Tissue-Specific Actions of Antidiabetic Thiazolidinediones on the Reduced Fatty Acid Oxidation in Skeletal Muscle and Liver of Zucker Diabetic Fatty Rats

Tomohiro Ide, Tomoko Nakazawa, Toshiro Mochizuki, and Koji Murakami

Fatty acid overload has been proposed as a cause of decreased responsiveness in the major insulin target tissues of the body such as muscle and liver tissue. We therefore investigated fatty acid oxidation in soleus muscle and liver isolated from Zucker diabetic fatty (ZDF) rats treated with thiazolidinediones, a new class of antidiabetic agents. $^{14}CO_2$ production from [^{14}C]palmitic (C16:0) acid was lower in the soleus muscle and liver of ZDF rats versus lean rats ($P < .05$). When administered orally to ZDF rats for 2 weeks, the thiazolidinediones troglitazone (300 mg/kg) and KRP-297 (10 mg/kg) increased palmitic acid oxidation in the soleus muscle of ZDF rats ($P < .05$). KRP-297, but not troglitazone, increased palmitic acid oxidation in the liver of ZDF rats ($P < .05$), and both troglitazone and KRP-297 inhibited triglyceride accumulation in the skeletal muscle of ZDF rats. Hepatic triglyceride accumulation in ZDF rats was inhibited by KRP-297, but not by troglitazone. A reduction of fatty acid oxidation in the liver of ZDF rats and an increase in response to KRP-297 were observed only when C16:0 and C18:0 fatty acids, not C8:0, were used as substrates. Thus, there were defects in fatty acid catabolic activity and triglyceride accumulation in the soleus muscle and liver of ZDF rats. These results indicate that KRP-297 has advantages over troglitazone in the amelioration of these lipid metabolic abnormalities in insulin resistance associated with obesity.

HYPERLIPIDEMIA is proposed as a cause of insulin resistance in non–insulin-dependent diabetes mellitus (NIDDM) associated with obesity.[1] Hyperglycemia is often preceded by hyperlipidemia and hyperinsulinemia in NIDDM patients and animal models of NIDDM associated with obesity.[2-4] The skeletal muscle and liver are major insulin-resistant tissues in the body. Lipid/heparin infusion, which elevates plasma free fatty acid levels, can induce defects in insulin-stimulated glucose uptake in skeletal muscle and insulin-suppressed glucose output in the liver of rats and humans.[5-9] The mechanism by which an elevation of plasma fatty acids induces skeletal muscle insulin resistance remains unclear, but may include an increase in cellular lipid levels.[9,10] In any event, the fatty acid catabolic activity in insulin-resistant tissues, such as skeletal muscle and liver is still poorly understood.

Thiazolidinediones, a new class of antidiabetic agents, are known to enhance insulin sensitivity in the skeletal muscle and liver of animals and patients with NIDDM.[11,12] Thiazolidinediones improve hyperlipidemia, as well as hyperglycemia and hyperinsulinemia. Although the mechanism by which thiazolidinediones improve insulin resistance has not been established, a peroxisome proliferator–activated receptor γ (PPARγ) was recently identified as a molecular target of these agents.[13,14] On the other hand, we recently reported a new thiazolidinedione, KRP-297, ie, (±)-5-[(2,4-dioxothiazolidin-5yl)methyl]-2-methoxy-*N*-[[4-(trifluoromethyl)phenyl[methyl] benzamide, that functions as a dual agonist of both PPARα and PPARγ.[15,16] This compound was also effective in improving the impaired glucose uptake in skeletal muscle of insulin-resistant animals.[17] PPARs are orphan members of the nuclear receptor superfamily and are known to regulate the expression of numerous genes involved in lipid metabolism and adipocyte differentiation.[18,19] PPARα has been shown to be expressed abundantly in tissues with high lipid catabolic activity such as the liver, skeletal muscle, kidney, heart, and brown adipose tissue.[20,21] PPARγ2 is expressed exclusively in adipose tissue, while PPARγ1 is expressed ubiquitously.[20,22] Thus, PPARα and PPARγ isoforms appear to function as regulators of lipid metabolism in a tissue-specific fashion.

To better characterize the effect of thiazolidinediones on insulin resistance, we measured lipid oxidation in liver and muscle tissue from insulin-resistant Zucker diabetic fatty (ZDF) rats treated with troglitazone or KRP-297. We report the tissue-specific actions of thiazolidinediones on reduced fatty acid oxidation and triglyceride accumulation in skeletal muscle and liver of ZDF rats.

MATERIALS AND METHODS

Animals

Male ZDF/Gmi-*fa/fa* rats and lean littermates (+/?) were obtained from Genetic Models International (Indianapolis, IN). All rats received a standard diet (Japan Clea, Tokyo) and tap water ad libitum. All institutional guidelines for animal care and use were applied in this study. ZDF and lean rats (n = 5) were 6 weeks old at the start of treatment. KRP-297 (10 mg/kg), troglitazone (300 mg/kg), or vehicle (0.5% gum arabic solution) were administered orally for 2 weeks. At the end of the treatment period, plasma samples were collected and the soleus muscle and liver were removed.

Fatty Acid Oxidation in Muscle and Liver

Measurement of $^{14}CO_2$ from [1-^{14}C]-octanoate, [1-^{14}C]-palmitic acid, or [1-^{14}C]-stearic acid was performed as previously described.[23] Soleus muscles were isolated from the hindlimbs, and the tendon of each was ligated with silk surgical thread and attached to a plastic holder. The muscles (84 to 123 mg) were placed in a glass vial containing 1 mL Krebs-Ringer phosphate buffer (pH 7.4) supplemented with 0.2% bovine serum albumin and 0.1 mmol/L palmitic acid (1 mCi/mL[1-^{14}C]-palmitic acid). Liver slices (81 to 183 mg) were also placed in glass vials containing 1 mL Krebs-Ringer phosphate buffer (pH 7.4) supplemented with 0.2% bovine serum albumin and 0.2 mmol/L octanoate (1 mCi/mL [1-^{14}C]-octanoate), 0.2 mmol/L palmitic acid (1 mCi/mL [1-^{14}C]-palmitic acid), and 0.2 mmol/L stearic acid (1 mCi/mL [1-^{14}C]-stearic acid). The tube was gassed with O_2:CO_2 (95%:5%), connected via a thick rubber tube to a scintillation vial with a circular glass filter

From the Central Research Laboratories, Kyorin Pharmaceutical, Tochigi, Japan.

Submitted July 1, 1999; accepted August 31, 1999.

Address reprint requests to Tomohiro Ide, Central Research Laboratories, Kyorin Pharmaceutical, 2399-1 Nogi-machi, Shimotsuga-gun, Tochigi 329-0114.

0026-0495/00/4904-0003$10.00/0

saturated with Soluen-350 (Packard Instrument, Meriden, CT), and incubated at 37°C in a metabolic shaker for 2 hours. The reaction was terminated by injecting 1 mL 8% $HClO_4$ through the rubber tube with a needle, and each tube was then incubated for an additional 1 hour to absorb the $^{14}CO_2$ into Soluen-350. $^{14}CO_2$ radioactivity in the glass filter was counted by adding 10 mL ACS II (Amersham, Buckinghamshire, UK) in a liquid scintillation counter (Packard Instrument).

Tissue Triglycerides

The muscle and liver were dissected and placed in liquid nitrogen, and these tissue samples were stored at −80°C until assay. The tissues were homogenized with chloroform:methanol (2:1, vol/vol), and the lipids were extracted. The triglyceride content was determined enzymatically using a commercial kit (Roche Diagnostics, Tokyo, Japan).

Plasma Assays

Plasma glucose was determined with a glucose oxidase–based method, the glucose B-test (Wako Pure Chemical industries, Osaka, Japan). Plasma free fatty acid levels were determined using an acyl-coenzyme A (CoA) oxidase–based method, the NEFA-C test (Wako Pure Chemical Industries). Plasma insulin and triglyceride levels were determined by insulin immunoassay (Morinaga Institute of Biological Science, Yokohama, Japan, and Kyowa Medex, Shizuoka, Japan).

Chemicals

KRP-297 and troglitazone were synthesized by Kyorin Pharmaceutical (Tochigi, Japan). [1-^{14}C]-octanoate, [1-^{14}C]-palmitic acid, and [1-^{14}C]-stearic acid were obtained from New England Nuclear (Boston, MA).

Statistical Analysis

All results are presented as the mean ± SE. Significant differences in mean values between lean and ZDF control rats were assessed using the unpaired Student's *t* test. The statistical significance of differences between ZDF control rats and antidiabetic-treated ZDF rats was assessed by Dunnett's test.

RESULTS

At 8 weeks of age, plasma insulin, free fatty acid, and triglyceride levels of ZDF rats were increased, but there was no significant difference in plasma glucose levels between ZDF and lean rats (Table 1). When administered orally to ZDF rats for 2 weeks, troglitazone (300 mg/kg) and KRP-297 (10 mg/kg) both normalized the elevated plasma glucose of ZDF rats, but KRP-297 was more effective than troglitazone in decreasing the plasma insulin, free fatty acid, and triglyceride levels of these rats. Neither KRP-297 nor troglitazone had any effect on the wet weight of the liver or soleus muscle or the body weight of ZDF rats. We measured $^{14}CO_2$ production from [1-^{14}C]-palmitic (C16:0) acid in soleus muscle and liver tissue isolated from ZDF and lean rats (Fig 1A). Palmitic acid oxidation was lower in ZDF control rats versus lean rats ($P < .05$). KRP-297 and troglitazone increased palmitic acid oxidation in the soleus muscle of ZDF rats by 90% ($P < .05$) and 81% ($P < .05$), respectively. Hepatic palmitic acid oxidation was also lower in ZDF control rats versus lean rats ($P < .05$; Fig 1B). KRP-297 increased hepatic palmitic acid oxidation in ZDF rats, but troglitazone had no significant effect.

To better characterize the defects of fatty acid oxidation in ZDF rats, we next studied the effect of fatty acid chain length on fatty acid oxidation using stearic (C18:0) acid and octanoate (C8:0) (Fig 2A and B). Consistent with the results obtained in assays using palmitic acid, hepatic stearic acid oxidation was lower in ZDF control rats than in lean rats ($P < .05$; Fig 2A). However, there was no difference in hepatic octanoate oxidation between ZDF control and lean rats (Fig 2B). KRP-297 increased stearic acid oxidation but not octanoate oxidation in the liver of ZDF rats.

We next studied the effect of troglitazone and KRP-297 on the triglyceride content of skeletal muscle and liver from ZDF rats (Fig 3A and B). ZDF rats exhibited triglyceride accumulation in the skeletal muscle and liver, and troglitazone and KRP-297 inhibited triglyceride accumulation in the skeletal muscle ($P < .05$) and KRP-297, but not troglitazone, inhibited triglyceride accumulation in the liver of ZDF rats ($P < .05$).

Table 1. Biochemical Characteristics of Lean Rats, ZDF Rats, and ZDF Rats Treated With KRP-297 or Troglitazone

Characteristic	Vehicle-Treated		KRP-297–Treated ZDF Rats	Troglitazone-Treated ZDF Rats
	Lean Rats	ZDF Rats		
Initial body weight (g)	125 ± 7	191 ± 8*	189 ± 11	191 ± 14
Final body weight (g)	196 ± 8	288 ± 6*	313 ± 14	304 ± 16
Food intake (g)	16 ± 0.4	26 ± 0.5*	31 ± 1.2†	28 ± 1.0
Liver weight (g)	7.7 ± 0.4	14.7 ± 0.5*	13.7 ± 0.7	14.9 ± 0.9
Soleus muscle weight(mg)	106 ± 5	107 ± 1	103 ± 3	113 ± 4
Glucose (mg/dL)	106 ± 4	155 ± 19	99 ± 2†	106 ± 5†
Insulin (ng/mL)	0.2 ± 0.1	16.3 ± 1.5*	3.4 ± 0.5†	7.3 ± 1.2†
Free fatty acid (mEq/L)	1.29 ± 0.08	4.98 ± 0.51*	1.26 ± 0.11†	2.36 ± 0.18†
Triglyceride (mg/dL)	18 ± 1	431 ± 37*	58 ± 16†	105 ± 12†

NOTE. Either KRP-297 (10 mg/kg/d) or troglitazone (300 mg/kg/d) was administered orally to ZDF rats for 2 weeks. Data are the mean ± SE; n = 5 for all groups.

*$P < .05$, lean *v* ZDF rats.

†$P < .05$, vehicle-treated ZDF rats *v* antidiabetic agent–treated ZDF rats.

DISCUSSION

The accumulation of lipids such as fatty acids, their CoA esters, and triglycerides has been proposed to induce tissue insulin resistance.[1,9,10] Recent studies have shown that insulin resistance is strongly correlated with fatty acyl-CoA ester and triglyceride concentrations in the skeletal muscle and liver of insulin-resistant animals and humans.[24-27] The triglyceride content of the skeletal muscle and liver was indeed higher in ZDF rats versus lean rats, although the fatty acid and CoA ester content was not measured. We previously reported a reduced fatty acid oxidation and triglyceride accumulation in the liver of insulin-resistant Zucker fatty rats.[16] In this study, we provide evidence that ZDF rats exhibit reduced fatty acid oxidation in the skeletal muscle, as well as the liver. It has been reported that the fatty acid transport rate is increased slightly in cardiac myocytes but is unchanged in hepatocytes of ZDF rats.[28] Our results suggest that a reduction of fatty acid oxidation, in addition to an elevation of plasma lipid levels, may contribute to

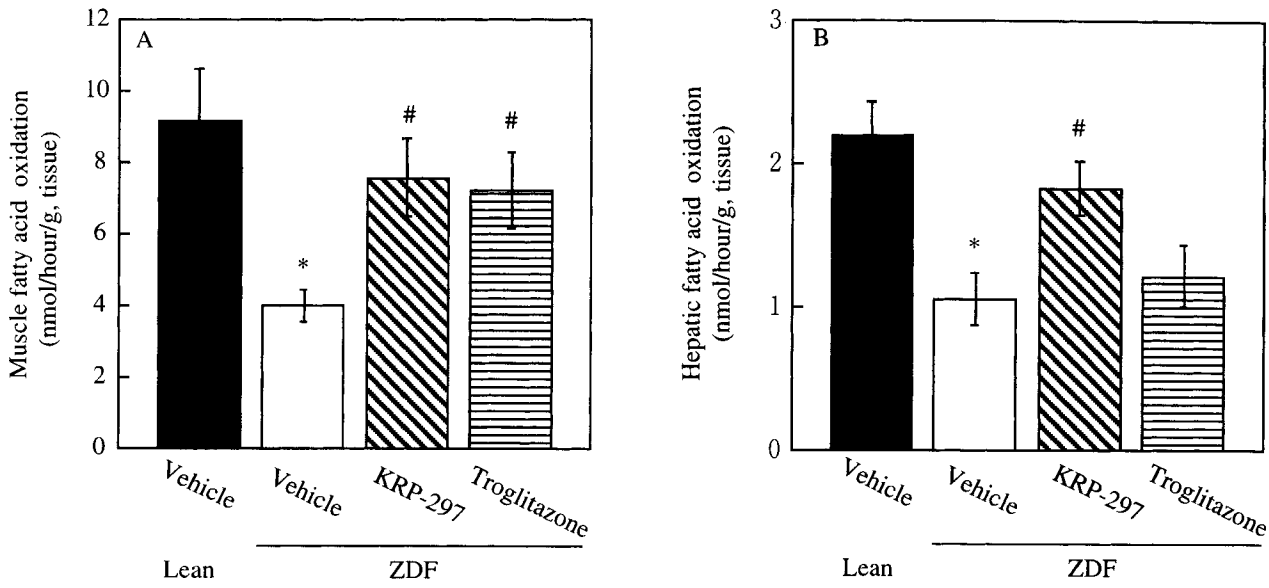

Fig 1. Fatty acid oxidation in muscle (A) and liver (B) of ZDF rats treated with KRP-297 and troglitazone. Either KRP-297 (10 mg/kg/d) or troglitazone (300 mg/kg/d) was administered orally to ZDF rats for 2 weeks. Tissue $^{14}CO_2$ production from [1-^{14}C]-fatty acid (C16:0) was measured. Data are the mean ± SE; n = 5 for all groups. *$P < .05$, lean *v* ZDF rats; #$P < .05$, vehicle-treated ZDF rats *v* antidiabetic agent–treated ZDF rats.

intracellular triglyceride accumulation in the skeletal muscle and liver of ZDF rats. On the contrary, Sreenan et al[29] reported no differences in muscle fatty acid oxidation among lean rats, severely diabetic ZDF rats, and troglitazone-treated ZDF rats. The plasma glucose level and age of the ZDF rats were apparently different between our study (155 ± 19 mg/dL; 8 weeks old) and theirs (407 ± 25 mg/dL; 13 to 14 weeks old), suggesting that the severity of diabetic syndromes may influence fatty acid oxidative activity.

When administered orally to ZDF rats, KRP-297, a dual agonist of both PPARα and PPARγ, increased fatty acid oxidation and inhibited triglyceride accumulation in the skeletal muscle and liver, while troglitazone, a PPARγ-selective agonist, had these effects only in skeletal muscle. Most recently, troglitazone was reported to inhibit triglyceride accumulation in the liver of ZDF rats[29] when examined at approximately a 10-fold higher dose. In this study, it should be noted that KRP-297 increased fatty acid oxidation and inhibited triglyceride accumulation in the liver of ZDF rats, even though KRP-297 was as effective as troglitazone in those in the muscles. PPARγ1 is relatively ubiquitously expressed in numerous tissues, including skeletal muscle, while PPARα is abundantly expressed in

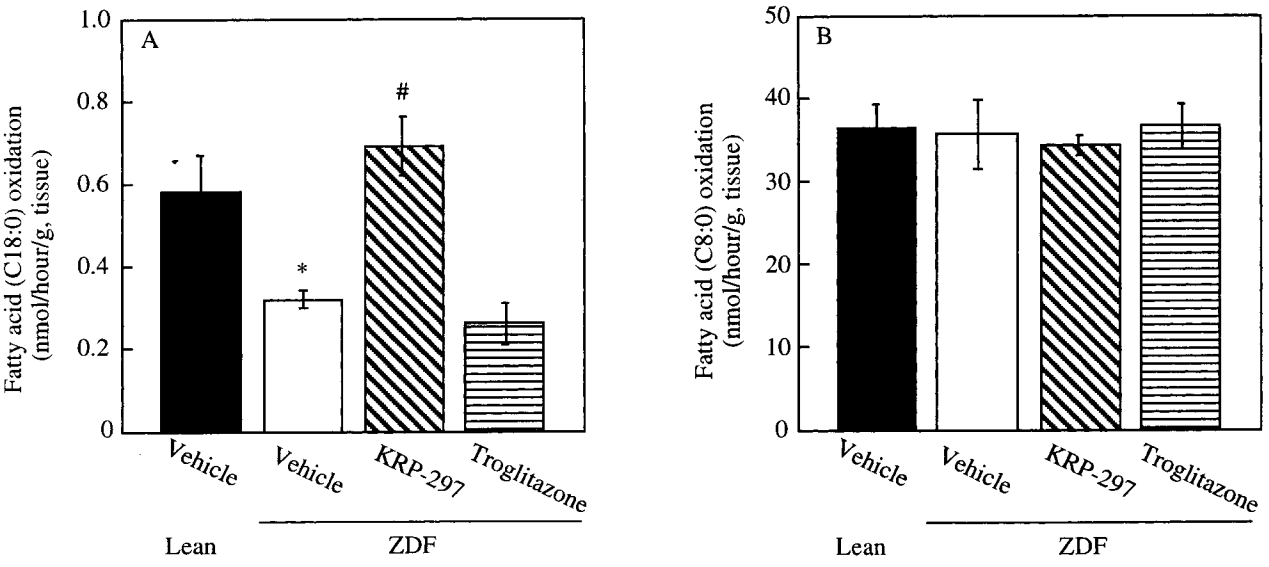

Fig 2. Effect of fatty acid chain length on fatty acid oxidation. Either KRP-297 (10 mg/kg/d) or troglitazone (300 mg/kg/d) was administered orally to ZDF rats for 2 weeks. Hepatic $^{14}CO_2$ production from [1-^{14}C]-fatty acid (C18:0, A) and [^{14}C]-fatty acid (C8:0, B) was measured. Data are the mean ± SE; n = 5 for all groups. *$P < .05$, lean *v* ZDF rats; #$P < .05$, vehicle-treated ZDF rats *v* antidiabetic agent–treated ZDF rats.

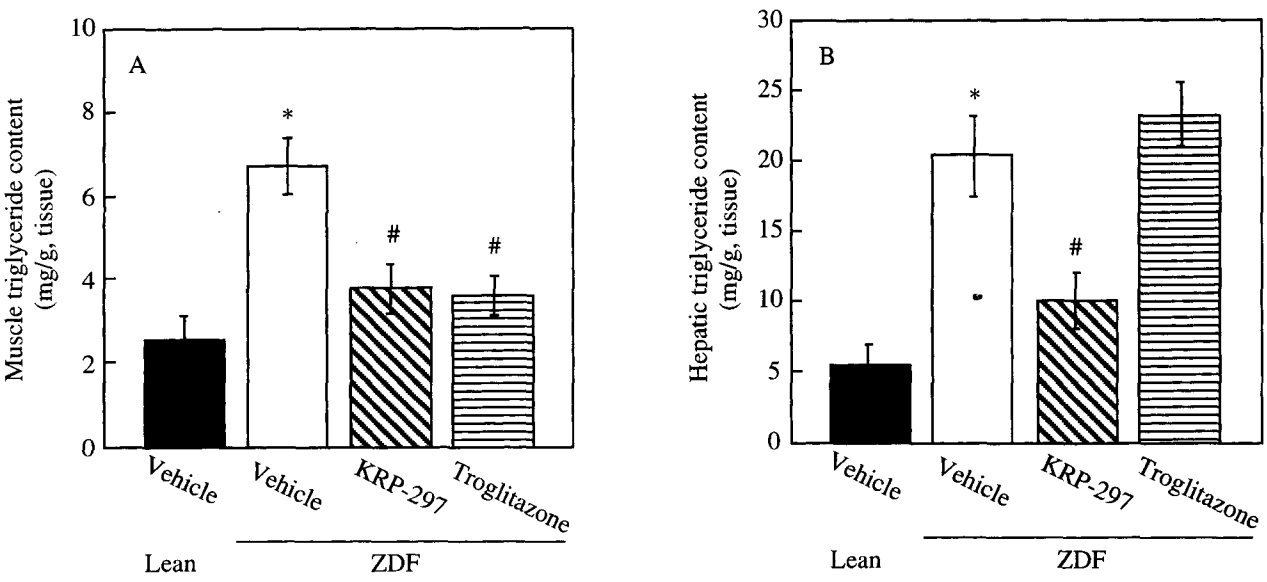

Fig 3. Triglyceride content in muscle (A) and liver (B) of ZDF rats treated with KRP-297 or troglitazone. Either KRP-297 (10 mg/kg/d) or troglitazone (300 mg/kg/d) was administered orally to ZDF rats for 2 weeks, and the tissue triglyceride content was measured. Data are the mean ± SE; n = 5 for all groups. *$P < .05$, lean *v* ZDF rats; #$P < .05$, vehicle-treated ZDF rats *v* antidiabetic agent–treated ZDF rats.

the liver.[20-22] We previously reported that KRP-297 increases the gene expression of a PPARα-regulated enzyme in rat hepatocytes and in Zucker fatty rats,[16] indicating that biological effects of KRP-297 in the liver might be due to PPARα activation. To clarify whether the biological effects of troglitazone and KRP-297 in skeletal muscle are mediated through PPAR activation, studies on PPAR-regulated gene expression in skeletal muscle are needed.

KRP-297 was more effective in reducing plasma insulin, free fatty acid, and triglyceride levels. The hypolipidemic action of KRP-297 might be explained by the dual activation of PPARα and PPARγ. Lefebvre et al[30] demonstrated an additive hypolipidemic effect of combined treatment with a PPARγ-selective activator, BRL-49,653, and a PPARα-selective activator, fenofibrate, in normal rats, consistent with our results in ZDF rats. Although it remains unclear whether PPARα activation improves hyperinsulinemia, based on our data, we speculate that PPARα activation may be responsible for ameliorating in vivo insulin resistance by reducing lipid levels in insulin-responsive tissues.[1,9,10]

A reduction of fatty acid oxidation in the liver of ZDF rats was observed when palmitic acid and stearic acid, but not octanoate, were used as substrates, and KRP-297 increased the oxidation of palmitic acid and stearic acid but not octanoate. Palmitic acid and stearic acid are known to be transported into mitochondria by carnitine palmitoyltransferase I and oxidized, unlike short-chain fatty acids such as octanoate.[31] These findings indicate that KRP-297 might ameliorate the impaired fatty acid transport into mitochondria in the liver of ZDF rats. Further studies are needed to determine whether defects are observed for fatty acid oxidation in the muscle as in the liver.

Randle et al[32] proposed that an increased availability of fatty acids may lead to an elevation of glucose-6-phosphate, which allosterically inhibits hexokinase, thereby reducing glucose transport and/or phosphorylation. However, more recent studies have shown that increased plasma lipid levels induce insulin resistance in skeletal muscle via a mechanism not involving hexokinase inhibition by glucose-6-phosphate.[9] Most recently, an elevation of plasma fatty acid levels was shown to impair the insulin signaling cascade, tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), and IRS-1–associated phosphatidylinositol 3-kinase activity,[10] although it is unclear whether fatty acids affect the insulin signaling cascade directly or indirectly. Our findings suggest that defects in fatty acid oxidative activity may be responsible for the triglyceride accumulation causing insulin resistance in the insulin-responsive tissues. However, we cannot exclude an effect of the glucose–fatty acid cycle on insulin resistance, because in vivo fatty acid utilization and oxidation may increase under conditions in which plasma fatty acid levels are elevated.

In conclusion, insulin-resistant rats with moderate hyperglycemia exhibited reduced fatty acid oxidation and triglyceride accumulation in the skeletal muscle and liver. These abnormalities were ameliorated by thiazolidinedione treatment in a tissue-specific fashion. KRP-297, a dual agonist of both PPARα and PPARγ, improved the reduced fatty acid oxidation and inhibited triglyceride accumulation in the skeletal muscle and liver, while the effect of troglitazone, a PPARγ-selective agonist, was observed only in skeletal muscle. Moreover, KRP-297 was more effective than troglitazone in ameliorating both hyperinsulinemia and hyperlipidemia. These results suggest that reduced fatty acid oxidative activity and lipid accumulation in the skeletal muscle and liver may play a role in the development of insulin resistance in patients with NIDDM associated with obesity.

ACKNOWLEDGMENT

We thank Dr T. Kadowaki for helpful discussions and M. Tsunoda and M. Suzuki for technical assistance.

REFERENCES

1. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 45:3-10, 1996
2. MacGarry JD: What if Mikowski had been ageusic? An alternate angle on diabetes. Science 258:766-770, 1992
3. MacGarry JD: Disordered metabolism in diabetes: Have we underemphasized the fat component? J Cell Biol 55S:29-38, 1994 (suppl)
4. Lee Y, Hirose H, Ohneda M, et al: β-Cell lipotoxicity in the pathogenesis of non–insulin-dependent diabetes mellitus of obese rats: Impairment in adipocyte–β-cell relationships. Proc Natl Acad Sci USA 91:10878-10882, 1994
5. Jenkins AB, Storlien LH, Chisholm DJ, et al: Effects of nonesterified fatty acid availability on tissue-specific glucose utilization in rats in vivo. J Clin Invest 82:293-299, 1988
6. Kim JK, Wi JK, Youn JH: Plasma free fatty acids decrease insulin-stimulated skeletal muscle glucose uptake by suppressing glycolysis in conscious rats. Diabetes 45:446-453, 1996
7. Boden G, Chen X, Ruiz J, et al: Mechanisms of fatty acid induced inhibition of glucose uptake. J Clin Invest 93:2438-2446, 1994
8. Lewis GF, Vranic M, Harley P, et al: Fatty acids mediated the acute extrahepatic effects of insulin on hepatic glucose production in humans. Diabetes 46:1111-1119, 1997
9. Roden M, Price TB, Perseghin G, et al: Mechanism of free fatty acid–induced insulin resistance in humans. J Clin Invest 97:2859-2865, 1996
10. Dresner A, Laurent D, Marcucci M, et al: Effects of free fatty acids on glucose transport and IRS-1–associated phosphatidylinositol 3-kinase activity. J Clin Invest 103:253-259, 1999
11. Saltiel AR, Olefsky JM: Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 45:1661-1669, 1996
12. Stevenson RW, McPherson RK, Persson LM, et al: The antihyperglycemic agent englitazone prevents the defect in glucose transport in rats fed a high fat diet. Diabetes 45:60-66, 1996
13. Lehmann JM, Moore LB, Smith-Oliver TA, et al: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator activated receptor γ (PPARγ). J Biol Chem 270:12953-12956, 1995
14. Forman BM, Tontonoz P, Chen J, et al: 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. Cell 83:803-812, 1995
15. Nomura M, Kinoshita S, Satho H, et al: (3-substituted benzyl) Thiazolidine-2,4-dione as structurally new antihyperglycemic agents. Bioorg Med Chem Lett 9:533-538, 1999
16. Murakami K, Tobe K, Ide T, et al: A novel insulin sensitizer acts as a coligand for peroxisome proliferator–activated receptor-α (PPARα) and PPARγ. Effect of PPARα activation on abnormal lipid metabolism in liver of Zucker fatty rats. Diabetes 47:1841-1847, 1998
17. Murakami K, Tsunoda M, Ide T, et al: Amelioration by KRP-297, a new thiazolidinedione, for impaired glucose uptake in skeletal muscle from obese insulin-resistant animals. Metabolism 48:1450-1454, 1999
18. Schoonjans K, Steals B, Auwerx J: Role of the peroxisome proliferator–activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. J Lipid Res 37:907-925, 1996
19. Tontonoz P, Hu E, Spiegelman BM: Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor γ. Curr Biol 5:571-576, 1995
20. Brasissant O, Foufelle F, Scotto C, et al: Differential expression of peroxisome proliferator–activated receptors: Tissue distribution of PPAR α, β and γ in the adult rat. Endocrinology 137:354-366, 1996
21. Su JL, Simmons CJ, Wisely B, et al: Monitoring of PPAR alpha protein expression in human tissue by the use of PPAR alpha–specific Mabs. Hybridoma 17:47-53, 1998
22. Zierath JR, Ryder JW, Doebber T, et al: Role of skeletal muscle in thiazolidinedione insulin sensitizer (PPARγ agonist) action. Endocrinology 139:5034-5041, 1998
23. Irikura T, Takagi K, Okada K, et al: Reduction of fructose-induced hypertriglycemia and fatty liver in rats by 4(4′-chlorobenzyloxy-)benzoyl nicotinate (KCD-232). Agric Biol Chem 48:977-983, 1984
24. Oakes ND, Camilleri S, Furler SM, et al: The insulin sensitizer, BRL49653, reduces systemic fatty acid supply and utilization and tissue lipid availability in rat. Metabolism 46:935-942, 1997
25. Chen MT, Kaufman LN, Spennetta T, et al: Effects of high-fat feeding to rats on the interrelationship of body weight, plasma insulin, and fatty acyl-coenzyme A esters in liver and skeletal muscle. Metabolism 41:564-569, 1992
26. Koyama K, Chen G, Lee Y, et al: Tissue triglycerides, insulin resistance, and insulin production: Implications for hyperinsulinemia of obesity. Am J Physiol 273:E708-E713, 1997
27. Pan DA, Lillioja S, Kriketos AD, et al: Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 46:983-988, 1997
28. Berk PD, Zhou SL, Kiang CL, et al: Uptake of long chain free fatty acids is selectively upregulated in adipocytes of Zucker rats with genetic obesity and non–insulin-dependent diabetes mellitus. J Biol Chem 272:8830-8835, 1997
29. Sreenan S, Keck S, Fuller T, et al: Effects of troglitazone on substrate storage and utilization in insulin-resistant rats. Am J Physiol 276:E1119-E1129, 1999
30. Lefebvre A-M, Peinado-Onsurbe J, Leitersdorf I, et al: Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. Arterioscler Thromb Vasc Biol 17:1756-1764, 1997
31. McGarry JD, Foster DW: The metabolism of (−)-octanoylcarnitine in perfused livers from fed and fasted rats: Evidence for a possible regulatory role of carnitine acyltransferase in the control of ketogenesis. J Biol Chem 249:7984-7990, 1974
32. Randle PJ, Garland PB, Hales CN, et al: The glucose fatty acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963