to 8 weeks of treatment with either 1,500 mg/day of CL 316,243 (n = 10) or placebo (n = 4). Insulin-mediated glucose disposal (IMGD), nonoxidative glucose disposal (NOGD), oxidative glucose disposal (OGD) (indirect calorimetry), and splanchnic glucose output (SGO; 3-[H^3]glucose) were determined during a 100-min hyperinsulinemic-euglycemic glucose clamp (40 mU · m^{-2} · min^{-1}) before and after 4 and 8 weeks of treatment. The 24-h energy expenditure (24-EE), 24-h respiratory quotient (24-RQ), and the oxidation rates of fat and carbohydrate were determined in a respiratory chamber before and after 8 weeks. After 4 weeks, treatment with CL 316,243 increased IMGD (+45%, P < 0.01) in a plasma concentration–dependent manner (r = 0.76, P < 0.02). This effect was due to an 82% increase in NOGD (P < 0.01), while OGD and SGO remained unchanged. The effects on insulin action were markedly diminished after 8 weeks; this was significantly related to an unexpected decline in the plasma concentrations of CL 316,243 (–36%, P = 0.08). At this time, 24-RQ was lowered (P < 0.001), corresponding to a 23% increase in fat oxidation (P < 0.01) and a 17% decrease in carbohydrate oxidation (P = 0.05). The 24-EE after 8 weeks did not differ from baseline, and there was no change in body weight or body composition. Plasma concentrations of glucose, insulin, and leptin were unaffected by treatment, while free fatty acid concentrations increased by 41% (P < 0.05), again linearly with the achieved plasma concentration of CL 316,243 (r = 0.67, P < 0.05). Treatment with CL 316,243 had no effect on heart rate or blood pressure and caused no cases of tremors. We conclude that treatment of lean male subjects with CL 316,243 increases insulin action and fat oxidation, both in a plasma concentration–dependent manner. This is the first study to demonstrate unequivocal metabolic effects of a highly selective β_3-adrenoceptor agonist in humans. *Diabetes* 47:1555–1561, 1998

Administration of β_3-adrenoceptor agonists produces weight reduction (1–5) and improves insulin action (3,6–8) in various rodent models of obesity and type 2 diabetes. These remarkable antiobesity and antidiabetic effects have led to a search for β_3-adrenergic agonists for use in humans (9–14). Over the past 15 years, several partially selective agonists have been tested in human clinical trials (15–18), but unfortunately, all of the previous compounds have suffered from one or more unacceptable pharmacokinetic or pharmacodynamic problems, including drug toxicity, short plasma half-life, or lack of full activity at the human β_3-adrenoceptor (10–12). In addition, none of the previous agents was highly β_3 selective, resulting in two important limitations. First, administration of these early compounds produced undesired β_1- and/or β_2-adrenoceptor effects, such as tachycardia (β_1) or hand tremor (β_2) (19–21). Second, and of greatest importance, there has yet been no proof that selective β_3-adrenergic stimulation exerts metabolic effects in humans.

CL 316,243 is a novel dioxolane dicarboxylate phenethanolamine with a high selectivity for the β_3-adrenoceptor (relative selectivity in vitro β_1:β_2:β_3 = 0:1:100,000 [22,3]). Its in vitro binding to the human β_3-adrenoceptor is practically identical to that of the rodent receptor. However, in contrast to the rodent receptor, where CL 316,243 is a full agonist, it is only a partial (60%) agonist at the human β_3-adrenoceptor, with a 50% effective dose (ED_{50}) of 1,800 ng/dl for in vitro cAMP production and lipolysis (3,22). The plasma half-life of CL 316,243 is 16 h, and its bioavailability is poor, with ~10% of an oral dose being absorbed.

In rodents, CL 316,243 exerts both antiobesity and antidiabetic effects (3,4,7,8,23). The antiobesity effect is thought to result primarily from an increase in energy expenditure, mediated by stimulation of brown adipose tissue (BAT) thermogenesis (3,4,24), but effects on plasma leptin concentrations and food intake have also been reported (25). The

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ANOVA, analysis of variance; BAT, brown adipose tissue; ECG, electrocardiogram; ED_{50}, 50% effective dose; 24-EE, 24-h energy expenditure; EMBS, estimated metabolic body size; FFA, free fatty acid; FFM, fat-free mass; IMGD, insulin-mediated glucose disposal; NOGD, nonoxidative glucose disposal; OGD, oxidative glucose disposal; R_a, rate of glucose appearance; 24-RQ, 24-h respiratory quotient; SGO, splanchnic glucose output; SMR, sleeping metabolic rate; WAT, white adipose tissue.

antidiabetic properties in rodents result mainly from an increase in insulin-mediated glucose disposal (IMGD) (8,25).

In humans, CL 316,243 has been shown to exert lipolytic activity on white adipose tissue (WAT) in vitro (26), a finding recently confirmed by preliminary in vivo results (27). However, as yet, there has been no demonstration of a systemic metabolic effect after treatment with CL 316,243 in humans. The aim of the present study, therefore, was to investigate the effects of CL 316,243 on insulin action, energy expenditure, substrate oxidation, body composition, leptin concentrations, and vital signs in healthy young lean male subjects.

RESEARCH DESIGN AND METHODS

Subjects. Fourteen healthy young lean male volunteers (Table 1) were enrolled into this randomized placebo-controlled double-blind study. Baseline examinations included a medical history, physical examination, 12-channel electrocardiogram (ECG) recording, routine laboratory tests, and a 75-g oral glucose tolerance test for the exclusion of impaired glucose tolerance or diabetes (World Health Organization criteria [28]). The study protocol was approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board. After giving written informed consent, volunteers were randomly assigned (2:1 design) to 8 weeks of treatment with either 1,500 mg/day of CL 316,243 once a day (CL group, n = 10) or placebo (P group, n = 4). Subjects reported every day, except for the weekend days, to receive and ingest their study medication under observation. At baseline and after 4 and 8 weeks of treatment, subjects were admitted to the clinical unit for 3–4 days for assessment of body composition, insulin action, and energy metabolism (see below). During the inpatient stay, all subjects received a weight-maintaining diet and abstained from strenuous exercise before the measurements. Body weight, heart rate, blood pressure, body temperature, and any potential adverse events were assessed every 2 weeks.

Body weight and body composition. Total body weight was measured using a precision scale (ACME, San Leandro, CA) and was corrected for the weight of the hospital gown. Percentages of body fat, fat mass, and fat-free mass (FFM) were estimated at weeks 0 and 8 by total body dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) (29).

Insulin action. At baseline and after 4 and 8 weeks of treatment, volunteers underwent a euglycemic-hyperinsulinemic glucose clamp (30). A primed (30 µCi) and continuous (0.3 µCi/min) 3-^{3}H-glucose infusion was used to determine the basal rate of splanchnic glucose output (SGO) using the non–steady-state equation of Steele (31). After 120 min, a primed continuous insulin infusion (40 mU · m^{-2} · min^{-1}) was started and maintained for 100 min, during which plasma glucose concentration was maintained constant at ~5.0 mmol/l by means of a variable 20% glucose infusion. Blood was drawn every 5 min throughout the clamp for determination of plasma glucose concentration and every 10 min throughout the last 40 min for determination of plasma insulin concentration and 3-^{3}H-glucose specific activity. During the euglycemic clamp, the rate of SGO equals the difference between the rate of exogenously infused glucose and the appearance rate of glucose (R_a) determined from Steele's equation. These data were calculated for each 10 min of the last 40 min of the clamp and then averaged to calculate the rate of IMGD. Indirect calorimetry using a ventilated hood system (32) was performed in conjunction with urinary nitrogen excretion determination (33) throughout the clamp to determine the rates of stimulated oxidative glucose disposal (OGD) and nonoxidative glucose disposal (NOGD). IMGD, OGD, NOGD, and SGO were normalized to the estimated metabolic body size (EMBS), calculated as FFM plus 17 kg (34).

Energy metabolism. At baseline and after 8 weeks of treatment, 24-h energy expenditure (24-EE), sleeping metabolic rate (SMR), and 24-h respiratory quotient (24-RQ) were determined in a respiratory chamber as described previously (35). In brief, volunteers entered the chamber at 7:45 A.M. after an overnight fast and remained therein until 7:00 A.M. the following morning. All subjects were fed three meals (at 8:00 A.M., 11:30 A.M., and 5:00 P.M.) and an evening snack (at 8:00 P.M.) composed of 50, 30, and 20% of calories from carbohydrate, fat, and protein, respectively. Spontaneous physical activity was measured by a radar system, and SMR was calculated as the mean metabolic rate of all 15-min periods during which physical activity was <1.5%. The 24-EE and SMR were adjusted for fat mass and FFM, while the 24-RQ was adjusted for percent body fat and energy balance, as described previously (35). From 24-EE, 24-RQ, and 24-h urinary nitrogen excretion, the oxidation rates of fat, carbohydrate, and protein were calculated.

Analytic procedures. Plasma glucose was determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA), and plasma insulin concentrations were determined using a commercial radioimmunometric assay (Concept 4; ICN Biomedicals, Cosa Mesa, CA). The 3-^{3}H-glucose specific activity was measured as described by others (36) using perchloric acid to precipitate proteins. Fasting plasma free fatty acid (FFA) (colorimetric assay; Wako Chemicals, Richmond, VA) and fasting plasma leptin concentrations (solid-phase sandwich enzyme immunoassay; Amgen, Thousand Oaks, CA) were measured at baseline and after 4 and 8 weeks. Plasma concentration of CL 316,243 was determined with a validated high-performance liquid chromatography assay using a reverse-phase column and ultraviolet detection.

Statistical analyses. Statistical analyses were performed using the procedures of SAS Institute (Cary, NC). Within-group comparisons were made by paired Student's t test procedures (changes from baseline to 4/8 weeks of treatment within each group) and by repeated measures analyses of variance (ANOVAs) for variables measured more than two times (overall effect of treatment). ANOVAs were performed for comparisons between groups. Data in the text and tables are given as means ± SD.

RESULTS

Results for the CL group (n = 10) at baseline and after 4 and 8 weeks of treatment are given in Tables 1 and 2. In the P group (n = 4), age (22.0 ± 5.0 years), body weight (65.9 ± 10.3 kg), and body composition (14 ± 4% body fat), as well as vital signs, biochemical measurements, and all measurements of insulin action and energy expenditure at baseline were similar to those of the CL group.

TABLE 1
Physical characteristics and vital signs in 10 healthy young lean male subjects before and after 4 and 8 weeks of treatment with 1,500 mg/day of CL 316,243

	Week			
	0	4	8	P
Age (years)	22.7 ± 2.8			
Body composition				
Body weight (kg)	73.3 ± 9.0		73.3 ± 8.6	NS
Body fat (%)	15 ± 5		15 ± 5	NS
Fat mass (kg)	11.2 ± 4.3		11.4 ± 4.3	NS
Fat-free mass (kg)	62.1 ± 7.9		61.9 ± 8.1	NS
Vital signs				
Heart rate (bpm)	64 ± 11	60 ± 9	66 ± 8	NS
Systolic blood pressure (mmHg)	118 ± 15	122 ± 8	119 ± 14	NS
Diastolic blood pressure (mmHg)	70 ± 20	75 ± 8	65 ± 8	NS

Data are means ± SD. P values represent the result from repeated measures ANOVA (treatment effect). Treatment with CL 316,243 had no effects on body weight and body composition or on heart rate and blood pressure. Data for the placebo group are given in the text.

TABLE 2
Achieved plasma concentrations of CL 316,243 and changes in selected biochemical measurements as well as in various measurements of insulin action and energy metabolism in 10 healthy young lean male subjects treated for 8 weeks with 1,500 mg/day of CL 316,243

	Week			
	0	4	8	*P*
Plasma concentration of CL 316,243 (ng/dl) (n = 9)		1,978 ± 959	1,275 ± 482	NS
Biochemical measurements				
Fasting plasma glucose (mmol/l)	4.9 ± 0.4	5.0 ± 0.7	4.9 ± 0.5	NS
Fasting plasma insulin (μU/ml)	7 ± 5	5 ± 2	4 ± 3	NS
Fasting plasma FFAs (μmol/l)	141 ± 79	198 ± 95*	201 ± 57*	<0.05
Fasting plasma leptin (ng/ml)	1.0 ± 0.5	1.0 ± 1.0	0.8 ± 0.6	NS
Basal SGO (mg · kg^{-1} EMBS · min^{-1})	1.8 ± 0.2	2.0 ± 0.4	1.9 ± 0.2	NS
Insulin action				
IMGD (mg · kg^{-1} EMBS · min^{-1})	5.6 ± 2.0	8.1 ± 2.3†	6.1 ± 1.8	<0.01
NOGD (mg · kg^{-1} EMBS · min^{-1})	3.2 ± 1.8	5.8 ± 2.1†	4.0 ± 1.4	<0.05
OGD (mg · kg^{-1} EMBS · min^{-1})	2.4 ± 0.5	2.2 ± 0.8	2.1 ± 0.5	NS
Energy metabolism				
24-EE (kcal/day)	2,052 ± 120		2,066 ± 62	NS
SMR (kcal/day)	1,504 ± 81		1,508 ± 126	NS
24-RQ	0.86 ± 0.02		0.83 ± 0.03‡	<0.01
Body temperature (°F)	97.4 ± 0.5		97.5 ± 0.3	NS

Data are means ± SD. *P* values represent the result from repeated measures ANOVA (overall treatment effect). Note the large SD for the CL plasma concentration, reflecting the high interindividual variability as illustrated also in Fig. 2. Data for the placebo group are given in the text. *P < 0.08; †P < 0.01; ‡P < 0.001, changes from baseline to 4 and 8 weeks.

Plasma concentrations of CL 316,243. The fasting plasma concentrations of CL 316,243 achieved at 4 and 8 weeks in 9 of the 10 treated subjects (1 sample lost) are shown in Table 2. The mean concentration of CL 316,243 at 8 weeks (1,275 ± 482 ng/dl) was 36% lower than at 4 weeks (1,978 ± 959 ng/dl), but this difference did not reach statistical significance (P = 0.08).

Body weight, body composition, vital signs, and side effects. The 8 weeks of treatment with CL 316,243 or placebo had no effects on body weight (± 0.0 kg [CL group] vs. + 0.1 kg [P group], NS) or body composition (fat mass: 0.2 kg [CL group] vs. 0.5 kg [P group], NS; FFM: –0.2 kg [CL group] vs. –0.4 kg [P group], NS) (Table 1). CL 316,243 treatment did not affect heart rate or systolic and diastolic blood pressures (Table 1). ECG intervals (PR-, QRS-, and QT-) were unchanged, and there were no cases of cardiac arrhythmia. None of the volunteers treated with CL 316,243 developed hand or other tremors.

Insulin action. Treatment with CL 316,243 for 4 weeks resulted in a 45 ± 41% increase (P < 0.01) in IMGD (Table 2, Fig. 1*A*). This increase was linearly related to the achieved plasma concentrations of CL 316,243 (r = 0.76, P < 0.02; Fig. 2). The increased IMGD was exclusively accounted for by an 82 ± 88% increase (P < 0.01) in NOGD, whereas OGD was unchanged (Table 2, Fig. 1*A*). No significant changes in IMGD or NOGD were seen in the P group (IMGD: from 5.09 ± 0.95 to 5.83 ± 2.74 mg · kg^{-1} EMBS · min^{-1}, NS; NOGD: from 2.66 ± 0.99 to 3.31 ± 2.21 mg · kg^{-1} EMBS · min^{-1}, NS). However, when the changes in insulin action were compared between the two groups, the difference did not reach statistical significance (P = 0.09 for both IMGD and NOGD). Between 4 and 8 weeks of treatment with CL 316,243, IMGD and NOGD declined, and at 8 weeks, neither of both measurements differed from the respective values before treatment (Table 2, Fig. 1*A*). Again, there was a linear relationship between the changes in IMGD (during weeks 4 and 8) and the changes in plasma CL 316,243 concentration during this time (P = –0.69, P < 0.05; Fig. 2). Basal SGO did not change during treatment in either group (Table 2) and was entirely suppressed at the end of the clamp in all subjects.

Energy metabolism. The 24-EE, SMR, and body temperature did not change after 8 weeks of treatment with CL 316,243 (Table 2, Fig. 1*B*) or placebo (24-EE: from 2,018 ± 65 to 1,996 ± 61 kcal/day, NS; SMR: from 1,488 ± 49 to 1,513 ± 54 kcal/day, NS; body temperature: from 97.1 ± 0.4 to 97.3 ± 0.7°F, NS). However, the 24-RQ was lowered in all 10 subjects treated with CL 316,243 (Fig. 1*B*, Table 2). This decline in 24-RQ corresponds to a 23 ± 20% increase in fat oxidation (from 87 ± 18 to 108 ± 24 g/day, P < 0.01) and a 17 ± 14% decrease in carbohydrate oxidation (from 239 ± 40 to 199 ± 56 g/day, P < 0.01), while protein oxidation was unchanged (Fig. 1*B*). The decrease in 24-RQ (r = –0.69, P = 0.07) and the increase in fat oxidation (r = 0.50, P < 0.05) were both correlated with the achieved plasma concentrations of CL 316,243. No changes in 24-RQ (0.02 ± 0.02, NS) or substrate oxidation were observed in the P group, and group comparisons were significant for 24-RQ (P < 0.001), fat oxidation (P = 0.005), and carbohydrate oxidation (P < 0.05). Neither energy intake nor energy balance during the 24-h respiratory chamber period differed between week 0 and week 8 in either group or between groups. However, as expected from the lowering in 24-RQ, the calculation of substrate balances during the 24 h in the chamber revealed a negative fat balance (–228 ± 227 kcal/day, P < 0.05) in the CL 316,243–treated subjects.

Fasting plasma concentrations of glucose, insulin, FFAs, and leptin. As shown in Table 2, fasting plasma glucose was unchanged by treatment with CL 316,243, while fasting plasma insulin levels tended to decrease (–41 ± 82%, NS). Fasting plasma FFAs were increased by 41% by treatment (P < 0.05; Table 2), and this increase was linearly correlated with the achieved plasma concentrations of CL 316,243 at

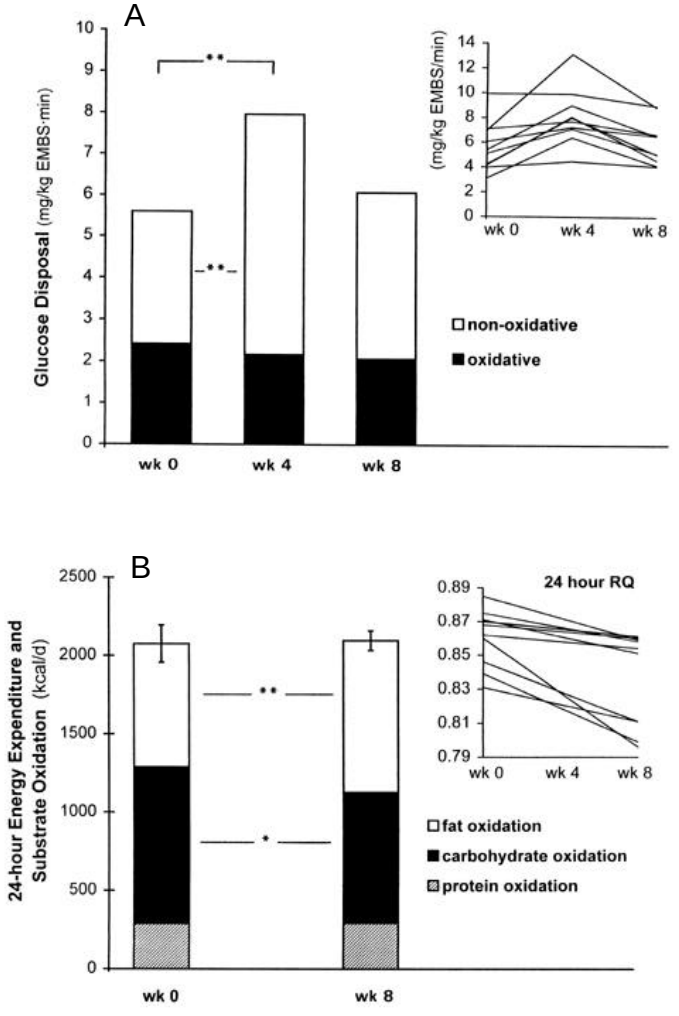

FIG. 1. Changes in insulin action (*A*) and energy metabolism (*B*) after 4 and 8 weeks of treatment with the selective β3-adrenoceptor agonist CL 316,243 in 10 healthy young lean male subjects. *A*: The heights of the columns represent total IMGD, while the subcolumns illustrate the respective proportions of OGD and NOGD. EBMS was calculated in our laboratory as FFM + 17 kg (34). **P < 0.01, significant changes from baseline for total IMGD and NOGD, respectively. Individual results for IMGD (M) are given in the upper right corner. *B*: The heights of the columns represent 24-EE, while the subcolumns illustrate the respective contributions of fat, carbohydrate, and protein oxidation. *P < 0.05 and **P < 0.01, significant changes from baseline for carbohydrate and fat oxidation, respectively. Individual results for 24-RQ are given in the upper right corner.

week 4 (r = 0.67, P < 0.05; Fig. 2). Interestingly, the change in plasma FFA concentrations from week 4 to 8 was negatively correlated with the change in CL 316,243 concentrations during this time (r = –0.57, P < 0.05; Fig. 2). Fasting plasma leptin concentrations were unaffected by treatment with CL 316,243 (Table 2).

DISCUSSION

After the first identification of β3-adrenoceptor agonists in the early eighties (1,37) and the observation that this class of β-adrenergic compounds was remarkably effective in correcting both obesity and diabetes in rodents (1–8), optimism was raised for discovering β3-adrenoceptor agonists for use in humans. Today, the gene encoding for the human β3-adrenoceptor has been isolated (38) and found to be expressed in human tissues (39,40). However, the β3-adrenergic agonists tested in clinical studies over the past 15 years have had little pharmacological activity (9–12,15–18), and none has been highly selective for the β3-adrenoceptor. This leads to the question whether the expression of biologically active β3-adrenoceptors in humans is sufficient to produce relevant metabolic effects (41,42). Recent results from both in vitro and in vivo studies using novel agents with high β3-selectivity strongly suggest that biologically active β3-adrenoceptors are present in humans (43,44), but to date, no study reporting metabolic effects of a selective β3-adrenoceptor agonist in humans has been published.

The present study confirms the presence of functional β3-adrenoceptors in humans and demonstrates, for the first time, that administration of a selective β3-adrenoceptor agonist exerts metabolic actions in humans. Treatment with the highly β3-selective compound CL 316,243 for 4 weeks resulted in a 45% increase in IMGD in lean male subjects, reflecting an important effect on glucose metabolism. In addition, the 24-RQ was significantly lowered after 8 weeks of treatment, demonstrating a marked stimulation of fat oxidation. Importantly, both effects were related to the achieved plasma concentrations of CL 316,243, thus indicating that these metabolic actions are in fact the result of β3-adrenergic stimulation.

Several questions emerge from these findings, particularly in regard to the putative mechanisms and sites of action of CL 316,243 in humans. With respect to the observed improvement in insulin action, the effect of the drug was entirely due to an increase in NOGD, whereas glucose oxidation was unaffected by treatment. This is in accordance with findings in rodents (8,23), in which CL 316,243 exerts its effects on insulin action exclusively by increasing NOGD, and also with earlier studies in humans using nonselective β3-adrenergic agents (45,46). Because the earlier compounds tested in humans had stimulatory effects not exclusively on β3-adrenoceptors but also on β1- and β2-adrenoceptors, it was uncertain whether the observed effects on insulin action were a β3-effect alone. In the present study, a highly selective β3-agonist was used, and therefore, it can be concluded that insulin action can be augmented by β3-adrenoceptor stimulation alone in humans.

Interestingly, BAT and WAT were the only tissue sites responsible for the CL 316,243–induced increase in glucose disposal in lean rodents (8). In contrast, skeletal muscle glucose uptake was significantly increased in obese diabetic Zucker rats treated with CL 316,243 (23), but this may have been a secondary effect, since CL 316,243 also induced a marked decline in fasting plasma glucose and FFA concentrations in these animals. In our study, the increase in IMGD was achieved in parallel with an increase in plasma FFA concentrations. This is surprising, since insulin action is usually deteriorated by elevated FFA concentrations. Moreover, it remains controversial whether functional β3-adrenoceptors are expressed in skeletal muscle in humans, and in fact, several studies have failed to detect β3-adrenoceptor mRNA expression in human (38) and rodent (47) skeletal muscle. In contrast, there is clear evidence for the expression of functional β3-adrenoceptors in human WAT (26,43,44), and it has been shown in rodents that CL 316,243 increases the number and binding affinity of insulin receptors as well as the expression of glucose transporters in white adipocytes (3). More

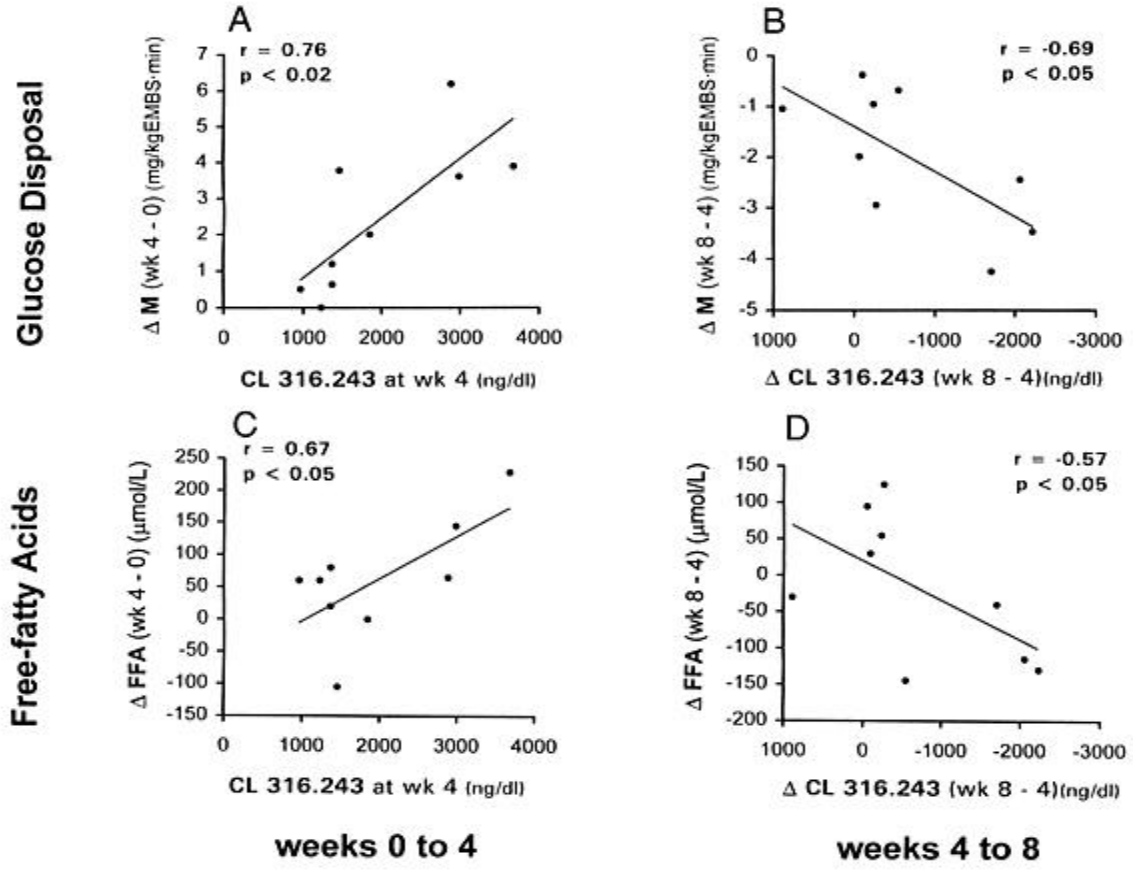

FIG. 2. Relationship between the achieved plasma concentrations of CL 316,243 and the changes in IMGD (M) (*A* and *B*) and fasting plasma FFA concentrations (*C* and *D*) between 0 and 4 weeks (*A* and *C*) and between 4 and 8 weeks (*B* and *D*) in 9 of the 10 subjects treated with CL 316,243.

recently, it has been reported that in rodents, CL 316,243 stimulates uncoupling protein–3 expression in WAT (48) and induces the appearance of brown adipocytes in areas considered traditionally to be WAT (49,50). The significance of these findings as a possible explanation for the improved insulin action in humans remains to be elucidated.

A finding of particular interest in our study was the observation that the increased glucose disposal and FFA concentrations at 4 weeks were both linearly related to the achieved plasma concentrations of CL 316,243. Upon closer scrutiny, the most prominent increase in insulin action and fasting plasma FFA concentrations after 4 weeks occurred in the three subjects in whom the achieved plasma concentrations of CL 316,243 exceeded the in vitro ED_{50} for cAMP production and lipolysis of 1,800 ng/dl (22,51). In these subjects, the increase in IMGD and FFA concentrations reached 83 and 140%, respectively. Considering the poor oral bioavailability of CL 316,243 (~10% absorption), it seems likely that larger pharmacodynamic effects would have been observed in the remaining subjects if higher plasma concentrations of CL 316,243 had been achieved. Importantly, the observed effects on insulin action were obtained in lean young men, i.e., in subjects with an a priori high insulin sensitivity. Results in rodents suggest that the effects of β_3-adrenoceptor agonists on insulin action are greater in obese diabetic than in lean animals (23), a finding supported by a study in humans using a nonselective compound (45). The increase in glucose disposal seen after 4 weeks of treatment was markedly diminished after 8 weeks, and this change in IMGD was, again, correlated with the change in plasma CL 316,243 concentrations, which declined by 36% during this time. Interestingly, the marked lowering of the 24-RQ was found after 8 weeks, i.e., when the prevailing plasma concentrations of the compound were already decreased.

The decline in plasma concentrations of CL 316,243 between 4 and 8 weeks was unexpected and was not anticipated from earlier clinical studies. We have no explanation for this, but noncompliance can be ruled out, as subjects were observed taking their medications. As pointed out, CL 316,243 is poorly absorbed in humans, which may have favored fluctuations in the bioavailability of the compound. Alternatively, the metabolism of the drug might have accelerated over time. It is unlikely that a downregulation of β_3-adrenoceptors accounted for the observed decline in insulin action between 4 and 8 weeks, as the β_3-adrenoceptor lacks phosphorylation sites at its intracellular domain, and it has been demonstrated that in rodents, the β_3-adrenoceptor is upregulated rather than downregulated by chronic β-adrenergic stimulation (52). However, the regulation of the human β_3-adrenoceptor may differ from that of the rodent receptor.

The observed decline in 24-RQ after 8 weeks indicates a shift from carbohydrate to fat oxidation by more than 200 kcal/day or ~10% of daily energy intake. Noteworthy is that these changes took place under carefully controlled dietary conditions, i.e., neither caloric intake nor macronutrient composition differed between the chamber experiments before and

after treatment. Furthermore, the 24-RQ of each subject was adjusted for energy balance, another major determinant of 24-RQ. Hence, it can be concluded that these changes in substrate oxidation reflect β_3-adrenergic stimulation.

In rodents, treatment with β_3-adrenoceptor agonists is known to increase fat oxidation. Although this is believed to be accounted for primarily by the enhanced BAT thermogenesis in these animals (53), we saw no effect of CL 316,243 on thermogenesis, as 24-EE, SMR, and body temperature were unaffected by treatment. We also found no effect of CL 316,243 on the plasma concentrations of leptin. This is in good agreement with findings in lean young rats (54), while in rodent models of diet-induced obesity, a marked suppression of leptin by treatment with CL 316,243 has been reported (54). Despite the lack of effects on thermogenesis and plasma leptin concentration and the failure to produce a measurable reduction of fat mass after 8 weeks, this study does not rule out a potential antiobesity effect of CL 316,243 in humans. First, it has been reported that the antidiabetic effects of β_3-adrenoceptor agonists in animals can be achieved with doses that do not increase thermogenesis or cause weight reduction (10). Second, in lean rodents, administration of β_3-adrenoceptor agonists produces only minor weight reductions, irrespective of whether thermogenesis is increased or not (10). Thus, treatment with CL 316,243, besides stimulating fat oxidation, may decrease fat stores over time, either preferentially in obese subjects or in lean subjects at higher plasma concentrations. Noteworthy is that the 24-h fat balance was negative in the CL 316,243–treated subjects at the end of the study, but this finding cannot be directly extrapolated to changes in fat mass, since dietary intake was not controlled during the 8 weeks of treatment.

We found no measurable cardiovascular effects or hand tremors after treatment with 1,500 mg/day of CL 316,243, confirming the high β_3-selectivity of this compound found in preclinical evaluation (22,51).

In conclusion, treatment of lean male human subjects with CL 316,243, a highly selective and partial β_3-adrenoceptor agonist, increases insulin action and fat oxidation in a plasma concentration–dependent manner. However, in the absence of changes in energy expenditure, CL 316,243 had no effect on body weight or composition. The observed metabolic actions were not accompanied by cardiovascular effects or hand tremors. This is the first study to demonstrate metabolic actions of a highly selective β_3-adrenoceptor agonist in humans. Its findings should encourage the clinical development and testing of new β_3-adrenoceptor agonists that are more fully active, have a higher bioavailability, and are developed against the human receptor as putative drugs for the treatment of obesity and diabetes in humans.

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