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Subcutaneous Fat Accumulation and Thiazolidinedione Derivatives : Sex Differences and Their Relation to Glycemic Control

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ABSTRACT

We investigated changes in body fat distribution during long-term treatment with troglitazone and its effect on glycemic control. We administered troglitazone at 400 mg/day to 40 outpatients with type 2 diabetes. Twenty-one drug-naive patients (8 men and 13 women) received troglitazone monotherapy for 16 months, and 19 patients (10 men and 9 women) who had been receiving glibenclamide continued this drug and also received troglitazone for 16 months. Levels of glycosylated hemoglobin significantly decreased and body mass index significantly increased in the men and women receiving troglitazone alone and in women receiving both glibenclamide and troglitazone. No significant changes were observed in the area of visceral fat in any treatment group. A significant increase in subcutaneous fat was observed in the men and women receiving troglitazone alone and in women receiving both glibenclamide and troglitazone. Although no sex difference was observed in the percentages of patients with reduced levels of gycosylated hemoglobin or increased subcutaneous fat among those receiving troglitazone, a significantly higher percentage of women had these changes among patients receiving both glibenclamide and troglitazone. The apparent sex difference in the degree of glycemic control produced by thiazolidinedione therapy in our subjects can be attributed to the increase in subcutaneous fat after treatment and to a pretreatment difference in the subcutaneous fat.

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Key words : thiazolidinedione derivative, peroxisome proliferator-activated receptor γ, insulin resistance, adipocytokine, sex difference

Introduction

Thiazolidinedione derivatives enhance glucose uptake in skeletal muscle and inhibit excessive glucose production in the liver[1]. How these drugs improve insulin resistance at the molecular level, however, remains unclear. In vitro studies have shown that thiazolidinedione promotes the differentiation of preadipocytes into adipocytes. Peroxisome proliferation-activated receptor (PPAR) γ is an important factor in this process of differentiation, and thiazolidinedione acts as a ligand for activation[2-5]. A recent in vitro study has found that thiazolidinedione promotes the differentiation of preadipocytes into adipocytes only in subcutaneous fat, suggesting a relationship between this effect and

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the decrease in insulin resistance seen with the drug[6]. Moreover, the thiazolidinedione derivative troglitazone reduces the size of adipocytes in genetically obese rats, demonstrating the relation of this drug to decreases in insulin resistance[7]. We have reported that treatment with troglitazone markedly promotes subcutaneous fat accumulation in mildly obese Japanese patients with type 2 diabetes mellitus[8]. Subsequently, many clinical studies have investigated thiazolidinedione derivatives and body fat distribution[9–12]. Sex differences exist in body fat distribution, that is, subcutaneous fat is easily accumulated in women whereas visceral fat is more likely to accumulate in men. We investigated the changes in body fat distribution during long-term administration of troglitazone and the relationship between glycemic control and accumulation of subcutaneous fat.

Patients and Methods

Patients

Troglitazone (400 mg/day) was administered to 40 outpatients with type 2 diabetes mellitus in whom plasma glucose was poorly controlled with conventional treatment. All patients had received nutritional counseling and been encouraged to exercise. Twenty-one drug-naive patients (8 men and 13 women) received troglitazone monotherapy for 16 months, and 19 patients (10 men and 9 women) who were already receiving glibenclamide (1.25–7.5 mg/day) continued receiving this drug and also received troglitazone for 16 months. All patients gave their informed consent for participation in the study.

Study design

The level of glycosylated hemoglobin (HbA1c), body mass index (BMI), and body fat distribution[13] at the level of the umbilicus as assessed with abdominal computed tomography were determined before start of treatment and after the end of treatment. Serum levels of leptin[14] and tumor necrosis factor-alpha [TNF-α][15], and plasma level of plasminogen activator inhibitor-1 [PAI-1][16] were measured before and after treatment. Hepatic function was evaluated each month by measuring levels of alanine aminotransferase, aspartate aminotransferease, and total bilirubin. All patients with type 2 diabetes mellitus had earlier received dietary education and were instructed to continue the diets they had followed before troglitazone treatment. Patients whose diets or exercise was substantially changed during the observation period and those who were admitted for treatment were excluded from the study population. The mean change in HbA1c value was calculated in all patients, who were then provisionally classified as high responders or low responders on the basis of whether the change in HbA1c value was greater or less than the mean change in HbA1c in all patients (−0.93%). In addition, patients were classified as high or low responders on the basis of whether they showed a change in subcutaneous fat area greater or less than the mean change in all patients (+39.6 cm^2).

Measurements

Plasma levels of glucose were determined with the hexokinase method; those of HbA1c were determined with high-performance liquid chromatography (HPLC); and hepatic function was assessed with enzyme method. Levels of leptin and PAI-1 were determined with radioimmunoassay (LINCO Research Inc, St. Charles, MO, USA) and with enzyme-linked immunosorbent assay (EIA) (Monozyme Inc, Hørsholm, Denmark), and TNF-α, with high sensitivity EIA (R & D Systems Inc, Minneapolis, MN, USA.).

Statistical analysis

All values are expressed as means±SD. Sex differences in each group before the start of treatment were analyzed with Student's t-test; differences between variables before and after treatment were assessed with the paired t-test; and differences between the 4 groups (men and women of the 2 groups) were assessed with one-way analysis of variance. Differences between high responders and low responders in each group were analyzed with the chi-square test. Differences with a p value less than 5% were considered significant ($p<0.05$).

Results

The clinical characteristics of subjects are presented in Table 1. The subcutaneous fat area in women was significantly greater than that in the men in both groups, whereas the visceral/subcutaneous (V/S) fat ratio in women was significantly lower than that in men. The BMI increased significantly in both men ($p<0.001$) and women ($p<0.01$) treated with troglitazone alone but increased only in women treated with both glibenclamide and troglitazone ($p<0.01$, Table 2). The level of HbA1c decreased significantly in both men and women treated with troglitazone alone and in women treated with both glibenclamide and troglitazone ($p<0.05$; Table 2). The area of visceral fat did not change significantly in any subjects (Table 3), but the area of subcutaneous fat increased significantly in both men ($p<0.05$) and women

Table 1. Patient profile for the troglitazone group and glibenclamide+troglitazone group before the treatment with troglitazone

	Troglitazone		Glibenclamide+Troglitazone	
	men	women	men	women
No of subjects	8	13	10	9
Age (year)	59.8±8.2	56.4±9.9	63.5±9.4	58.9±5.8
Duration of diabetes (year)	3.5±3.4	3.6±3.1	9.4±5.2	10.6±6.6
BMI (kg/m²)	25.8±1.5	27.3±5.4	23.1±2.3	27.8±4.7*
HbA1c (%)	7.7±1.2	8.1±1.9	9.3±1.2	8.8±0.9
Subcutaneous fat area (cm²)	138.5±63.0	235.7±83.6**	89.3±48.8	224.1±116.8**
Visceral fat area (cm²)	142.4±57.3	113.4±41.6	93.0±49.2	123.7±43.0
V/S ratio	1.15±0.52	0.51±0.21**	1.07±0.48	0.63±0.28*

*$p<0.05$, **$p<0.01$, vs. men

Table 2. Changes in HbA1c and BMI before and after treatment with troglitazone

	HbA1c (%)		BMI (kg/m²)	
	Before treatment	After treatment	Before treatment	After treatment
Troglitazone				
men	7.7±1.2	7.0±0.9*	25.8±1.5	27.0±1.7***
women	8.1±1.9	7.0±0.7*	27.3±5.4	28.8±5.3**
Glibenclamide+Troglitazone				
men	9.3±1.2	9.3±1.9	23.1±2.3	23.5±2.3
women	8.8±0.9	7.7±1.1*	27.8±4.7	30.0±5.9**

*$p<0.05$, **$p<0.01$, ***$p<0.001$, vs. before treatment

Table 3. Changes in body fat distribution before and after treatment with troglitazone

	Visceral fat area (cm²)		Subcutaneous fat area (cm²)		V/S ratio	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Troglitazone						
men	142.4±57.3	128.0±52.5	138.5± 68.0	163.6± 78.6*	1.15±0.52	0.85±0.37*
women	113.4±41.6	106.2±48.3	235.7± 83.6	284.2±100.2***	0.51±0.21	0.38±0.15**
Glibenclamide+Troglitazone						
men	93.0±49.2	100.6±48.8	89.3± 48.8	108.1± 49.2	1.07±0.48	1.04±0.55
women	123.7±43.0	128.1±52.3	224.1±116.8	270.8±145.4*	0.63±0.28	0.52±0.19

*$p<0.05$, **$p<0.01$, ***$p<0.001$, vs. before treatment

($p<0.001$) receiving troglitazone and in women receiving both glibenclamide and troglitazone ($p<0.05$).

When the changes in HbA1c, BMI, subcutaneous fat area, and visceral fat area after the treatment were compared between the 4 groups (Fig. 1), the degree of change in the first 3 variables were slightly, although not significantly, greater in women than in the men in both treatment groups. Visceral fat area decreased slightly but not significantly in both men and women treated with troglitazone but increased slightly but not significantly in men and women treated with both glibenclamide and troglitazone.

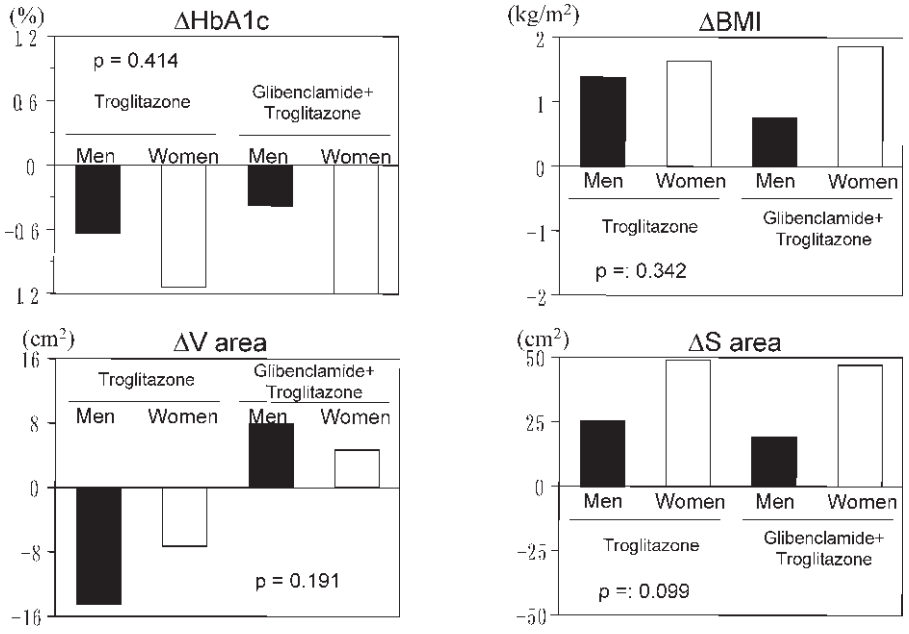

Fig. 1. Change in HbA1c, BMI, visceral fat (V), and subcutaneous fat (S) in patients with type 2 diabetes mellitus. Bars show mean values of HbA1c, BMI, V area, and S area.

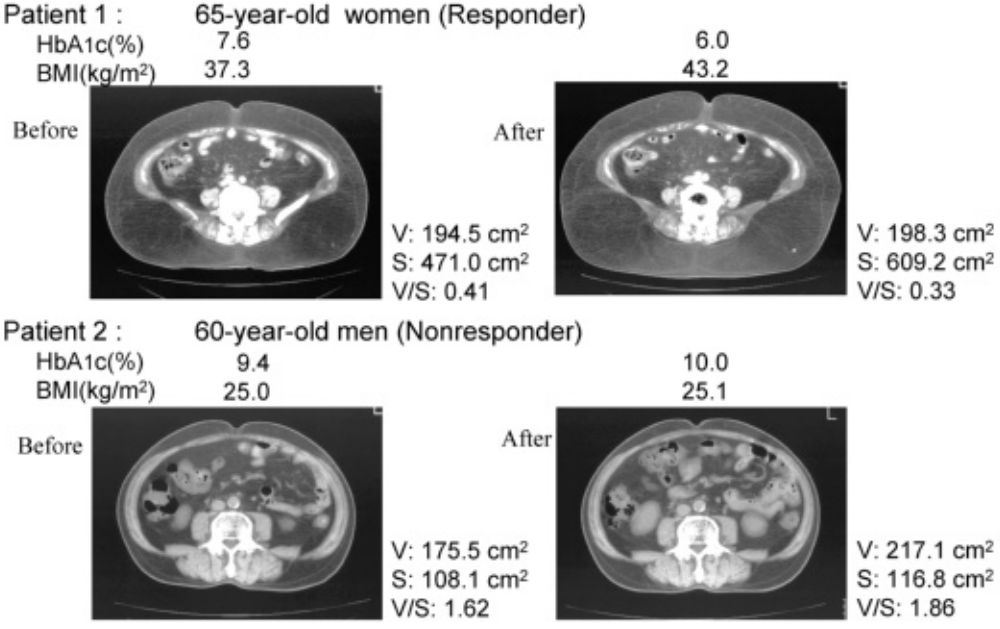

Fig. 2. Typical abdominal computed tomographic images of a responder in whom the HbA1c level decreased and a nonresponder in whom the HbA1c level did not decrease with troglitazone treatment.

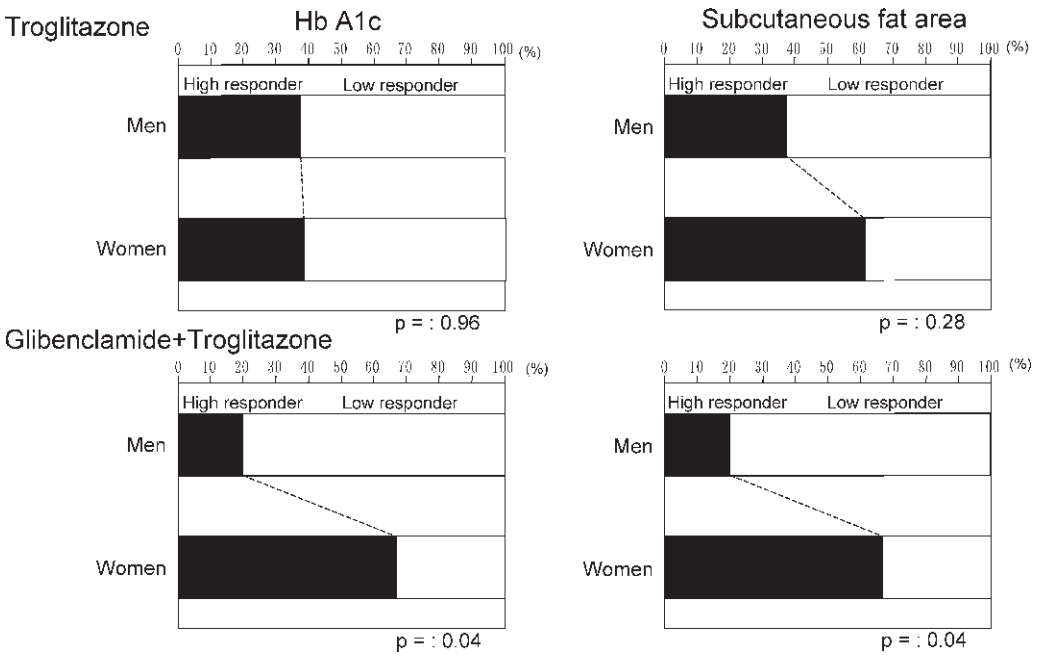

Fig. 3. Sex differences between high and low responders with regard to HbA1c level and subcutaneous fat accumulation.

Figure 2 shows typical abdominal computed tomographic images at the level of the umbilicus in patients in whom HbA1c values decreased (responders) or did not decrease (nonresponders). Patient 1, who was a typical responder, was a 65-year-old woman. The amount of subcutaneous fat in this patient was extremely large before treatment (subcutaneous fat-type obesity) and had increased markedly by the end of treatment. Patient 2, who was a typical nonresponder, was a 60-year-old man. The volume of subcutaneous fat before the start of treatment in this patient was small (visceral fat type obesity) and had increased only slightly by the end of treatment.

Among patients receiving troglitazone, the percentages of patients who were high responders according to HbA1c values (37.5% of men and 38.5% of women) and on the basis of subcutaneous fat accumulation (37.5% of men and 61.5% of women) did not differ according to sex (Fig. 3). In contrast, among patients receiving both glibenclamide and troglitazone, the percentage of high responders was significantly greater for women than for men on the basis of both HbA1c levels (66.7% and 20.0%, $p<0.05$) and subcutaneous fat accumulation (66.7% and 20.0%, $p<0.05$, Fig. 3).

There were no significant changes in levels of leptin, TNF-α, or PAI-1 in patients receiving troglitazone (leptin: 6.0±5.4 ng/ml [before treatment] and 5.0±3.4 ng/ml [after treatment]; TNF-α 24.6±26.3 and 11.6±8.8 pg/ml, PAI-1: 56.1±31.9 and 53.2±30.8 ng/ml] or in patients receiving both glibenclamide and troglitazone (leptin: 4.4±3.7 and 2.8±1.5 ng/ml; TNF-α: 11.6±16.6 and 9.1±8.1 pg/ml; PAI-1: 59.7±34.7 and 45.4±17.0 ng/ml). No hepatic dysfunction or other adverse effects were observed in either group.

Discussion

To clarify the action of thiazolidinedione derivatives in decreasing insulin resistance, Okuno et al.[7] conducted an experiment in which troglitazone was administered to Zucker fatty rats. They found that no change occurred in the total weight of white adipose tissue despite an increase in the DNA content. However, they did find that adipocytes forming adipose tissue had become smaller after treatment. Specifically, the number of small adipocytes (2,500 μm^2 or smaller) increased 400% whereas the number of large adipocytes (5,000 μm^2 or larger) decreased by

50%. Okuno et al. concluded that this morphologic change is involved in the decrease in TNF-α and leptin associated with troglitazone treatment.

In the present study we found that treatment with troglitazone alone significantly increased subcutaneous fat volume and significantly decreased HbA1c levels in both men and women. A significant increases in subcutaneous fat and a significant decrease in HbA1c levels occurred only in women receiving both glibenclamide and troglitazone. No significant changes in the levels of the adipocytokines leptin, TNF-α or PAI-1 were observed in either group. This lack of change in the levels of secreted adipocytokines did not agree with findings of animal studies in which troglitazone decreased the number of large adipocytes[7]. Changes in blood adipocytokine levels over time should be investigated. No change was observed in PAI-1 levels[16]; this result was expected because no significant change was observed in the closely related volume of visceral fat.

A noteworthy result of our study was that among subjects treated with both glibenclamide and troglitazone a significantly higher percentage of women than men were defined as high responders according to changes in levels of HbA1c and area of subcutaneous fat. This result clearly demonstrates a sex differences in glycemic control and subcutaneous fat accumulation. Although the reason for this sex difference in glycemic control in these patients is not clear, it might be attributed to the conspicuous increase in subcutaneous fat volume during treatment as well as to the significantly higher subcutaneous volume before the start of treatment in the women.

Results of in vitro experiments[6] and experiments in Zucker fatty rats[7] suggest that troglitazone promotes differentiation of preadipocytes into adipocytes through PPAR-γ activity, especially in the subcutaneous adipose tissue, and thus markedly increases the number of small adipocytes while decreasing the number of large adipocytes. Because large adipocytes secrete more leptin[14] and TNF-α[15] and release more free fatty acids than do small adipocytes, the levels of these adipocytokines and free fatty acids decreased with the decrease in number of large adipocytes. We believe that the direction of energy accumulation in the body changed from visceral fat[17] toward subcutaneous fat and that this change might be substantially involved in the decrease in insulin resistance associated with thiazolidinedione derivatives.

The greater efficacy of troglitazone in Japanese patients with type 2 diabetes mellitus has been reported in those whose BMI was 25 kg/m^2 or more and whose fasting insulin level was 10 μU/mL or more[18]. The fasting insulin level was not measured in this study, so the sex differences in troglitazone efficacy might be attributed to the differences in fasting insulin level observed among the subjects. Of particular note is that in patients who received both glibenclamide and troglitazone, baseline BMIs were significantly higher in women than in men, a difference that might be responsible for the sex differences in troglitazone efficacy. Obese Japanese men with subcutaneous fat-type obesity tend to have higher fasting insulin values and a significantly larger area under the insulin curve on the 75 g oral glucose tolerance test than do men with visceral fat-type obesity[17]. That is, patients with diabetes who have high BMI and fasting insulin levels and show a favorable response to troglitazone treatment likely have accumulated subcutaneous fat. We previously investigated the relation between pancreatic reserve function for insulin secretion and body fat distribution in animals and obese patients with diabetes[19] and found that extremely obese white Americans or Europeans are most likely to have subcutaneous fat-type obesity while most mildly obese Japanese have visceral fat-type obesity. Therefore, the efficacy of troglitazone could differ between obese patients with diabetes in the United States or Europe and those in Japan.

In conclusion, we observed a sex difference in the glycemic control afforded by thiazolidinedione derivatives in mildly obese Japanese patients with type 2 diabetes mellitus. We attribute this difference to an increase in subcutaneous fat after treatment with thiazolidinedione derivatives and to a pretreatment difference in the subcutaneous fat.

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