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Metabolism Clinical and Experimental 56 (2007) 1418 – 1424
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

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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Received 5 July 2006; accepted 5 June 2007

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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doi:10.1016/j.metabol.2007.06.005

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.

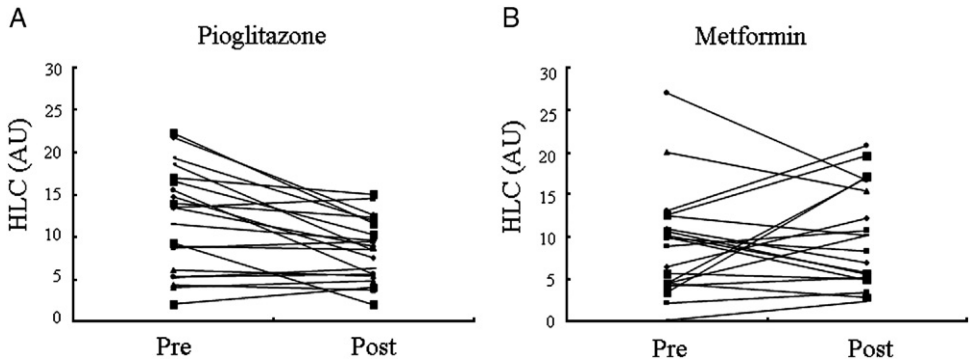

Fig. 1. Effects of treatment with pioglitazone (A) or metformin (B) for 6 months on HLC.

acquisition in the steady-state sequence was used to obtain in-phase and out-of-phase images of liver (flip angle, 75°; repetition time, 120 milliseconds; echo time, 1.8 and 4.2 milliseconds for out-of phase and in-phase images, respectively; matrix, 256 × 160; field of view, 32 × 24; acquisition time, 15 seconds). Hepatic lipid content was determined from the index of the fast spoiled gradient recall acquisition in the steady-state sequence as ([intensities of in-phase] − [intensities of out-of-phase])/([intensities of in-phase] + [intensities of out-of-phase]). During measurements, each subject lay supine within the bore of the magnet [14]. Identical volumes of soleus muscle and liver of each subject were scanned in the pre- and posttreatment MRS analyses with the use of anatomical landmark visualizing images.

A series of T1-weighted transaxial scans for determination of visceral and subcutaneous fat areas was acquired from a region extending from 4 cm above to 4 cm below the fourth and fifth lumbar interspace (16 slices, each with a thickness of 10 mm). Areas of visceral and subcutaneous fat were measured with the NIH Image software. The area of subcutaneous fat was analyzed by selecting the outer and inner margins of subcutaneous adipose tissue of interest from the cross-sectional images and counting the number of pixels between these outer and inner margins. The area of visceral (intra-abdominal) fat was determined from histograms specific to the visceral region. The histograms were summed over the range of pixel values designated as fat.

2.3. Assay of glucose, insulin, adiponectin, and RBP4

Plasma glucose concentration was determined by the glucose oxidase method. Serum insulin concentration was measured with a sandwich enzyme immunoassay system (Tosoh, Tokyo, Japan). Serum total adiponectin level was measured with a sandwich enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical, Tokyo, Japan) as previously described [15]; the interassay coefficient of variation was <10%, but all samples were assayed at the same time. Serum RBP4 level was also measured with a sandwich enzyme-linked immunosorbent assay kit (AssayPro, St Charles, MO) following manufacturer's instructions. The intraassay and interassay coefficients of variation were <2.6% and <5.0%, respectively. Insulin sensitivity was assessed from the homeostasis model assessment of insulin resistance (HOMA-IR) index [16]. Serum levels of total cholesterol, triglyceride, and high-density lipoprotein cholesterol were measured by enzymatic methods with the use of an autoanalyzer (TBA-200FR; Toshiba, Tokyo, Japan).

2.4. Statistical analysis

Data are presented as means ± SD. A paired *t* test was used to compare mean values between pre- and posttreatment with pioglitazone or metformin. Differences between the 2 groups were evaluated by 2-way analysis of variance for repeated measures. The linear relation between variables was assessed by calculation of the Pearson correlation

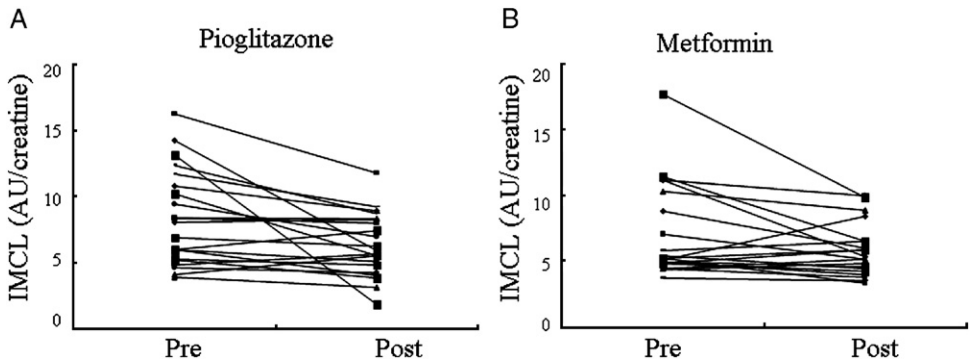

Fig. 2. Effects of treatment with pioglitazone (A) or metformin (B) for 6 months on IMCL.

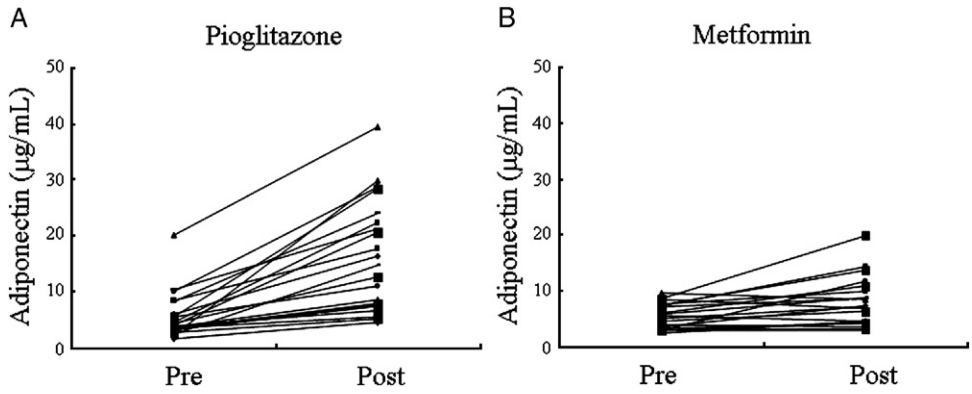

Fig. 3. Effects of treatment with pioglitazone (A) or metformin (B) for 6 months on serum adiponectin concentration.

coefficient. A P value < .05 was considered statistically significant. Statistical analysis was performed with the use of the StatView program (version 5.0J; SAS Institute, Cary, NC).

3. Results

Baseline clinical and laboratory characteristics, including body mass index (BMI), did not differ significantly between the pioglitazone treatment group and the metformin treatment group (Table 1). The BMI in the pioglitazone group did not change significantly during the intervention period, whereas metformin treatment resulted in a significant BMI decrease. The change in BMI during the intervention period however did not differ significantly between the 2 treatment groups.

Although the areas of visceral fat and subcutaneous fat were not significantly affected by treatment with pioglitazone or metformin, pioglitazone induced a significant decrease in the ratio of visceral to subcutaneous fat area. Pioglitazone significantly reduced HLC and IMCL, whereas metformin reduced only IMCL (Table 1, Figs. 1 and 2).

Pioglitazone increased insulin sensitivity as assessed by the HOMA-IR index, whereas this index was not affected by metformin treatment (Table 1). The serum adiponectin concentration was increased by both pioglitazone and metformin, but the effect of pioglitazone was substantially greater than that of metformin (Table 1, Fig. 3). The serum RBP4 concentration was significantly reduced by pioglitazone; although it also tended to be reduced after metformin treatment, this effect was not statistically significant (Table 1, Fig. 4).

For the pioglitazone group, the HOMA-IR index was significantly correlated with the area of visceral fat before treatment ($r = 0.46$, $P < .05$) but not after treatment ($r = 0.05$, $P > .05$), whereas it was inversely correlated with serum adiponectin concentration after treatment ($r = -0.52$, $P < .05$) (Fig. 5A). Hepatic lipid content was inversely correlated with serum adiponectin concentration before ($r = -0.43$, $P < .05$) and after ($r = -0.58$, $P < .01$) treatment (Fig. 5B). The serum concentration of RBP4 was not correlated with the HOMA-IR index or HLC in the pioglitazone group either before or after treatment.

For the metformin group, the HOMA-IR index was significantly correlated with visceral fat area before ($r = 0.65$, $P < .01$) and after ($r = 0.64$, $P < .01$) treatment; but it was not correlated with serum adiponectin concentration after treatment ($r = -0.36$, $P > .05$). Hepatic lipid content was inversely correlated with serum adiponectin concentration

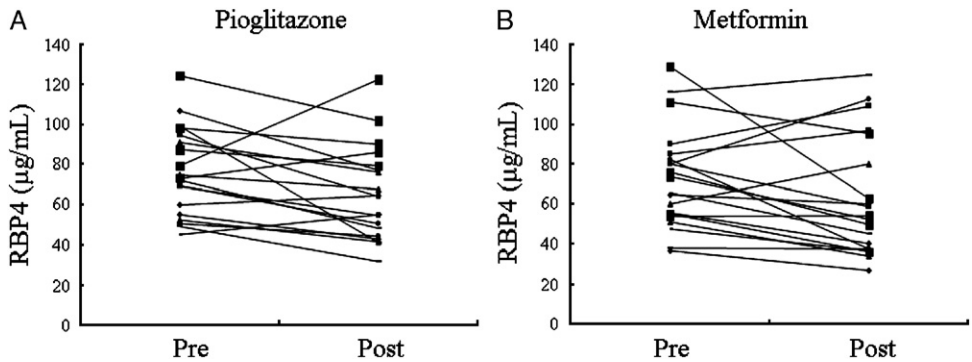

Fig. 4. Effects of treatment with pioglitazone (A) or metformin (B) for 6 months on serum RBP4 concentration.

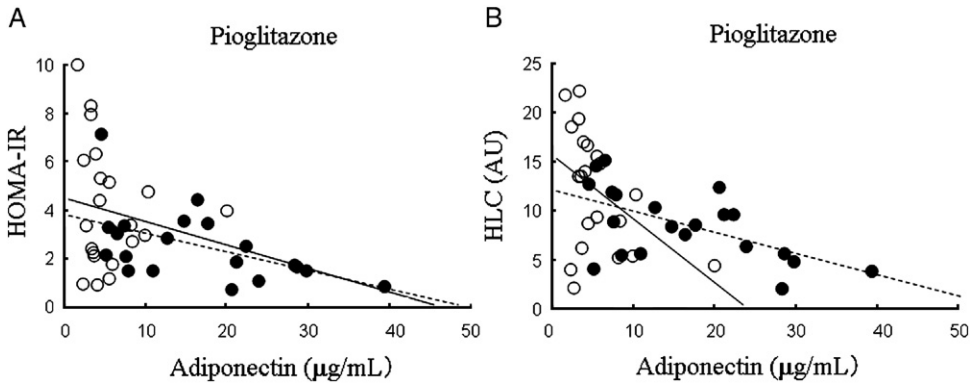

Fig. 5. Relations between serum adiponectin concentration and the HOMA-IR index (A) or HLC (B) in the pioglitazone group. Open circles and solid lines, before pioglitazone treatment; closed circles and dotted lines, after pioglitazone treatment. A, Before pioglitazone treatment: $r = -0.17$, $P > .05$; after pioglitazone treatment: $r = -0.52$, $P < .05$. B, Before pioglitazone treatment: $r = -0.43$, $P < .05$; after pioglitazone treatment: $r = -0.58$, $P < .01$.

before treatment ($r = -0.48$, $P < .05$) but not after treatment. The serum concentration of RBP4 was not correlated with the HOMA-IR index or HLC in the metformin group either before or after treatment.

4. Discussion

We have shown that pioglitazone reduced both IMCL and HLC, whereas metformin reduced only IMCL, in individuals with type 2 diabetes mellitus. As far as we are aware, our study is the first to compare the effects of pioglitazone with those of metformin on intracellular lipid content in both liver and skeletal muscle at the same time. To examine whether sulfonylurea prescribed with pioglitazone or metformin affected these results, we further investigated the differences among pioglitazone or metformin monotherapy groups. In the patients treated with pioglitazone, HLC (12.7 ± 6.1 vs 8.7 ± 3.6 AU, $P < .01$) and IMCL (8.5 ± 3.5 vs 6.9 ± 2.5 AU/creatine, $P < .01$) were still increased significantly even in pioglitazone monotherapy cases (n =7). In the patients treated with metformin, only IMCL was still decreased in metformin monotherapy cases (9.0 ± 4.7 vs 6.1 ± 2.3 AU/creatine, $P < .05$) compared between pre- and posttreatment (n =11).

Thiazolidinediones, which are ligands of the nuclear transcription factor peroxisome proliferator–activated receptor γ, increase insulin sensitivity in peripheral tissues and liver [17]. This effect is generally accompanied by weight gain and an increase in the amount of subcutaneous fat, whereas the amount of visceral fat and the ratio of visceral to subcutaneous fat decrease [18-22]. Thiazolidinediones activate adenosine monophosphate–activated protein kinase (AMPK) and increase the serum concentration of adiponectin, which also activates AMPK in both muscle and liver [23-25]. Phosphorylation of acetyl–coenzyme A (CoA) carboxylase by AMPK results in rapid inhibition of the activity of this enzyme, leading to a decreased tissue content of malonyl-CoA. Malonyl-CoA is an inhibitor of carnitine palmitoyltransferase 1, the rate-limiting enzyme in β-oxidation. The net effect of AMPK activation is therefore the stimulation of fatty acid oxidation in both muscle and liver [26]. Thiazolidinediones may thus reduce IMCL and HLC through activation of AMPK. Indeed, these drugs have previously been shown to increase hepatic insulin sensitivity [27,28] and to reduce HLC in individuals with type 2 diabetes mellitus or nonalcoholic steatohepatitis [21,29-32]. Troglitazone or pioglitazone also reduced IMCL within the vastus lateralis muscle as assessed by muscle biopsy in patients with type 2 diabetes mellitus or individuals with impaired glucose tolerance [33,34].

Metformin is another insulin-sensitizing agent that acts primarily on hepatic glucose production and has additional effects on peripheral insulin sensitivity [35]. Although the precise molecular mechanisms of metformin action remain unclear, this drug activates AMPK in muscle liver [36-38]. Both rosiglitazone and metformin were recently shown to increase AMPK activity in liver, whereas only rosiglitazone reduced the uptake of fatty acids into liver in rats with fatty acid–induced insulin resistance [39]. Metformin was shown to increase hepatic insulin sensitivity without affecting liver fat in patients with type 2 diabetes mellitus [40]. This drug was also found to reduce IMCL within the vastus lateralis muscle in patients with type 2 diabetes mellitus [33] but not in individuals with impaired glucose tolerance [34].

In contrast to thiazolidinediones, metformin has not been found to change the serum concentration of adiponectin in most human studies [28,40-43]. We have now shown that pioglitazone markedly increased the serum adiponectin concentration in our study subjects, whereas the effect of metformin on serum adiponectin was small but significant. In more detail, the serum adiponectin concentration was markedly increased in the pioglitazone treatment group with and also without sulfonylurea, whereas it was increased in the metformin treatment group just only with sulfonylurea. The sulfonylurea prescribed with metformin in all cases was glimepiride, which was previously reported to increase

adiponectin concentration [44,45]. In the metformin treatment group, the small but statistically significant increase in adiponectin concentration (5.4 ± 2.3 vs 8.1 ± 4.4 μg/mL, $P < .01$) might therefore be due to the effect of concomitant glimepiride treatment. We also found that the serum adiponectin concentration was inversely correlated with the HOMA-IR index and HLC after pioglitazone treatment in subjects with type 2 diabetes mellitus. Although the reason why metformin only reduced IMCL in the present study is unclear, it is possible that not only AMPK activation but also a substantial increase in serum adiponectin concentration or a decrease in fatty acid uptake into liver play an important role in the reduction in HLC.

The serum concentration of RBP4 has previously been shown to be increased in individuals with type 2 diabetes mellitus [46,47]. It was also found to correlate with insulin resistance in individuals with obesity, impaired glucose tolerance, or type 2 diabetes mellitus; and physical training resulted in a decrease in the serum RBP4 level only in subjects who showed amelioration of insulin resistance [48]. Rosiglitazone treatment also reduced the increased level of RBP4 messenger RNA in adipose tissue and normalized the increased serum RBP4 concentration in mice deficient in the glucose transporter GLUT4 specifically in adipose tissue [13]. We have now shown that pioglitazone treatment was associated with a small but significant decrease in the serum RBP4 concentration in subjects with type 2 diabetes mellitus. Although the decrease in RBP4 concentration was observed in the overall pioglitazone treatment group, it was not observed in the pioglitazone monotherapy group (73.4 ± 24.8 vs 60.2 ± 27.3 μg/mL, $P > .05$) nor in the pioglitazone and sulfonylurea combination therapy group (81.2 ± 16.8 vs 73.5 ± 13.7 μg/mL, $P > .05$). This lack of statistical significance might due to the smaller sample size. The overall effect was likely due to pioglitazone itself because no change in lifestyle (including exercise) of the study subjects was instituted during the intervention period and a change in RBP4 concentration was not observed in the metformin group. These data suggest that RBP4 may play a role in insulin resistance in individuals with type 2 diabetes mellitus, although the mechanism of its action remains unclear.

In conclusion, our findings suggest that pioglitazone may improve insulin sensitivity both by affecting the serum concentrations of adipokines and by reducing the intracellular lipid content of both liver and skeletal muscle in individuals with type 2 diabetes mellitus.

Acknowledgment

This study was supported in part by a grant for the 21st Century COE Program “Center of Excellence for Signal Transduction Disease: Diabetes Mellitus as Model” from the Ministry of Education, Culture, Sports, Science, and Technology of Japan as well as by a grant from the Cooperative Link of Unique Science and Technology for Economy Revitalization.

References

[1] Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest 2000;106:171-6.
[2] Perseghin G, Scifo P, Cobelli FD, et al. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a ^{1}H-^{13}C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. Diabetes 1999;48:1600-6.
[3] Krssak M, Petersen KF, Dresner A, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ^{1}H NMR spectroscopy study. Diabetologia 1999;42:113-36.
[4] Jacob S, Machann J, Rett K, et al. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. Diabetes 1999;48:1113-9.
[5] Virkamaki A, Korsheninnikova E, Seppala-Lindroos A, et al. Intramyocellular lipid is associated with resistance to in vivo insulin actions on glucose uptake, antilipolysis, and early insulin signaling pathways in human skeletal muscle. Diabetes 2001;50:2337-43.
[6] Banerji MA, Buckley MC, Chaiken RL, et al. Liver fat, serum triglycerides and visceral adipose tissue in insulin-sensitive and insulin-resistant black men with NIDDM. Int J Obes Relat Metab Disord 1995;19:839-40.
[7] Goto T, Onuma T, Takabe K, Kral JG. The influence of fatty liver on insulin clearance and insulin resistance in non-diabetic Japanese subjects. Int J Obes Relat Metab Disord 1995;19:841-5.
[8] Ryysy L, Hakkinen AM, Goto T, et al. Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. Diabetes 2000;49:749-58.
[9] Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab 2002;87:3023-8.
[10] Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease. A feature of metabolic syndrome. Diabetes 2001;50:1844-50.
[11] Szczepaniak LS, Babcock EE, Schick F, et al. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am J Physiol 1999;276:E977-89.
[12] Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev 2005;26:439-51.
[13] Yang Q, Graham TE, Mody N, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436:356-62.
[14] Maeda K, Ishihara K, Miyake K, et al. Inverse correlation between serum adiponectin concentration and hepatic lipid content in Japanese with type 2 diabetes. Metabolism 2005;54:775-80.
[15] Hotta K, Funahashi T, Arita Y, et al. Plasma concentration of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595-9.
[16] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 1985;28:412-9.
[17] Yki-Jarvinen H. Thiazolidinediones. N Engl J Med 2004;351:1106-18.
[18] Miyazaki Y, Glass L, Triplitt C, et al. Effect of rosiglitazone on glucose and non-esterified fatty acid metabolism in type II diabetic patients. Diabetologia 2001;44:2210-9.
[19] Miyazaki M, Mahankali A, Matsuda M, et al. Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. Diabetes Care 2001;24:710-9.
[20] Miyazaki Y, Mahankali A, Matsuda M, et al. Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab 2002;87:2784-91.

[21] Carey DG, Cowin GJ, Galloway GJ, et al. Effect of rosiglitazone on insulin sensitivity and body composition in type 2 diabetic patients. Obes Res 2002;10:1008-15.
[22] Adams M, Montague CT, Prins JB, et al. Activators of peroxisome proliferator–activated receptor–γ have depot-specific effects on human preadipocyte differentiation. J Clin Invest 1997;100:3149-53.
[23] Fryer LG, Parbu-Patel A, Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 2002;277:25226-32.
[24] Saha AK, Avilucea PR, Ye JM, et al. Pioglitazone treatment activates AMP-activated protein kinase in rat liver and adipose tissue in vivo. Biochem Biophys Res Commun 2004;314:580-5.
[25] Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2004;8:1288-95.
[26] Winder WW, Hardie DG. AMP-activated protein kinase, a metabolic master switch: possible role in type 2 diabetes. Am J Physiol 1999;277: E1-E10.
[27] Bajaj M, Suraamornkul S, Pratipanawatr T, et al. Pioglitazone reduces hepatic fat content and augments splanchnic glucose uptake in patients with type 2 diabetes. Diabetes 2003;52:1364-70.
[28] Bajaj M, Suraamornkul S, Piper P, et al. Decreased plasma adiponectin concentrations are closely related to hepatic fat content and hepatic insulin resistance in pioglitazone-treated type 2 diabetic patients. J Clin Endocrinol Metab 2004;89:200-6.
[29] Mayerson AB, Hundal RS, Dufour S, et al. The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. Diabetes 2002;51: 797-802.
[30] Katoh S, Hata S, Matsushima M, et al. Troglitazone prevents the rise in visceral adiposity and improves fatty liver associated with sulfonylurea therapy—a randomized controlled trial. Metabolism 2001;50:414-7.
[31] Caldwell SH, Hespenheide EE, Redick JA, et al. A pilot study of a thiazolidinedione, troglitazone, in nonalcohol steatohepatitis. Am J Gastroenterol 2001;96:519-25.
[32] Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, et al. Interim result of a pilot study demonstrating the early effects of the PPAR-γ ligand rosiglitazone on insulin sensitivity, aminotransferases, hepatic steatosis and body weight in patients with non-alcoholic steatohepatitis. J Hepatol 2003;38:434-40.
[33] Mathieu-Costello O, Kong A, Ciaraldi TP, et al. Regulation of skeletal muscle morphology in type 2 diabetic subjects by troglitazone and metformin: relationship to glucose disposal. Metabolism 2003;52: 540-6.
[34] Rasouli N, Raue U, Miles LM, et al. Pioglitazone improves insulin sensitivity through reduction in muscle lipid and redistribution of lipid into adipose tissue. Am J Physiol Endocrinol Metab 2005;288:E930-4.
[35] Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med 2002;137:25-33.
[36] Zhou G, Myers R, Li Y, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001;108:1167-74.
[37] Musi N, Hirshman MF, Nygren J, et al. Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes 2002;51:2074-81.
[38] Shaw RJ, Lamia KA, Vasquez D, et al. The kinase LKB1 mediates glucose homeostasis in the liver and therapeutic effects of metformin. Science 2005;310:1642-6.
[39] Ye JM, Dzamko N, Cleasby ME, et al. Direct demonstration of lipid sequestration as a mechanism by which rosiglitazone prevents fatty-acid–induced insulin resistance in the rat: comparison with metformin. Diabetologia 2004;47:1306-13.
[40] Tiikkainen M, Häkkinen A-M, Korsheninnikiva E, et al. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. Diabetes 2004;53:2169-76.
[41] Maeda N, Takahashi M, Funahashi T, et al. PPARγ ligands increase expression and plasma concentration of adiponectin, an adipose-derived protein. Diabetes 2001;50:2094-9.
[42] Yu JG, Javorschi S, Hevener AL, et al. The effects of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. Diabetes 2002;51:2968-74.
[43] Phillips SA, Ciaraldi TP, Kong APS, et al. Modulation of circulating and adipose tissue adiponectin levels by antidiabetic therapy. Diabetes 2003;52:667-74.
[44] Tsunekawa T, Hayashi T, Suzuki Y, et al. Plasma adiponectin plays an important role in improving insulin resistance with glimepiride in elderly type 2 diabetic subjects. Diabetes Care 2003;26:285-9.
[45] Fukuen S, Iwaki M, Yasui A, et al. Sulfonylurea agents exhibit peroxisome proliferator–activated receptor gamma agonistic activity. J Biol Chem 2005;25:23653-9.
[46] Basualdo CG, Wein EE, Basu TK. Vitamin A (retinol) status of first nation adults with non–insulin-dependent diabetes mellitus. J Am Coll Nutr 1997;16:39-45.
[47] Abahusain MA, Wright J, Dickerson JWT, de Vol EB. Retinol, alpha-tocopherol and carotenoids in diabetes. Eur J Clin Nutr 1999;53:630-5.
[48] Graham TE, Yang Q, Bluher M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006;354:2552-63.