Troglitazone Reduces Plasma Leptin Concentration but Increases Hunger in NIDDM Patients

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OBJECTIVE — Troglitazone, which improves peripheral insulin resistance of experimental diabetic animals and diabetic patients, affects *ob* gene expression in the adipose tissue of rodents. The present study was undertaken to examine a hypothesis that clinical administration of troglitazone may reduce circulating leptin levels and affect eating behavior in NIDDM patients.

RESEARCH DESIGN AND METHODS — Troglitazone was administered at a dosage of 200 mg twice daily for 12 weeks in 20 poorly controlled NIDDM patients. Chronological changes in glycemic control, serum lipids, immunoreactive leptin (IRL) levels, and BMI were measured. Body fat weight was also assessed by bioelectric impedance.

RESULTS — Troglitazone significantly decreased fasting plasma glucose, serum immunoreactive insulin, and HbA_{1c} levels. Serum levels of IRL and triglyceride were significantly reduced by troglitazone administered for 4, 8, and 12 weeks. Troglitazone administration significantly increased the BMI in NIDDM patients, and two-thirds of the patients complained of increased hunger after the start of troglitazone administration.

CONCLUSIONS — Troglitazone significantly reduces circulating leptin levels at clinical doses. It may affect the eating behavior of poorly controlled NIDDM patients through the improvement of glycemic control and/or the reduction of circulating leptin.

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Since the anorexigenic peptide, leptin, was found in the genetically obese (*ob/ob*) mouse (1), evidence has accumulated that leptin plays an important role in the regulation of eating behavior in rodents. Leptin acts on the hypothalamus to inhibit food intake and to stimulate energy expenditure (2,3). A strong positive correlation of circulating leptin levels with BMI, percentage of body fat, and body fat weight in humans has been reported (4,5). Several bioactive substances, such as hormones and cytokines, affect circulating leptin levels in animals and humans (6–9). Insulin is an especially important determinant of circulating leptin levels because chronic insulin infusion, but not acute infusion, increases *ob* gene expression and circulating leptin levels (6,7). However, whether changes in circulating leptin levels may actually affect satiety in humans has still not been determined.

Thiazolidinediones improve peripheral insulin resistance in NIDDM patients with hyperinsulinemia (10,11). These agents are available for the treatment of NIDDM patients. Recently, thiazolidinedione derivatives have been reported to decrease *ob* gene expression in 3T3-L1 adipocytes and genetically obese animals (12,13). However, whether clinical administration of these agents may affect circulating leptin levels and eating behavior has not been explored in NIDDM patients. The present study was undertaken to examine the hypothesis that troglitazone, one of thiazolidinediones, may modulate circulating leptin levels in poorly controlled NIDDM patients. In addition, we observed changes in BMI, body fat weight, serum lipids levels, and appetite possibly caused by troglitazone administered for 12 weeks.

RESEARCH DESIGN AND METHODS

Subjects

Twenty poorly controlled NIDDM patients (9 men and 11 women) were included in the present study. The average age and BMI were 55.47 ± 2.96 years and 24.85 ± 1.26 kg/m^2, respectively. Before troglitazone treatment was begun, 9 (45%) of the patients were treated with only diet therapy, and 11 (55%) were treated with diet and glibenclamide. Caloric intake was not changed during the whole observation period. Body fat weight was measured by bioelectric impedance using TBF-101 (Tanita, Tokyo). Blood samples were obtained from the cubital vein in the early morning after overnight fasting. After centrifugation, the obtained serum and plasma were frozen at −20°C until assay.

Protocol

Troglitazone was administered at a dosage of 200 mg twice daily for 12 weeks in 20 poorly controlled NIDDM patients. Chronological changes in fasting plasma glucose (FPG) and HbA_{1c}, serum immunoreactive insulin (IRI), serum immunoreactive leptin (IRL), lipids levels, and BMI were measured. Body fat weight was also assessed by bioelectric impedance.

Assays

Serum IRL levels were measured using a commercially available radioimmunoassay

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Abbreviations: apo, apolipoprotein; FPG, fasting plasma glucose; HOMA, homeostasis model assessment; IRI, immunoreactive insulin; IRL, immunoreactive leptin; RIA, radioimmunoassay; TC, total cholesterol; TG, triglyceride.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

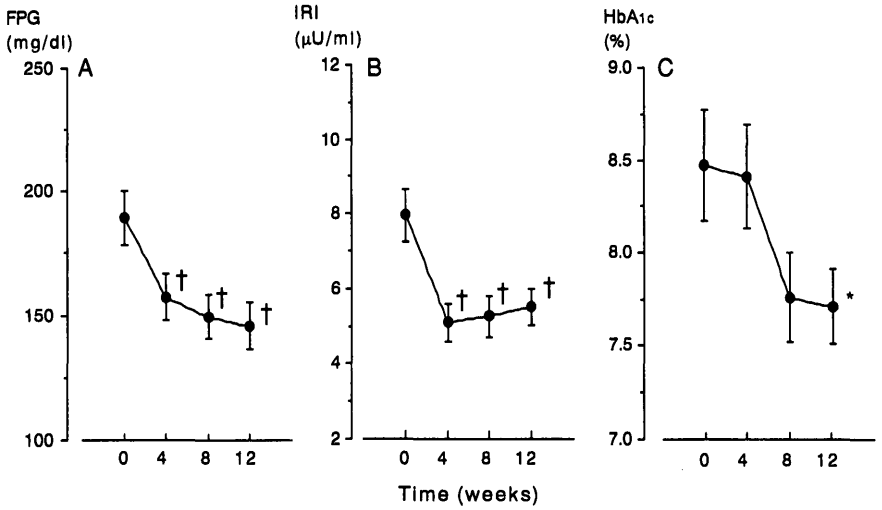

Figure 1—*Changes in FPG (A), serum IRI (B), and HbA_{1c} (C) levels by troglitazone (400 mg/day) administered for 12 weeks in poorly controlled NIDDM patients. *P < 0.05 and †P < 0.01 versus values before the start of troglitazone administration.*

(RIA) kit (Linco Research, St. Charles, MO). Within-assay variation was 3.4–8.3%, and between-assay variation was 3.0–6.2%. FPG and HbA_{1c} levels were measured by the glucose oxidase method and high-performance liquid chromatography, respectively. Serum IRI levels were measured using a commercially available RIA kit (Phadeceph Insulin RIA kit; Pharmacia Japan, Tokyo).

Homeostasis model assessment (HOMA) was used to assess changes in pancreatic β-cell function and insulin resistance before and at 12 weeks after the start of troglitazone treatment (14). Assuming that normal subjects aged <35 years who are of normal weight have 100% β-cell function and an insulin resistance of 1, the values for a patient can be assessed from the fasting insulin and glucose concentrations by the formulas β-cell function (%) = 20 × [insulin]/([glucose] − 3.5) and resistance = insulin/(22.5$e^{-\ln[glucose]}$).

Serum levels of total cholesterol (TC), HDL cholesterol, and triglyceride (TG) were spectrophotometrically measured by diagnostic kits provided by Kyowa Medix (Tokyo) (15). Serum levels of apolipoprotein (apo) A-I, apoB, and apoE were spectrophotometrically measured by diagnostic kits (APO A-I, B, E, AUTO.N "DAIICHI") provided by Daiichi Pure Chemicals (Tokyo).

Statistical analysis

All data were expressed as means ± SEM. The statistical analysis of the means was performed by two-way analysis of variance with one repeated measurement. The difference of the means was determined by paired *t* test.

RESULTS — Figure 1 demonstrates chronological changes in FPG, HbA_{1c}, and IRI levels for 12 weeks after the start of troglitazone administration. Administration of troglitazone for 12 weeks obviously improved hyperglycemia, hyperinsulinemia, and HbA_{1c} levels in poorly controlled NIDDM patients. Changes in both pancreatic β-cell function and insulin resistance by troglitazone administration were assessed by HOMA (14) (Fig. 2). Insulin resistance was obviously improved by troglitazone administration for 12 weeks. However, pancreatic β-cell function was not affected by the treatment.

We determined changes in serum IRL levels and in BMI associated with 12 weeks of troglitazone administration. Serum IRL levels were significantly reduced by troglitazone administration at 4, 8, and 12 weeks (Fig. 3). BMI was significantly increased at 4, 8, and 12 weeks after the start of troglitazone treatment, but an increase of total body fat weight assessed by bioelectric impedance was not statistically significant.

Table 1 demonstrates changes in serum TC, HDL cholesterol, TG, and apoA-I, apoB, and apoE levels produced by troglitazone administration for 12 weeks. Serum levels of TC and TG were chronologically reduced, but the reduction was statistically significant only in TG levels. Serum HDL cholesterol levels tended to increase with troglitazone administration, but the difference was not significant. No obvious changes were observed in serum levels of apoA-I, apoB, and apoE.

Figure 4 presents the incidence of increased hunger in poorly controlled NIDDM patients treated with troglitazone. Two-thirds of the patients complained of increased hunger at 4 weeks after the start of troglitazone administration.

CONCLUSIONS — The present study has reconfirmed that troglitazone administration for 12 weeks improves insulin resistance and has added a new finding that in poorly controlled NIDDM patients, immediate reduction of circulating IRL levels by troglitazone administration is accompanied by a significant increase in BMI. On the

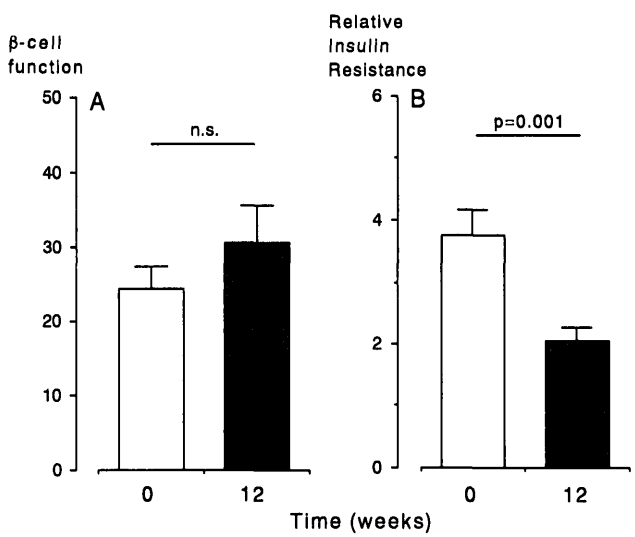

Figure 2—*Changes in pancreatic β-cell function (A) and insulin resistance (B) assessed by HOMA (14) after administration of troglitazone (400 mg/day) for 12 weeks in poorly controlled NIDDM patients.*

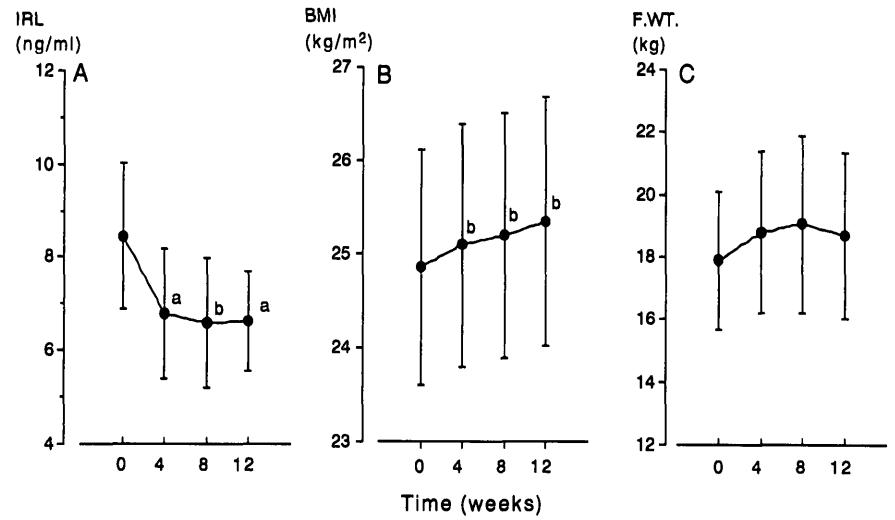

Figure 3—*Changes in fasting serum IRL level (A), BMI (B), and total body fat weight (F.WT.) (C) associated with administration of troglitazone (400 mg/day) for 12 weeks in poorly controlled NIDDM patients. P < 0.05 (a) and P < 0.01 (b) versus values before the start of troglitazone administration.*

whole, the poor glycemic control of NIDDM patients was obviously improved despite an increase in BMI.

Thiazolidinedione derivatives have recently been reported to inhibit *ob* gene expression in both in vitro and in vivo experiments (12,13). In obese nondiabetic patients (average BMI, 32.4 kg/m²), administration of troglitazone, 200 mg twice daily for 12 weeks, produced no significant change in fasting plasma leptin levels despite a 40–50% reduction in fasting and postmeal plasma insulin levels (16). In obese patients (average BMI, 42.9 kg/m²) with polycystic ovary syndrome, leptin concentrations remained unchanged after treatment with 200 or 400 mg troglitazone daily for 3 months (17). Therefore, thiazolidinedione derivatives appear not to affect circulating leptin levels in obese patients whose BMI is >30 kg/m². However, the clinical influence of thiazolidinedione derivatives on *ob* gene expression and circulating leptin levels has not been determined in poorly controlled NIDDM patients. We confirmed that troglitazone administration at clinical doses obviously reduces circulating IRL levels in poorly controlled NIDDM patients. The discrepancy between previous observations (16,17) and the present results can be explained by the difference in the BMI and the recent glycemic control state of the subjects included in each study.

In addition, insulin levels decreased after 12-week troglitazone treatment. Insulin is associated with circulating leptin (18,19). Hickey et al. have documented an improvement in insulin sensitivity and a reduction in circulating leptin levels in female patients after 12 weeks of aerobic exercise training (20). Another possible explanation is that the reduction of serum leptin levels by troglitazone is partially due to the reduction of serum insulin levels and the improvement of insulin sensitivity in poorly controlled NIDDM patients.

In the present study, the BMI was significantly increased by 12 weeks of troglitazone administration. Also, as indicated by their complaints during the study, two-thirds of the poorly controlled NIDDM patients treated with troglitazone experienced increased hunger (Fig. 4). Increased appetite might possibly be associated with body weight and body fat gain caused by troglitazone administration. In contrast, because thiazolidinedione derivatives do not affect serum leptin concentrations in obese subjects (16,17), changes in feelings of hunger in commonly obese NIDDM patients should be determined in further studies. Conversely, it is well known that sympathetic nerve activity involves the regulation of body weight (21). Another possible explanation for the increase in BMI is a reduction in sympathetic nerve activity resulting from the reduction in leptin.

The cause of feelings of increased hunger associated with troglitazone is not fully determined in the present study. One possible explanation is the reduction of circulating leptin levels by troglitazone administration. The observed reduction of circulating leptin, the anorexigenic message to the hypothalamic feeding centers, might stimulate the appetite in poorly controlled NIDDM patients. However, troglitazone failed to affect the satiety and body weight gain in obese nondiabetic patients (16). Second, because studies have demonstrated that both glucose and insulin involve the hypothalamic regulation of feeding behavior and sympathetic activities in rodents (22,23), the improvement of hyperglycemia and hyperinsulinemia produced by troglitazone administration may involve increases in hunger and BMI.

In addition, it was demonstrated that hypertriglyceridemia of poorly controlled NIDDM patients was also improved by troglitazone administration. Peripheral insulin resistance involves changes in circulating lipid levels (24,25). Metformin, which is another potential candidate for intervention in the insulin resistance syndrome, induced a better maintenance of FPG and TC levels but had no significant effect on serum TG levels (26). In contrast, other investigators have reported a significant reduction of serum TG and TC levels associated with glycemic control by metformin (27). These data indicate that an improvement of insulin resistance should have beneficial effects on lipid metabolism in NIDDM patients. Troglitazone has been thought to have beneficial effects on the

Table 1—*Chronological changes in serum lipid levels after the start of treatment with troglitazone*

	Time (weeks)			
	0	4	8	12
TC	238.6 ± 13.2	224.1 ± 9.9	225.3 ± 10.5	227.8 ± 9.8
HDL cholesterol	54.8 ± 3.8	57.6 ± 4.3	60.1 ± 4.5	57.1 ± 3.8
TG	187.5 ± 32.9	122.3 ± 14.3	107.2 ± 12.3*	122.6 ± 12.2
ApoA-I	135.4 ± 7.4	131.9 ± 5.7	129.4 ± 6.5	127.0 ± 5.3
ApoB	114.9 ± 6.4	108.4 ± 7.4	112.2 ± 7.2	110.8 ± 7.1
ApoE	7.3 ± 0.7	6.7 ± 0.5	7.1 ± 0.5	7.1 ± 0.5

Data are means ± SEM and are expressed in milligrams per deciliter. *$P < 0.05$ versus the value before the start of treatment with troglitazone.

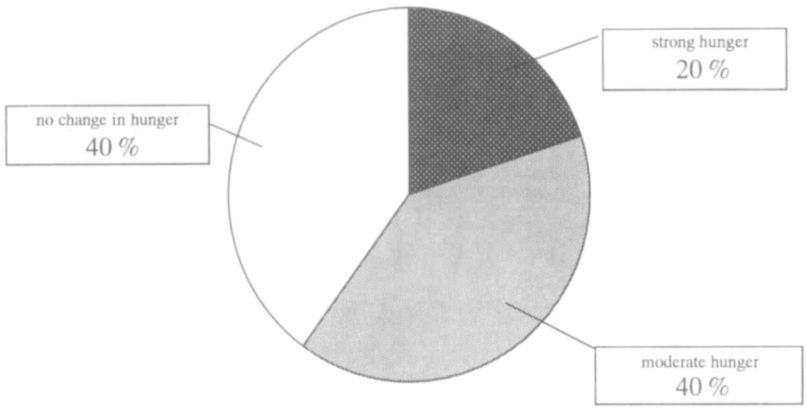

Figure 4—*The incidence of increased hunger in poorly controlled NIDDM patients treated with troglitazone (400 mg/day). The hunger score was determined by the complaints of patients at 4 weeks after the start of the treatment. 1 (strong hunger): A patient complains of marked hunger that subsequently induces an obvious increase of daily food intake. 2 (moderate hunger): A patient complains of moderate hunger that is not accompanied by an increase of daily food intake. 3 (no change in hunger): A patient did not complain of any change in hunger.*

dyslipidemia of NIDDM patients (28). The present results demonstrate clinically that improvement of insulin resistance by troglitazone administration reduces serum TG levels in poorly controlled NIDDM patients, but they fail to show significant changes in serum levels of TC, HDL cholesterol, apoA-1, apoB, and apoE. If patients treated with troglitazone are studied in larger numbers, poorly controlled NIDDM patients may demonstrate a significant reduction of serum TC levels.

It was demonstrated that in addition to improving insulin resistance, the administration of troglitazone at clinical dosages reduces circulating leptin levels in poorly controlled NIDDM patients. The reduction by troglitazone of circulating leptin levels may affect eating behavior in NIDDM patients.

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