Insulin Sensitization in Diabetic Rat Liver by an Antihyperglycemic Agent

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This study aimed to demonstrate directly that the thiazolidinedione pioglitazone acts as an insulin sensitizer. We tested the hypothesis that pioglitazone treatment of diabetic rats alters liver function such that responsiveness of selected genes to subsequent insulin regulation is enhanced. Although flux through gluconeogenic/glycolytic pathways involves regulation of many enzymes, we presently report the effects of insulin on expression of two key enzymes in these metabolic pathways, ie, phosphoenolpyruvate carboxykinase (PEPCK) and glucokinase (GK). Rats were either studied as nondiabetic controls or injected with streptozotocin as a model for insulin-deficient diabetes. Diabetic animals were treated without or with pioglitazone and subsequently examined for acute responses to insulin. Pioglitazone treatment of diabetic animals significantly enhanced the effects of insulin to reverse elevated blood glucose. Although the mean level of liver mRNA transcripts encoding PEPCK was increased to nearly 300% in diabetic animals as compared with nondiabetic controls (100%), it was significantly lower in pioglitazone-treated diabetic rats (119% of control) than in diabetic rats without pioglitazone (223% of control) after insulin treatment. By contrast, mRNA transcripts encoding GK were not detectable in diabetic animals, but were increased markedly by insulin treatment in all animal groups. Insulin-enhanced expression of GK was significantly greater in liver from animals that were treated earlier with pioglitazone (291% of control) than in liver from those that were untreated (214% of control). An amplified acute response of liver to insulin thus established pioglitazone as an insulin sensitizer. Our findings further showed that such sensitization can be developed even in the insulin-deficient state. These observations underscore the potential for agents with this action to reverse the insulin-resistant state characteristic of non–insulin-dependent diabetes.

NON–INSULIN-DEPENDENT diabetes mellitus is characterized by two separate abnormalities. The first is aberrant pancreatic insulin secretion. The second is insulin resistance in peripheral tissues, ie, reduced glucose uptake, particularly in muscle, and overproduction of glucose in liver.[1-6] The order of onset for such derangements during development of diabetes remains controversial,[7-14] but the overt diabetic condition is only apparent when pancreatic and peripheral defects coexist. With severe fasting hyperglycemia, the ability of insulin to suppress hepatic glucose output becomes impaired[5,7]; such liver insulin resistance may thus exacerbate the diabetic condition.

Since hepatic glucose overproduction is a key abnormality in diabetes, it is important to identify characteristic molecular alterations in the liver that may underlie the condition and to develop strategies to compensate for such changes. To this end, we recently described the actions of pioglitazone, a treatment agent known to decrease blood glucose, on the activity and expression of the major rate-limiting enzyme for liver gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK).[15] Our results showed that PEPCK activity and mRNA abundance were concordantly elevated twofold in insulin-resistant obese mice and returned to normal when animals were treated with pioglitazone. Since this therapy led to decreased hepatic gluconeogenesis while insulin levels were also decreased, it appeared that pioglitazone acted to restore sensitivity to insulin's inhibitory actions on PEPCK transcription.[15,16] Also, with insulin infusion in fatty rats, hepatic glucose production was decreased threefold in pioglitazone-treated animals as compared with untreated controls.[17] Further, pioglitazone-like agents only reduced hepatic glucose output in insulin-deficient diabetic rats when insulin levels were at least partially restored at the same time.[18] Together, these studies support the concept that pioglitazone and related analogs act as insulin sensitizers in liver.

We aim now to demonstrate directly that pioglitazone acts as an insulin sensitizer. We tested the hypothesis that pioglitazone treatment of insulin-deficient diabetic animals alters liver function, which results in enhanced regulation of liver enzymes in response to subsequent insulin injection. For such experiments, we investigated insulin-regulated expression of two enzymes in the gluconeogenic and glycolyic pathways. Specifically, we measured the abundance of mRNAs that encode PEPCK and glucokinase (GK). Since both PEPCK and GK are reported to be regulated by insulin at the transcriptional level,[19] steady-state levels of their mRNAs should generally reflect gene expression.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (160 to 180 g) were purchased from Charles River Laboratories (Portage, MI) and were housed individually in shoebox cages and provided food and water ad libitum. Rats were injected in the tail vein with streptozotocin (65 mg/kg body weight) as an experimental model for insulin-deficient diabetes (type I). Diabetic animals were allowed to recover for 7 days, and were then grouped randomly for further treatment without or with pioglitazone for 4 days (at a dose of 20 mg/kg/d in chow). Other rats were maintained similarly for use as nondiabetic controls.

Experimental Design

On day 5 from the start of drug treatment, animals in each group (n = 12 per group) were compared for acute response to insulin

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(intravenous regular porcine insulin 10 to 40 U/kg body weight); designated control animals were injected with saline only. At indicated times (2 to 4 hours) animals were lightly anesthetized with ethyl ether for blood sampling from the orbital sinus for determinations of blood glucose and serum insulin levels. Samples of liver tissue were then excised and frozen quickly on dry ice for later RNA extraction. Animal and tissue handling procedures were reviewed and approved by animal use protocol committees at The Upjohn Company and the Edward J. Hines, Jr, Veterans Administration Hospital.

Glucose and Insulin Determinations

Blood glucose levels were determined with an Alpkem Glucose Analyzer (Alpkem, Clackamus, OR), and insulin levels were determined by radioimmunoassay.[20]

Northern Blot Analysis of RNA

Total RNA was extracted from liver tissue samples essentially according to the method reported by Chomczynski and Sacchi.[21] RNA was then size-fractionated on 1% agarose gels and transferred to nylon membranes according to the methods reported previously.[15] RNA was cross-linked to membranes with a UV Stratalinker (Stratagene, La Jolla, CA) and then hybridized to antisense riboprobes prepared using the supplier's protocol (Promega, Madison, WI). Riboprobes for PEPCK were prepared using an 846–base pair *Eco*RI/*Ava*I–excised fragment of PC116[22] (generously supplied by Dr Daryl Granner, Vanderbilt University, Nashville, TN) subcloned into pGEM-3Z with the 5′ end adjacent to the SP6 promoter. The construct cGK.Z1A[23] (supplied by Dr Daryl Granner) represents a 2.36-kb insert for rat GK that was cloned into the *Eco* RI site of pBluescript SK$^-$ and was used to prepare GK Riboprobes with RNA polymerase T3. Hybridization and rinse conditions were described previously, as were procedures for signal quantification and correction for minor RNA loading differences.[24]

Statistics

Differences between groups were analyzed by one- or two-way ANOVA, and differences between pairs of groups were analyzed by the multiple-comparison tests of Tukey or Student-Neuman-Keuls. Results were expressed as the mean ± SEM, and differences were considered significant at P less than .05.

RESULTS

For these experiments, rats were evaluated as nondiabetic controls or injected in the tail vein with streptozotocin as a model for insulin-deficient diabetes. Diabetic animals were grouped randomly for 4-day treatment without or with pioglitazone, and on day 5 animals in each group were compared for acute response to injected insulin (intravenous regular porcine 40 U/kg). Two hours after insulin injection, blood samples were obtained for measurement of glucose levels. Although insulin injection decreased blood glucose levels significantly in all animal groups, it was of particular note that pioglitazone treatment of diabetic animals significantly enhanced the effects of insulin to decrease blood glucose acutely (untreated *v* treated, $P < .05$), as shown in Fig 1.

Two hours after insulin injection, liver samples were taken for extraction of total RNA. Subsequent determinations of mRNA transcripts encoding the liver enzymes PEPCK and GK were performed by Northern blotting and hybridization. For each animal group, samples of ethidium bromide–stained RNA are shown to represent its intactness, ie, approximate 2:1 ratio of 28S:18S ribosomal RNA, as well as the consistency of loading (Fig 2A). Intensity of the 18S signal was used to correct for minor loading differences as described earlier. RNA message transcripts of 3.0-kb size that encode the gluconeogenic enzyme PEPCK were shown by high-stringency hybridization to be markedly overexpressed in the diabetic condition, and were generally decreased by insulin treatment in each of the animal groups (Fig 2B). Transcripts encoding the glycolytic enzyme GK (2.4 kb) were detected in nondiabetic control rats, but could not be detected in streptozotocin-diabetic animals; expression of GK transcripts was induced by insulin treatment in each of the animal groups (Fig 2C).

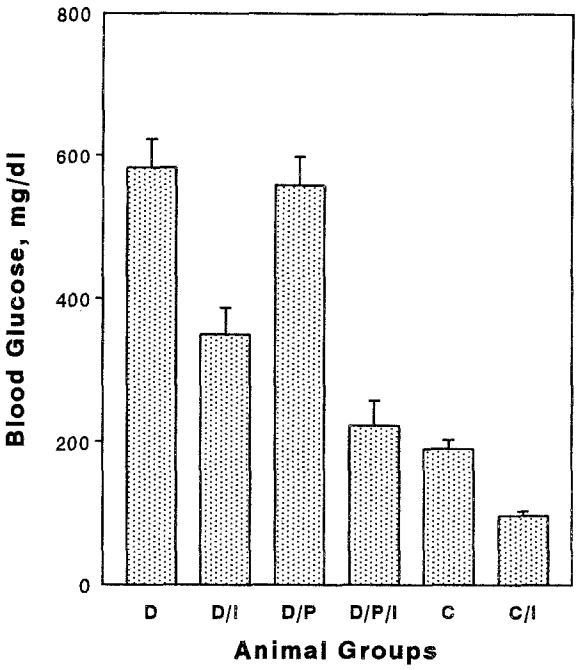

Fig 1. Pioglitazone effects on blood glucose levels. Blood glucose levels were determined 2 hours after insulin injection. D, diabetic rats; D/I, insulin-injected diabetic; D/P, pioglitazone-treated diabetic; D/P/I, insulin-injected, pioglitazone-treated diabetic; C, control nondiabetic; C/I, insulin-injected control. As expected, blood glucose levels were decreased significantly by insulin in all animal groups (D *v* D/I, D/P *v* D/P/I, C *v* C/I: $P < .05$). Treatment of streptozotocin-diabetic animals with pioglitazone significantly enhanced the action of insulin to decrease blood glucose (D/I *v* D/P/I, $P < .05$). By contrast, diabetic animals treated only with pioglitazone showed no correction of elevated blood glucose levels (D/P *v* D, P = NS).

Quantification of mRNA transcript signals for PEPCK and GK showed that pioglitazone augmented insulin's effects on gene expression. The mean level of mRNA encoding PEPCK was elevated to nearly 300% in untreated diabetic rats as compared with nondiabetic rats (100%). After insulin injection, the PEPCK mRNA level was significantly lower in pioglitazone-treated diabetic animals (119% of control) than in diabetic animals without such pioglitazone treatment (223% of control; untreated *v* treated, $P < .05$), as shown in Fig 3A. By contrast, mRNA transcripts encoding GK were not detectable in diabetic rats (with or without pioglitazone treatment), but abun-

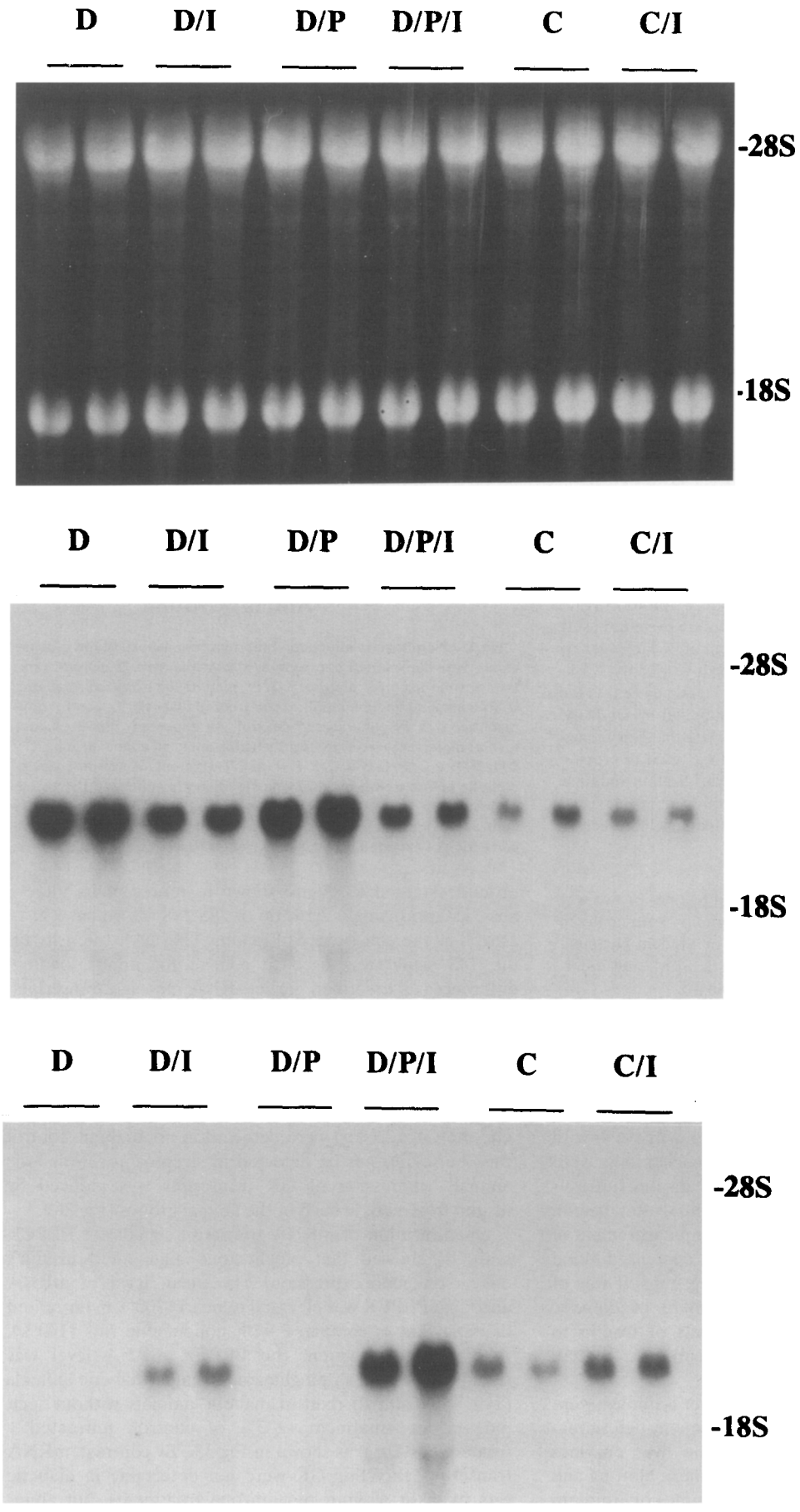

Fig 2. Representative Northern blots for analysis of RNA. Two hours after insulin injection, liver samples were removed and RNA was extracted for determination of levels of mRNA encoding PEPCK and GK. (A) Ethidium bromide–stained gel showing even loading of samples. (B) Size-fractionated total RNA (10 μg per lane) from individual animals was probed for abundance of transcripts encoding PEPCK; 2 representative samples are shown for each of the 6 animal groups indicated previously. (C) Size-fractionated RNA was probed for abundance of transcripts encoding GK. Abbreviations as in Fig 1.

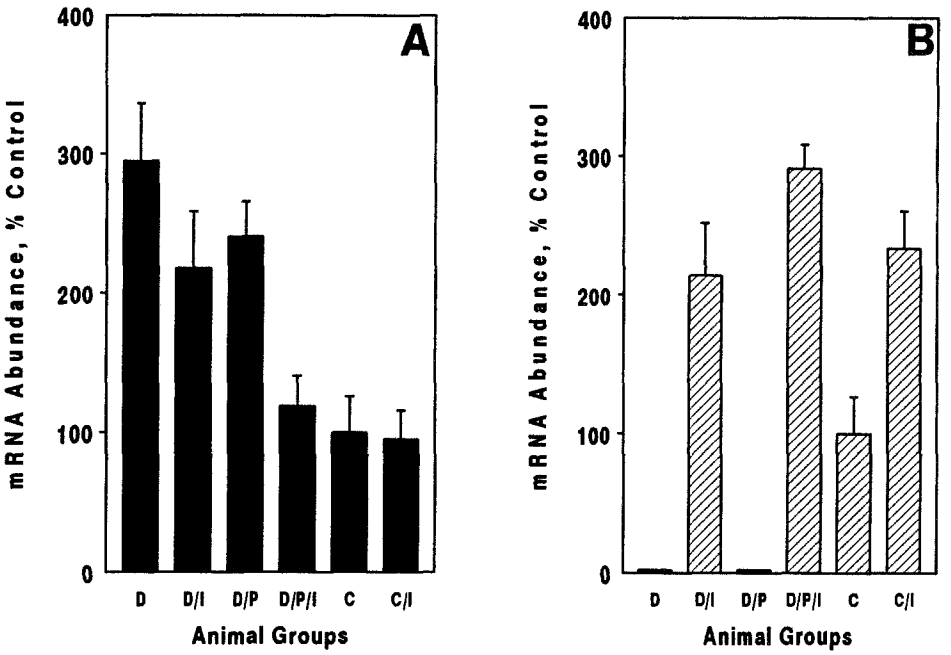

Fig 3. Enhanced responses to acute insulin injection in diabetic rats pretreated with pioglitazone as compared with rats not pioglitazone-treated. Two hours after insulin injection, liver samples were removed and RNA extracted for measurement of mRNA transcripts encoding PEPCK and GK (n = 12 animals per group). (A) Mean levels of liver transcripts encoding PEPCK 2 hours after insulin injection. D *v* C, $P < .05$; D *v* D/I, $P < .05$; D/I *v* D/P/I, $P < .05$. (B) Mean levels of liver transcripts encoding GK 2 hours after insulin injection. C *v* C/I, $P < .05$; D/I *v* D/P/I, $P < .05$. Abbreviations as in Fig 1.

dance of these transcripts was increased markedly by insulin injection in all animal groups (Fig 3B). Insulin-enhanced expression of GK was significantly greater in liver from animals that were treated earlier with pioglitazone (291% of control) than from those that were not treated (214% of control; untreated *v* treated, $P < .05$). Such GK expression was similar to the level achieved in nondiabetic control rats in response to acute insulin injection (234% of untreated control; control *v* insulin-treated, $P < .05$). Using a transcriptional run-on assay essentially as previously described,[25] we confirmed that both GK and PEPCK were regulated at the transcriptional level (data not shown).

The sensitizing effects of pioglitazone were underscored by showing the effects on GK mRNA abundance with increasing doses of insulin and with time after insulin injection (Tables 1 and 2). For such studies, abundance of GK transcripts induced by insulin injection at different doses was higher in animals that had been treated for 4 days with pioglitazone than in those that did not receive such treatment ($P = .05$) and tended to be higher in response to a single insulin dose over time (P = NS). Although we showed an augmented decrease of PEPCK mRNA abundance in response to insulin at the 2-hour time point for pioglitazone-treated animals (Fig 3), the complete time course (2 to 4 hours) was somewhat variable since measurable changes in mRNA from sample to sample resulted from both decreased transcription and degradation of existing RNA transcripts (data not shown).

DISCUSSION

In diabetes, elevated blood glucose is a consequence of increased hepatic glucose output in concert with reduced peripheral glucose utilization. Glucose output is largely derived from increased gluconeogenesis, and expression of the gluconeogenic enzyme PEPCK is normally inhibited by insulin. Liver glucose utilization occurs via glycolysis, and expression of the key glycolytic enzyme GK is normally stimulated by insulin.

In prior studies, we demonstrated that overexpression of

Table 1. GK mRNA Abundance (mean arbitrary density units ± SEM) in Response to Varying Doses of Insulin 2 Hours After Injection

Insulin Dose (U/kg)	Diabetic Liver GK mRNA	
	Controls	Pioglitazone-Treated
0	0.12 ± 0.06	0.15 ± 0.04
10	1.04 ± 0.39	1.99 ± 0.24
20	1.16 ± 0.42	1.58 ± 0.35
40	1.47 ± 0.40	2.70 ± 0.51

NOTE. Streptozotocin-induced diabetic rats were randomly grouped (n = 6 animals per group) as untreated controls or those receiving treatment with pioglitazone (20 mg/kg/d for 4 days), and abundance of mRNA transcripts encoding GK in liver was determined. By 2-way ANOVA and Tukey's post hoc procedures, the group treated with pioglitazone showed significantly higher GK mRNA abundance than the untreated group ($P = .05$).

Table 2. Time Course for Changes in GK mRNA Abundance (mean arbitrary density units ± SEM) in Response to Insulin (20 U/kg) Injection

Time After Insulin (h)	Diabetic Liver GK mRNA	
	Controls	Pioglitazone-Treated
0	0.11 ± 0.06	0.16 ± 0.08
2	1.65 ± 0.24	2.24 ± 0.44
3	4.09 ± 0.69	5.32 ± 0.80
4	3.78 ± 0.34	3.53 ± 0.69

NOTE. Streptozotocin-induced diabetic rats were randomly grouped (n = 6 animals per group) as untreated controls or those receiving treatment with pioglitazone (20 mg/kg/d for 4 days). Abundance of mRNA transcripts encoding GK in liver was determined at 2, 3, or 4 hours after injection of insulin (20 U/kg). Levels of GK transcripts tended to be higher in the pioglitazone-treated group, but differences did not reach statistical significance.

PEPCK in insulin-resistant diabetic animals could be corrected by treatment with pioglitazone, an antihyperglycemic agent.[15] Since the high levels of insulin in these diabetic animals were considerably decreased by pioglitazone treatment, we deduced that the agent was likely acting as an insulin sensitizer. The aim of the present study was to demonstrate directly such sensitization. In these experiments, we used hyperglycemic, insulin-deficient diabetic rats in which we observed that liver PEPCK was highly overexpressed and GK was markedly underexpressed. We showed that treatment of insulin-deficient diabetic rats with the thiazolidinedione compound pioglitazone improved blood glucose–lowering actions, as well as liver responsiveness to subsequent insulin treatment. Specifically, elevated liver PEPCK mRNA abundance in diabetic animals was decreased and diminished GK mRNA was restored in response to acute insulin treatment. We reported briefly that both PEPCK and GK were regulated at the transcriptional level. Such findings are consistent with prior reports of transcriptional regulation of these genes by insulin, although regulation by increasing mRNA stability is also possible.[19] The key observation in our study is that the improvements in PEPCK and GK expression were augmented in animals that received prior treatment with pioglitazone. Taken together, we showed that sensitization by pioglitazone could be developed even in the insulin-deficient state that preceded acute insulin replacement. Such findings provide strong support for the concept that pioglitazone acts as an insulin sensitizer.

Additional evidence is likewise consistent with the ability of thiazolidinediones to augment insulin responses. This relatively new class of drugs was discovered empirically by observation of antihyperglycemic effects in animal models of non–insulin-dependent diabetes mellitus. In obese rats and mice, in which insulin resistance is a conspicuous metabolic defect, analogs of this class decreased blood glucose and triglyceride levels.[26] Metabolic improvements occurred in concordance with decreased insulin levels, and the compounds were found to be inactive in the absence of insulin.[26,27] Therefore, it appeared that the principal effect was an improvement of peripheral target-tissue responses to insulin. Since such changes were not consistently accompanied by significant increases in either the number or affinity of insulin receptor-binding sites, the metabolic improvements were likely due to enhanced postbinding events' mediating insulin's actions.[27-30]

Although the precise molecular mechanisms underlying the insulin-sensitizing actions of pioglitazone are not yet understood, candidate mechanisms have been described. Kobayashi et al[30,31] recently reported that pioglitazone administration in vivo reversed the decreased tyrosine kinase activity of skeletal muscle insulin receptors in both Wistar fatty rats and high-fat–fed rats. Since hyperglycemia, hyperinsulinemia, and hyperlipidemia were also improved, it was unclear whether such pioglitazone effects on receptor function were direct or indirect. However, in vitro studies demonstrated that pioglitazone could directly ameliorate high glucose–induced desensitization of insulin receptor kinase in Rat 1 fibroblasts.[32] An additional mechanism has been proposed whereby pioglitazone induces expression of aFABP.[33] This protein is abundant in adipocytes and is a target for insulin receptor tyrosine kinase; it may thus play an intermediary role in insulin signal transmission.[34] Further studies will be necessary to understand fully the compensatory actions of pioglitazone and related agents on insulin's signaling pathways.

In summary, this study demonstrated that treatment of streptozotocin-diabetic rats with the thiazolidinedione agent pioglitazone leads to increased sensitivity to insulin for regulated expression of enzymes that are key to gluconeogenesis and glucose utilization in liver. This so-called sensitization is key to restoring insulin's normal inhibition of excessive hepatic glucose production. Overall, understanding such actions is important to facilitate treatment of the insulin-resistant condition that predominates in type II diabetes and also occurs in type I diabetes.[35,36]

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REFERENCES

1. Taylor R: Aetiology of non-insulin dependent diabetes. Br Med Bull 45:73-91, 1989

2. Kahn SE, Porte D: Islet dysfunction in non–insulin-dependent diabetes mellitus. Am J Med 85:4-8, 1988

3. DeFronzo RA: The triumvirate: Beta cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 37:667-687, 1988

4. Olefsky JM, Garvey WT, Henry RR, et al: Cellular mechanisms of insulin resistance in non–insulin-dependent (type II) diabetes. Am J Med 85:86-105, 1988

5. Kolterman OG, Gray RS, Griffen J, et al: Receptor and post-receptor defects contribute to the insulin resistance in noninsulin-dependent diabetes mellitus. J Clin Invest 68:967-969, 1981

6. Reaven GM: Insulin secretion and insulin action in non–insulin-dependent diabetes mellitus: Which defect is primary? Diabetes Care 7:17-24, 1984

7. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: A balanced overview. Diabetes Care 15:318-353, 1992

8. Martin BC, Warram JH, Krolewski AS, et al: Role of glucose and insulin resistance in development of type 2 diabetes mellitus: Results of a 25-year follow-up study. Lancet 340:925-929, 1992

9. Lillioja S, Mott DM, Spraul M, et al: Insulin resistance and insulin secretory dysfunction as precursors of non–insulin-dependent diabetes mellitus. N Engl J Med 329:1988-1992, 1993

10. Gulli G, Ferrannini E, Stern M, et al: The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two Mexican-American NIDDM parents. Diabetes 41:1575-1586, 1992

11. Warram JH, Martin BC, Krolewski AS, et al: Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. Ann Intern Med 113:909-913, 1990

12. Turner RC, Matthews DR, Clark A, et al: Pathogenesis of NIDDM—A disease of deficient insulin secretion. Bailliere's Clin Endocrinol Metab 2:327-342, 1988

13. O'Rahilly SP, Nugent Z, Rudenski AS: Beta cell dysfunction, rather than insulin insensitivity, is the primary defect in familial type 2 diabetes. Lancet 2:360-364, 1986

14. Unger RH: GLUT2 and the pathogenesis of type II diabetes. Diabetes Nutr Metab 5:65-69, 1992

15. Hofmann CA, Edwards CWI, Hillman RM, et al: Treatment of insulin-resistant mice with the oral antidiabetic agent pioglitazone: Evaluation of liver GLUT2 and phosphoenolpyruvate carboxykinase expression. Endocrinology 130:735-740, 1992

16. O'Brien RM, Granner DK: PEPCK gene as model of inhibitory effects of insulin on gene transcription. Diabetes Care 13:327-339, 1990

17. Ikeda H, Taketomi S, Sugiyama Y, et al: Effects of pioglitazone on glucose and lipid metabolism in normal and insulin resistant animals. Arzneimittelforschung 40:156-160, 1990

18. Tominga M, Igarashi M, Daimon M, et al: Thiazolidinediones (AD-4833 and CS-045) improve hepatic insulin resistance in streptozotocin-induced diabetic rats. Endocrine J 40:343-349, 1993

19. Pilkis SJ, Granner DK: Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. Annu Rev Physiol 54:885-909, 1992

20. Zaharto DS, Beck LV: Studies of a simplified plasma insulin immunoassay using cellulose powder. Diabetes 17:444-457, 1968

21. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159, 1987

22. Beale EG, Chrapkiewicz NB, Scoble HA, et al: Rat hepatic cytosolic phosphoenolpyruvate carboxykinase (GTP). J Biol Chem 260:10748-10760, 1985

23. Magnuson MA, Andreone TL, Printz RL, et al: Rat glucokinase gene: Structure and regulation by insulin. Proc Natl Acad Sci USA 86:4838-4842, 1989

24. Hofmann C, Lorenz K, Braithwaite SS, et al: Altered gene expression for tumor necrosis factor-alpha and its receptors during drug and dietary modulation of insulin resistance. Endocrinology 134:264-270, 1994

25. Schibler U, Hagenbuchle O, Wellauer PK, et al: Two promoters of different strengths control the transcription of the mouse alpha-amylase gene Amy-1 in the parotid gland and the liver. Cell 33:501-508, 1983

26. Colca JR, Morton DR: Antihyperglycaemic thiazolidinediones: Ciglitazone and its analogues, in Bailey CJ, Flatt PR (eds): New Antidiabetic Drugs. London, UK, Smith-Gordon, 1990, pp 255-261

27. Hofmann CA, Colca JR: New oral thiazolidinedione antidiabetic agents act as insulin-sensitizers. Diabetes Care 15:1075-1078, 1992

28. Sugiyama Y, Taketomi S, Shimura Y, et al: Effects of pioglitazone on glucose and lipid metabolism in Wistar fatty rats. Arzneim:Helforschung 40:263-268, 1990

29. Chang AY, Wyse BM, Gilchrist BJ: Ciglitazone, a new hypoglycemic agent. II. Effect on glucose and lipid metabolisms and insulin binding in the adipose tissue of C57BL/6J-ob/ob and −+/? mice. Diabetes 32:839-845, 1983

30. Iwanishi M, Kobayashi M: Effect of pioglitazone on insulin receptors of rat skeletal muscles from high-fat–fed rats. Metabolism 42:1017-1021, 1993

31. Kobayashi M, Iwanishi M, Egawa K, et al: Pioglitazone increases insulin sensitivity by activating insulin receptor kinase. Diabetes 41:476-483, 1992

32. Maegawa H, Tachikawa-Ide R, Ugi S, et al: Pioglitazone ameliorates high glucose induced desensitization of insulin receptor kinase in Rat 1 fibroblasts in culture. Biochem Biophys Res Commun 197:1078-1082, 1994

33. Kletzien RF, Foellmi LA, Harris PKW, et al: Adipocyte fatty acid–binding protein: Regulation of gene expression in vivo and in vitro by an insulin-sensitizing agent. Mol Pharmacol 12:558-562, 1992

34. Hresko RC, Hoffman RD, Flores-Riveros JR, et al: Insulin receptor tyrosine kinase–catalyzed phosphorylation of 422 (aP2) protein. Substrate activation by long chain fatty acid. J Biol Chem 265:21075-21085, 1990

35. Yki-Jarvinen H, Koivisto VA: Continuous subcutaneous insulin infusion therapy decreases insulin resistance in type I diabetes. J Clin Endocrinol Metab 58:659-666, 1984

36. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988