Correlation Between Lipid and Glycogen Contents in Liver and Insulin Resistance in High-Fat–Fed Rats Treated With the Lipoprotein Lipase Activator NO-1886

Masataka Kusunoki, Kazuhiko Tsutsumi, Tsutomu Hara, Hitoshi Ogawa, Takao Nakamura, Tetsuro Miyata, Fumihiko Sakakibara, Yoshitaka Fukuzawa, Takashi Suga, Shinichi Kakumu, and Yutaka Nakaya

Insulin resistance results in accumulation of triglyceride content and reduction of glycogen content in skeletal muscle. However, very few studies have measured lipid content and glycogen content in liver associated with insulin resistance. We studied the relationship between liver lipid content, liver glycogen, and insulin resistance in high-fat–fed rats, which are animal models of insulin resistance. High-fat–fed rats were hyperlipidemic, hyperglycemic, and hyperinsulinemic. Furthermore, the glucose infusion rates (GIR) were lower (normal rats, 10.35 ± 1.66; high-fat–fed rats, 4.86 ± 0.93 mg/kg/min; $P < .01$) and the triglyceride and cholesterol contents in liver were higher in the high-fat–fed rats than in normal rats. On the other hand, the glycogen content in liver was lower than in normal rats. There was an inverse relationship between liver triglyceride content and liver glycogen content. When the lipoprotein lipase (LPL) activator NO-1886 was administered to the high-fat–fed rats at a daily dose of 50 mg/kg body weight for 10 weeks, GIR (9.87 ± 3.76 mg/kg/min, $P < .05$ *v* high-fat–fed control group) improved, causing an improvement of the hyperlipidemia, hyperglycemia, and hyperinsulinemia. Furthermore, NO-1886 decreased triglyceride and cholesterol concentrations and increased glycogen content in liver of the high-fat–fed rats. In this study, we found that insulin resistance caused fatty liver and reduced glycogen content in liver. Administration of the LPL activator NO-1886 improved the insulin resistance, resulting in an improvement in the relationship between triglyceride and glycogen content in liver of high-fat–fed rats.

INSULIN RESISTANCE is characterized by high plasma insulin, which causes acceleration of very–low-density lipoprotein (VLDL) synthesis.[1] This results in hypertriglyceridemia and fatty liver in animals and humans with insulin resistance. In addition, insulin resistance decreases glycogen synthetase activity and causes a reduction of glycogen content in skeletal muscle.[2,3] However, few reports exist regarding the relationship between lipid and glycogen contents in liver of insulin resistant animals and humans. Conflicting results have been reported regarding glycogen synthesis in liver in diabetes, with some studies showing a reduction of glycogen synthesis[4] and others reporting that glycogen synthesis was not affected.[5]

The aims of this study were 2-fold. The first was to ascertain the relationship between lipid and glycogen contents in liver of high-fat–fed rats, an insulin-resistant animal model.[6] The second was to ascertain the effects of the lipoprotein lipase (LPL) activator NO-1886, which has previously been reported to increase LPL activity resulting in elevation of high-density lipoprotein cholesterol (HDL-C) and reduction of triglycerides in animals,[7-10] on lipid and glycogen contents in liver in the model rats. We determined that high-fat feeding caused insulin resistance in rats, which resulted in fatty liver and reduced glycogen contents in liver. We also determined that there was an inverse relationship between triglyceride contents and glycogen contents in liver.

From the First Department of Internal Medicine and Institute of Physical, Aichi Medical University, Aichi; Otsuka Pharmaceutical Factory, Inc, Tokushima; Faculty of Engineering, Yamagata University, Yamagata; School of Medicine, University of Tokyo, Tokyo; and the School of Medicine, Tokushima University, Tokushima, Japan.

Submitted September 21, 2001; accepted December 19, 2001.

Address reprint requests to Masataka Kusunoki, MD, First Department of Internal Medicine, Aichi Medical University, Nagakute-cho, Aichi-gun, Aichi 480-11, Japan.

0026-0495/02/5106-0014$35.00/0

doi:10.1053/meta.2002.32732

MATERIALS AND METHODS

Materials

Agent NO-1886, 4-diethoxyphosphorylmethyl-N-(4-bromo-2-cyanophenyl) benzamide, was synthesized in the New Drug Research Laboratory of Otsuka Pharmaceutical Factory, Inc, Naruto, Tokushima, Japan. All other chemicals used were high-grade commercially available products.

Animal Experiments

Male Sprague-Dawley rats weighing 500 to 600 g at the age of 9 months were obtained from Japan SLC, Inc, Shizuoka, Japan. The animals were maintained under a 12-hour light-dark cycle (light cycle from 7 AM to 7 PM) at a constant temperature of 23 ± 2°C. Rats were fed high-fat chow (26.7% safflower oil in standard laboratory chow; CRF-1, Oriental Yeast Co, Tokyo, Japan) containing NO-1886, which was equivalent to 50 mg/kg body weight of NO-1886, for 10 weeks (NO-1886 group). Control rats were fed a high-fat chow. Animals were allocated to either the control group, NO-1886 group, or normal group (which was fed standard chow) with stratification based on baseline body weight. Body weights at the start of the experiment were 552 ± 32, 528 ± 64, and 553 ± 27 g in the normal, control, and NO-1886 groups, respectively. There were no significant differences in body weight. The animals were given free access to food and tap water. At the end of the experimental period, after a 12-hour overnight fast, blood samples were collected at 9 AM from the tail vein for determination of plasma lipids, glucose, and insulin. A euglycemic glucose clamp study was then performed, after which the livers were removed. The liver samples were maintained in liquid nitrogen for measurement of lipids and glycogen in tissues. Normal-diet–fed Sprague-Dawley rats were used for comparison (normal group).

Euglycemic Glucose Clamp Studies

After blood samples were collected from the tail vein, the euglycemic glucose clamp study was performed following a 12-hour overnight fast, which was started after the final NO-1886 administration. Rats

Table 1. Plasma Lipid, Glucose, and Insulin Levels and GIR in NO-1886–Treated High-Fat–Fed Rats

		Plasma Lipids						
	N	Total Cholesterol (mg/dL)	HDL-C (mg/dL)	Triglycerides (mg/dL)	NEFA (μEq/L)	Glucose (mg/dL)	Insulin (μU/mL)	GIR (mg/kg/min)
Normal rats	5	60 ± 9	53 ± 8	131 ± 5†	462 ± 45†	161 ± 8*	49 ± 6†	10.35 ± 1.66*
High-fat–fed rats								
Control	5	74 ± 15	52 ± 5	184 ± 37	598 ± 100	192 ± 13	107 ± 29	4.86 ± 0.93
NO-1886	5	147 ± 16*	136 ± 16*	94 ± 13*	407 ± 52*	175 ± 7†	62 ± 24†	9.87 ± 3.76†

NOTE. Data are expressed as means ± SD.

Significantly different from the respective values in control rats: *$P < .01$, †$P < .05$.

were anesthetized with sodium pentobarbital (50 mg/kg body weight), and extension tubings were attached to the jugular vein by an adapter so that glucose and insulin could be infused simultaneously into the jugular vein. The carotid catheter was used for blood sampling. After a 30-minute basal period, a continuous infusion of human regular insulin (Novolin R; Novo Nordisk, Copenhagen, Denmark) at a rate of 60 pmol/kg/min was administered throughout the study. Blood samples were drawn at 10-minute intervals for immediate determination of plasma glucose (YSI 2300 glucose analyzer; YSI, Yellow Springs, OH) and plasma concentration was kept constant at 80 mg/dL by a variable infusion of 10% dextrose solution. Steady-state was generally achieved within 60 to 90 minutes. A maximal glucose clamp was then started. Insulin was infused and 20% dextrose was variably infused to maintain plasma glucose concentration at 80 mg/dL, and the glucose infusion rate (GIR; glucose mg/kg/min) was determined.

Analytical Methods

Plasma lipids, glucose, and insulin. Plasma total cholesterol, HDL-C, triglycerides, nonesterified free fatty acid (NEFA), and glucose were determined by conventional enzymatic methods. The cholesterol C-test Wako (Wako Pure Chemical Industries, Osaka, Japan) was used in the case of cholesterol, the Nescote HDL-C kit N (Nippon Shoji, Osaka Japan) for HDL-C, the triglyceride G-test Wako (Wako Pure Chemical Industries) for triglycerides, the NEFA C-test Wako for NEFA, and the glucose CII test Wako for glucose. Insulin was determined by conventional enzyme immunoassay, using the Glazyme insulin-EIA test (Wako Pure Chemical Industries).

Tissue lipids. Tissue samples of approximately 0.2 g, together with 2 mL of chloroform-methanol (2:1) solution,[11] were added into centrifuge tubes and homogenized by Polytron (PCU-2-110; KINEMATICA GmbH, Luzern, Switzerland). The tubes were then centrifuged at 3,000 rpm. An aliquot of chloroform-methanol extract was transferred to another tube and dried under a stream of nitrogen gas. These samples were redissolved in 100 μL isopropyl alcohol, after which cholesterol and triglyceride levels in the isopropyl alcohol were measured by conventional enzymatic methods.

Tissue glycogen. Glycogen was measured by the method of Hassid and Abraham with slight modification.[12] Approximately 0.2 g of tissue was dropped into a centrifuge tube containing 1 mL of 30% potassium hydroxide solution. The tissue was then digested by heating the tube in a boiling water bath for 20 minutes. When the tissue was dissolved, 0.5 mL of saturated sodium sulfate was added and the glycogen was precipitated by the addition of 3 mL of 95% ethanol. The tube and contents were heated again until the mixture began to boil, then cooled and centrifuged at 3,000 rpm. The mother liquor was decanted, and the test tube was allowed to drain. The precipitated glycogen was redissolved in 1 mL of distilled water and reprecipitated with 1.5 ML of 95% ethanol, the alcoholic supernatant liquid decanted, and the tube drained as before. The purified glycogen was redissolved in 1 mL of distilled water and glycogen in this solution was measured by Anthrone reagent.[12]

Statistical Analysis

The results are expressed as means ± SD. Comparisons among the 3 groups were analyzed for statistical significance using 1-way analysis of variance, followed by Dunnett's test multiple comparisons. Correlation analysis was performed using Spearman's test. *P* values less than .05 were considered significant.

RESULTS

Body Weight

At the end of the experiment, the body weights of the normal, control, and NO-1886 groups were 589 ± 43, 750 ± 45, and 662 ± 53 g, respectively. The mean body weight of the control group was higher than that of the normal group ($P < .01$) and the mean body weight of the NO-1886 group was lower than that of the control group ($P < .05$).

Plasma Lipid, Glucose, and Insulin Levels in High-Fat–Fed Rats

Plasma lipid, glucose, and insulin levels are listed in Table 1. Plasma triglycerides, NEFA, glucose, and insulin levels of high-fat–fed control rats were higher than in normal rats. NO-1886 increased plasma total cholesterol and HDL-C, and decreased triglycerides, NEFA, glucose, and insulin levels.

GIR Using a Euglycemic Glucose Clamp

GIR values are listed in Table 1. The GIR values of high-fat–fed control rats were 53% lower than those of normal rats. GIR values recovered to normal levels in high-fat–fed control rats after NO-1886 administration.

Triglyceride, Cholesterol, and Glycogen Contents of Rat Liver

Triglyceride, cholesterol and glycogen contents in liver are listed in Table 2. The triglyceride and cholesterol contents in liver of high-fat–fed control rats increased approximately 4.9- and 2.7-fold, respectively, compared with normal rats. The glycogen content in liver of high-fat–fed control rats was 53% lower than in normal rats. NO-1886 decreased the triglyceride and cholesterol contents and increased the glycogen content in liver of high-fat–fed rats compared to control.

Relationships Between Lipid Contents and Glycogen Contents in Liver and GIR Values

The relationships between lipid contents and glycogen contents in liver and GIR values were assessed in experimental animals (normal rats and high-fat–fed rats). Triglyceride con-

Table 2. Liver Trigyceride, Cholesterol, and Glycogen Levels in NO-1886–Treated High-Fat–Fed Rats

	N	mg/g wet liver		
		Triglycerides	Cholesterol	Glycogen
Normal rats	5	48.74 ± 13.80*	10.30 ± 2.57*	43.12 ± 5.50*
High-fat–fed rats				
Control	5	241.54 ± 27.56	27.84 ± 6.12	20.09 ± 4.87
NO-1886	5	157.45 ± 40.79*	18.44 ± 4.33†	33.75 ± 5.40*

NOTE. Data are expressed as means ± SD.

Significantly different from the respective values in control rats: *$P < .01$, †$P < .05$.

tents were inversely correlated with GIR values ($r = -0.790$), while glycogen contents were positively correlated with GIR values ($r = 0.687$). Cholesterol contents were inversely correlated with GIR values ($r = -0.550$). Triglyceride contents were inversely correlated with glycogen contents in liver ($r = -0.852$; Fig 1).

DISCUSSION

Insulin resistance is defined as a subnormal biologic response to a given concentration of insulin. Therefore, insulin-resistant subjects have higher plasma insulin levels than normal subjects. Hyperinsulinemia causes hypertriglyceridemia and fatty liver.[1] Furthermore, insulin resistance causes a reduction in the rate of muscle glycogen synthesis in people with type 2 diabetes.[2,3] There are many reports stating that insulin resistance causes fatty liver and reduction of glycogen synthesis in skeletal muscle. However, few reports have discussed the relationship between fatty liver and glycogen contents in liver of insulin-resistant animals. In this study, we observed the relationship between fatty liver and glycogen contents in liver of high-fat–fed rats by using the LPL activator NO-1886.

Storlien et al have previously reported that high fat-fed rats are a model of insulin resistance.[6,13] This model is also considered a model of obesity.[13]

The mean body weight of the group of rats fed a high-fat diet significantly increased compared with the normal chow group by the 10th week of feeding. However, the mean body weight of the NO-1886 group was lower than that of the control group after 10 weeks administration of NO-1886 in this study. Kusunoki et al previously reported that NO-1886 increases fat oxidation by increasing skeletal muscle LPL activity. This causes a suppression of fat accumulation, resulting in decreased body weight gain.[11]

In this study, the GIR values in these model rats were lower than in normal rats, and they were hyperinsulinemic, hyperglycemic, and hypertriglyceridemic. Furthermore, we found that the model rats had fatty liver and low hepatic glycogen contents. Thorburn et al[3] have reported that a reduction in glycogen synthase activity causes reduced glycogen formation in muscle in type 2 diabetic patients. Unfortunately, we did not determine glycogen synthase activity in this study. However, low glycogen content in the liver of this model may have been caused by low glycogen synthase activity, similar to type 2 diabetic patients.[3] On the other hand, Upton et al reported in Zucker diabetic fatty (ZDF) rats that glycogen synthase activity and glycogen contents in liver did not differ compared to control (nondiabetic).[5] We cannot explain the difference between our results and Upton's data.

NO-1886 administration resulted in GIR values returning to normal. This indicates that NO-1886 improved insulin resistance in this model. Specifically, NO-1886 improved not only hyperinsulinemia, hyperglycemia, and hypertriglyceridemia, but also improved lipid and glycogen contents in liver of high-fat–fed rats by improving insulin resistance.

NO-1886 decreased plasma free fatty acid and glucose levels, as well as lipid contents in liver of high-fat–fed rats. However, NO-1886 did not inhibit triglyceride and cholesterol synthesis, or free fatty acid release (data not shown). Also, NO-1886 did not decrease plasma glucose in insulin-deficient streptozotocin-induced diabetic model rats.[8] Therefore, these results show that insulin resistance causes fatty liver and reduction of glycogen content in liver. Furthermore, the triglyceride contents in liver were inversely correlated with GIR values and glycogen, while glycogen contents in liver were positively correlated with GIR values. On the other hand, the correlation between cholesterol content and glycogen content was weaker than with triglyceride contents. These results may indicate that improvement of insulin resistance causes a reduction of lipid contents and an elevation of glycogen contents in liver.

The antidiabetic agent, thiazolidinedione (MCC-555), increases glucose entry into insulin-sensitive tissues, resulting in a significant increase in hepatic glycogen contents and increased fat pad mass in ZDF rats.[5] On the other hand, NO-1886 decreases fat accumulation in high-fat–fed obesity rats.[13] Therefore, NO-1886 and thiazolidinedione may have different mechanisms for the improvement of insulin resistance. How-

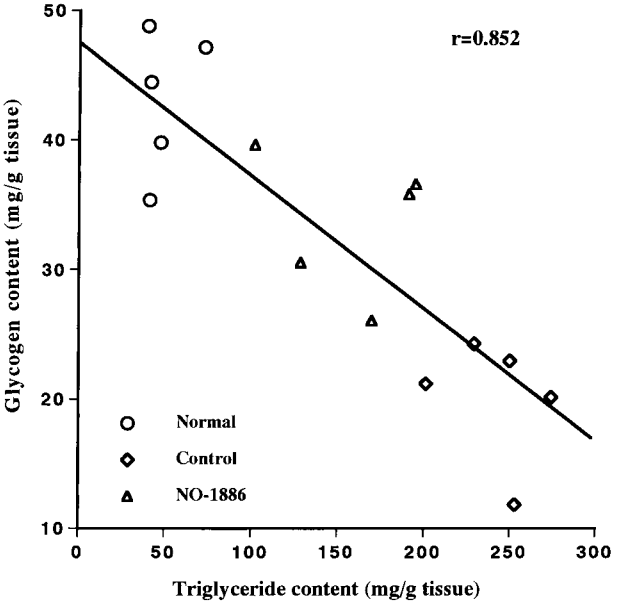

Fig 1. Relationship between liver triglyceride contents and liver glycogen contents in high-fat–fed rats. (○) Normal rat group; (◇) high-fat–fed control rat group; (△) NO-1886–treated high-fat–fed rat group.

ever, these results may indicate that the improvement of insulin resistance causes the increase in glycogen contents in diabetes.

In summary, the results of our study indicate that insulin resistance causes fatty liver and a reduction of glycogen contents in liver. Triglyceride contents were inversely correlated with glycogen contents in liver. On the other hand, the correlation between cholesterol content and glycogen content was weaker than with triglyceride contents. NO-1886 decreased lipid content and increased glycogen content in liver of high-fat–fed rats, an insulin-resistant animal model, by improving insulin resistance.

REFERENCES

1. Moller DE, David EM, Flier JS: Insulin resistance-mechanisms, syndromes and implications. N Engl J Med 325:938-948, 1991

2. Cline GW, Petersen KF, Krssak M, et al: Impaired glucose transport as a cause of decreased insulin stimulated muscle glycogen synthesis in type 2 diabetes. N Engl J Med 341:240-246, 1999

3. Thorbun AW, Gumbiner B, Bulacan F, et al: Multiple defects in muscle glycogen synthase activity contribute to reduced glycogen synthesis in non-insulin dependent diabetes mellitus. J Clin Invest 87:489-495, 1991

4. Taylor SI: Diabetes mellitus, in Scriver CR, Beaudet AL, Sly WS et al (eds): The Metabolic and Molecular Bases of Inherited Disease, vol 1. New York, NY, McGraw-Hill, 1995, pp 843-896

5. Upton R, Windowsons PS, Ishii S, et al: Improved metabolic status and insulin sensitivity in obese fatty (fa/fa) Zucker rats and Zucker diabetic fatty (ZDF) rats treated with thiazolidinedione, MCC-555. Br J Pharmacol 125:1708-1714, 1998

6. Storlien LH, Jenkins AB, Chisholm DJ, et al: Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipids. Diabetes 40:280-289, 1991

7. Tsutsumi K, Inoue Y, Shima A, et al: The novel compound NO-1886 increases lipoprotein lipase activity with resulting elevation of HDL-cholesterol, and long-term administration inhibits atherogenesis in the coronary arteries of rats with experimental atherosclerosis. J Clin Invest 92:411-417, 1993

8. Tsutsumi K, Inoue Y, Shima A, et al: Correction of hypertriglyceridemia with low high-density lipoprotein cholesterol by he novel compound NO-1886, a lipoprotein lipase-promoting agent, in STZ-induced diabetic rats. Diabetes 44:414-417, 1995

9. Hara T, Cameron-Smith D, Cooney GJ, et al: The actions of novel lipoprotein lipase activator, NO-1886, in hypertriglyceridemic fructose-fed rats. Metabolism 47:149-153, 1998

10. Hara T, Kusunoki M, Tsutsumi K, et al: A lipoprotein lipase activator, NO-1886, improved endothelium-dependent relaxation of rat aorta associated with aging. Eur J Pharmacol 350:75-79, 1998

11. Entenman C: General procedures for separating lipid components of tissue, Methods Enzymol 3:299-317, 1957

12. Hassid WZ, Abraham S: Chemical procedures for analysis of polysaccharides, Methods Enzymol 3:34-50, 1957

13. Kusunoki M, Hara T, Tsutsumi K, et al: The lipoprotein lipase activator, NO-1886, suppresses fat accumulation and insulin resistance in rats fed a high-fat diet. Diabetologia 43:875-880, 2000