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Effects of pioglitazone and metformin on intracellular lipid content in liver and skeletal muscle of individuals with type 2 diabetes mellitus

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Abstract

Both ectopic fat accumulation and changes of the amount of several adipocyte secreting proteins (adipokines) are thought to contribute to the development of insulin resistance associated with obesity and type 2 diabetes mellitus. We have now investigated the effects of 2 insulin-sensitizing drugs, pioglitazone and metformin, on body fat composition and serum adipokine concentrations in individuals with type 2 diabetes mellitus. A total of 41 diabetic patients were treated with pioglitazone (n =21) or metformin (n =20) for 6 months. Intramyocellular lipid content (IMCL) and hepatic lipid content as well as the areas of subcutaneous and visceral fat deposits in the abdomen were determined by nuclear magnetic resonance spectroscopy before and after drug treatment. The serum concentrations of adiponectin and retinol binding protein 4 were also determined by enzyme-linked immunosorbent assays. Pioglitazone treatment reduced both hepatic lipid content (12.0 ± 6.1 vs 8.4 ± 3.7 arbitrary units [AU], $P < .01$) and IMCL (8.4 ± 3.6 vs 6.3 ± 2.4 AU/creatine, $P < .01$), whereas metformin reduced only IMCL (7.0 ± 3.6 vs 5.8 ± 2.0 AU/creatine, $P < .05$). Although the areas of visceral and subcutaneous fat were not significantly affected by treatment with either drug, pioglitazone induced a significant reduction in the ratio of visceral to subcutaneous fat area (0.92 ± 0.41 vs 0.85 ± 0.41, $P < .05$). Pioglitazone treatment also resulted in a marked increase in serum adiponectin concentration (5.6 ± 4.1 vs 16.2 ± 9.9 μg/mL, $P < .0001$) and a small but significant decrease in serum retinol binding protein 4 concentration (73.4 ± 25.1 vs 65.1 ± 23.7 μg/mL, $P < .05$). These results suggest that pioglitazone may improve insulin sensitivity both by affecting serum adipokine concentrations and by reducing the intracellular triglyceride content of liver and skeletal muscle in individuals with type 2 diabetes mellitus.

1. Introduction

Insulin resistance plays a major role in the pathogenesis of type 2 diabetes mellitus, and the accumulation of fatty acid metabolites and triglyceride in skeletal muscle or liver is thought to contribute to the development of insulin resistance [1-10]. Proton nuclear magnetic resonance spectroscopy (^{1}H-MRS) allows the noninvasive measurement of intracellular triglyceride content in skeletal muscle (intramyocellular lipid content or IMCL) and liver (hepatic lipid content or HLC) [11]. Adipose tissue secretes various biologically active molecules, known as *adipokines*, which include adiponectin and retinol binding protein (RBP) 4. Adiponectin functions as an insulin-sensitizing adipokine [12], whereas RBP4 was recently shown to induce insulin resistance in mice [13].

Biguanides and thiazolidinediones are administered clinically to ameliorate insulin resistance associated with type 2 diabetes mellitus, but the precise mechanisms of action of these drugs remain unknown. We have now investigated the effects of the thiazolidinedione pioglitazone and the biguanide metformin on intracellular lipid content in skeletal muscle and liver as well as on the serum concentrations of adiponectin and RBP4 in individuals with type 2 diabetes mellitus.

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2. Subjects and methods

2.1. Subjects

The study subjects included 41 individuals (24 men, 17 women) with type 2 diabetes mellitus diagnosed according to the criteria of the Japanese Society of Diabetes (plasma glucose concentration of ≥7.6 mmol/L after fasting or of ≥11.1 mmol/L apparent 2 hours after an oral 75-g glucose load, confirmed on at least 2 occasions). They had not previously been treated with thiazolidinediones, biguanides, or insulin. All the subjects were Japanese and recruited from patients attending Kobe University Hospital. Individuals with renal failure (serum creatinine concentration of ≥1.5 mg/dL), severe liver dysfunction (aspartate aminotransferase or alanine aminotransferase level of ≥50 IU/mL), or severe heart failure were excluded from the study. Standard lifestyle modifications for type 2 diabetes mellitus, including exercise and dietary changes, had already been adopted by each subject several months before entry into the study and were maintained during the intervention period. Twenty-one subjects were treated with pioglitazone (30 mg/d) and 20 subjects with metformin (750 mg/d, the maximal dose allowed in Japan) for 6 months. Seven patients in the pioglitazone group and 11 patients in the metformin group were being treated with sulfonylureas at the start of the study, and this treatment was continued with no change in dosage during the intervention period. The study was performed with written informed consent from all subjects and was approved by the Ethics Committee of Kobe University Graduate School of Medicine. The basal characteristics of the participants are shown in Table 1.

2.2. Determination of IMCL, HLC, and intra-abdominal fat

Intracellular lipid content in skeletal muscle and liver was determined by ^{1}H-MRS as described previously [14]. In brief, single-voxel ^{1}H spectra were acquired from the soleus muscle with a conventional circumferential extremity coil on a 1.5-T magnetic resonance machine (Signa Echo Speed; GE Yokogawa Medical Systems, Hino, Japan). Volumes of interest were centered within the soleus muscle and positioned to avoid vascular structures and gross adipose tissue deposits. Given that the soleus muscle is composed mostly of slow-twitch oxidative fibers (fiber type 1) and that the triglyceride content of the soleus muscle has been shown to be the best predictor of whole-body insulin sensitivity [2], we considered this muscle to be representative. Localizer images were obtained to position the volume of interest. A point-resolved spectroscopy pulse sequence (repetition time, 3000 milliseconds; echo time, 50 milliseconds) was used, and 64 averages were accumulated with conventional water signal suppression (acquisition time, 252 seconds). The voxel size was 15 × 15 × 15 mm^3. The integrals of the methylene signals at 1.4 and 1.6 ppm were calculated with the National Institutes of Health (NIH) Image software (NIH, Bethesda, MD) to represent IMCL and extramyocellular lipid content, respectively. The integral of the creatine signal at 3.1 ppm served as an internal standard for quantitation of IMCL, which was expressed as arbitrary units (AU) relative to the amount of creatine. The fast spoiled gradient recall

Table 1
Basal characteristics and effects of pioglitazone and metformin treatment in the study subjects

Characteristic	Pioglitazone		*P*	Metformin		*P*	Pioglitazone vs Metformin
	Pre	Post		Pre	Post		*P* *
Sex (M/F)	12/9			12/8			
Age (y)	61.5 ± 11.8			56.7 ± 13.2			
BMI (kg/m^2)	25.5 ± 3.1	25.3 ± 3.0	.4424	26.0 ± 2.9	24.9 ± 2.8	**.0316**	.0849
Fasting plasma glucose (mmol/L)	7.7 ± 1.3	6.6 ± 1.3	**.0014**	7.0 ± 1.3	6.5 ± 1.1	.1449	.1019
Fasting serum insulin (pmol/L)	71.0 ± 43.9	52.0 ± 34.2	**.0135**	58.4 ± 47.4	57.6 ± 30.2	.9156	.0818
Serum total cholesterol (mmol/L)	5.8 ± 0.9	5.7 ± 1.0	.8865	5.5 ± 1.0	5.2 ± 1.1	.1744	.3147
Serum HDL cholesterol (mmol/L)	1.1 ± 0.2	1.3 ± 0.3	**<.0001**	1.1 ± 0.3	1.3 ± 0.4	**.0231**	.9379
Serum triglyceride (mmol/L)	1.8 ± 0.8	1.5 ± 0.6	.0503	2.0 ± 1.0	1.8 ± 1.0	.3347	.4206
Serum adiponectin (μg/mL)	5.6 ± 4.1	16.2 ± 9.9	**<.0001**	5.4 ± 2.3	8.1 ± 4.4	**.0023**	**.0001**
Serum RBP4 (μg/mL)	73.4 ± 25.1	65.1 ± 23.7	**.0289**	72.5 ± 25.2	62.5 ± 29.8	.0624	.8666
HOMA-IR index	4.1 ± 2.5	2.5 ± 1.4	**.0029**	3.0 ± 2.5	2.8 ± 1.6	.6909	**.0468**
SFA (cm^2)	152.7 ± 60.1	164.8 ± 73.2	.0676	153.7 ± 65.6	151.1 ± 65.6	.8307	.2755
VFA (cm^2)	125.5 ± 42.3	125.0 ± 47.0	.8804	102.6 ± 46.1	92.9 ± 43.2	.3187	.3608
VFA/SFA	0.92 ± 0.41	0.85 ± 0.41	**.0246**	0.71 ± 0.25	0.83 ± 1.00	.5660	.3576
HLC (AU)	12.0 ± 6.1	8.4 ± 3.7	**.0014**	9.0 ± 6.3	10.0 ± 5.9	.5015	**.0105**
IMCL (AU/creatine)	8.4 ± 3.6	6.3 ± 2.4	**.0077**	7.0 ± 3.6	5.8 ± 2.0	**.0454**	.3814
Body weight (kg)	66.4 ± 14.0	65.9 ± 13.5	.3325	67.5 ± 11.2	65.4 ± 10.8	**.0265**	.1061
Duration of diabetes (y)	10.2 ± 12.6			7.5 ± 7.8			

Data are means ± SD. There are no significant differences between the 2 groups at baseline. Significant *P* values are shown in bold. HDL indicates high-density lipoprotein; SFA, subcutaneous fat area; VFA, visceral fat area.

* *P* values are for comparison of the effects of treatment (change from baseline) between the 2 groups.