The Actions of a Novel Lipoprotein Lipase Activator, NO-1886, in Hypertriglyceridemic Fructose-Fed Rats

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High circulating fasting and prandial triglyceride levels are associated with both insulin resistance and the development of cardiovascular disease. The aim of this investigation was to study the effects of NO-1886, a novel lipoprotein lipase (LPL) activator, on triglyceride levels, fat oxidation, and glucose tolerance in fructose-fed rats, a hypertriglyceridemic model of insulin resistance. Adult male Wistar rats were fed for 4 weeks with a high-starch diet or a high-fructose diet without and with NO-1886 (50 mg · kg^{-1} · d^{-1} orally). Fructose feeding increased plasma triglyceride levels, an effect that was ameliorated by NO-1886 treatment (week 1/week 4: starch-fed, 2.4 ± 0.1/2.8 ± 0.2 mmol/L; fructose-fed, 3.6 ± 0.5/5.5 ± 0.5; fructose + NO-1886, 2.7 ± 0.2/3.6 ± 0.3). The mean 24-hour respiratory quotient (RQ) was significantly lower in the fructose + NO-1886 group compared with fructose-fed rats, indicating increased oxidation of fat. Fructose feeding elevated liver triglyceride levels by 74% ($P < .01$), an effect not altered by NO-1886. Red and white quadriceps hindlimb muscle triglyceride levels were not different between groups. Glucose tolerance (intravenous test in long-term cannulated rats) was mildly deteriorated and fasting insulin and glucose levels were elevated in fructose-fed rats, effects which were ameliorated by NO-1886. In conclusion, in the fructose-fed rat model of hypertriglyceridemia and insulin resistance, addition of a LPL activator reduced circulating triglyceride levels without causing increased muscle triglyceride accumulation or deterioration in glucose tolerance. LPL activators may prove to be a fruitful avenue to explore in the search for new therapeutic agents in the treatment of dyslipidemias and insulin resistance.

HYPERTRIGLYCERIDEMIA, characterized by elevated very–low-density lipoprotein (VLDL) triglyceride, is common in insulin-resistant states, including non–insulin-dependent diabetes mellitus (NIDDM) and obesity.[1] Elevated fasting and prandial triglyceride levels have been strongly implicated in the prevalent cardiovascular disease of individuals with NIDDM.[2,3]

The rate-limiting step in the removal of triglyceride from triglyceride-rich lipoproteins is catalyzed by the insulin-sensitive enzyme lipoprotein lipase (LPL), which is present at the capillary endothelial/luminal interface of many tissues, including adipose tissue and skeletal muscle. LPL hydrolyzes the triglyceride core of lipoproteins, liberating free fatty acids (FFAs) that are absorbed by diffusion into adjacent tissues.[4] In insulin-resistant states, there is no clear relationship between the plasma triglyceride concentration and LPL activity measured in the fasted state.[4,5] However, following insulin infusion, there is a blunting of the normal stimulation of adipose tissue LPL activity and the normal suppression of skeletal muscle LPL activity is lost.[6-8]

It is therefore possible that the normalization of LPL activity in insulin-resistant states provides a potential mechanism by which hypertriglyceridemia can be alleviated. However, it would be important that this improvement in circulating triglyceride levels did not come at the cost of impaired glucose metabolism either via replacement of carbohydrate fuels by lipid (the glucose/fatty acid cycle[9]) or via accumulation of storage triglyceride in tissues such as skeletal muscle. Skeletal muscle is the most important tissue for insulin-stimulated glucose metabolism, and increased levels of stored lipid have been closely associated with impaired insulin action in this tissue.[10]

Tsutsumi et al[11,12] have described a novel pharmacological stimulator of LPL activity, NO-1886, that enhances LPL expression in adipose tissue and increases LPL activity in postheparin plasma in rats. In parallel with the increased LPL activity, NO-1886 treatment in normal and streptozotocin-induced diabetic rats markedly decreases plasma triglyceride concentrations. These studies suggest that NO-1886 may provide a potential strategy for the amelioration of hypertriglyceridemia in insulin-resistant states. However, in the studies reported by Tsutsumi et al,[11,12] it was not established whether the LPL-mediated increase in the hydrolysis of plasma triglycerides results in an accumulation of triglycerides in peripheral tissues or an elevation in the rate of fat oxidation. It is therefore important to determine in a hypertriglyceridemic insulin-resistant state what impact LPL activation has on fuel utilization and insulin sensitivity.

Fructose feeding provides a dietary model of hypertriglyceridemia, insulin resistance, and hypertension due to the marked fructose stimulation of hepatic de novo lipogenesis and VLDL production.[13-15] The aim of this study was to investigate the actions of the novel LPL activator, NO-1886, on fat oxidation (determined by whole-animal indirect calorimetry) and insulin sensitivity (determined by an intravenous glucose tolerance test [IVGTT]) in the hypertriglyceridemic and insulin-resistant fructose-fed rat.

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Table 1. Experimental Diet Composition

Ingredient (g/kg diet)	Starch Diet	Fructose Diet
Fructose	—	330
Cornstarch	660	330
Casein	190	190
Safflower oil	45	45
Gelatin	10	10
Wheat bran	45	45
Methionine	1	1
Vitamin mix*	9	9
Mineral mix*	50	50

*Composition as described by Thorburn et al.[15]

MATERIALS AND METHODS

NO-1886, 4-diethoxyphosphorylmethyl-*N*-(4-bromo-2-cyanophenyl) benzamide, was a kind gift from the New Drug Research Laboratory of Otsuka Pharmaceutical (Tokushima, Japan). All research procedures were formally approved by the Animal Ethics Committee of the Royal Prince Alfred Hospital and complied with the guidelines for the Care and Use of Animals for Research Purposes (National Health and Medical Research Council, Australia).

Rats and Diets

Male Sprague-Dawley rats (~150 g) were housed in groups of four or five per cage on a 12-hour light-dark cycle. The rats were randomly assigned to two groups and fed ad libitum either a high-starch or high-fructose diet. The compositions of the diets are described in Table 1. Half of the fructose-fed group also received NO-1886 (50 $mg \cdot kg^{-1} \cdot d^{-1}$), which was thoroughly mixed into the food. All diets were fed for 4 weeks, and at weekly intervals blood (250 μL) in the postabsorptive state (9:00 AM) was taken from the tail vein by tipping the warmed tail. All results are reported for seven to eight rats per diet/diet + drug group.

Indirect Calorimetry

Measurements of the respiratory quotient (RQ) were made on pairs of rats by indirect calorimetry over 24 hours early in the fourth week after commencement of the diet. Rats from the same experimental group were paired and housed together in a metabolic cage with ad libitum access to water and food for the 24-hour period. Air was drawn through the cage at a rate of 3.0 L/min. The carbon dioxide and oxygen content of the gas efflux was measured using a Datex Deltatrac gas analyzer (Helsinki, Finland). The RQ for the duplicate animals was calculated at standardized conditions for temperature, atmospheric pressure, and water vapor using the equations of Frayn.[16] Urinary nitrogen excretion was standardized to 200 mg/d in all rats based on data from Rafecas et al.[17] Gas analyzers were calibrated before measurement with a standard gas mixture (CO_2/O_2 5%/95%).

IVGTT

After 28 days on the diets, the rats were anesthetized using pentobarbital-ketamine and an indwelling cannula was placed in the right jugular vein as described previously.[18] The rats were allowed to recover from the surgery for 4 days, and then were fasted overnight before administration of an intravenous glucose load (0.5 g/kg). Blood samples were collected at 0, 2, 5, 10, 15, 30, 45, and 60 minutes following glucose administration. The animals were killed at completion of the IVGTT by anesthetic overdose (pentobarbital 100 mg/kg intravenously). Tissues collected included the liver, epididymal white adipose tissue (WAT), heart, and quadriceps hindlimb skeletal muscle, separated into white (composed mainly of fast-twitch glycolytic fibers) and red (containing a mixture of slow-twitch oxidative and fast-twitch oxidative-glycolytic fibers) portions.[19] The tissues were immediately frozen in liquid nitrogen for subsequent measurement of tissue triglyceride content.

Analytical Methods

Plasma glucose levels were measured using a glucose oxidase-peroxidase method with 4-aminoantipyrine as the dye. Plasma triglyceride and FFA levels were measured using commercial enzymatic colorimetric methods (Boehringer, Mannheim, Germany, and Wako Chemicals, Osaka, Japan). The plasma insulin level was measured using a double-antibody radioimmunoassay with rat insulin standards and anti-rat insulin first antibody (Linco Research, St Louis, MO). Tissue triglyceride levels were measured by a colorimetric enzymatic procedure as for plasma triglycerides, after extraction of total tissue lipid with chloroform-methanol. The tissue triglyceride concentration is expressed as milligrams triglyceride per gram tissue wet weight.

Statistical Analysis

Results are expressed as the mean ± SEM. Statistical comparisons were made by ANOVA with Fisher's protected least significant difference (PLSD) posthoc comparisons. Continuous RQ measurements were analyzed using ANOVA with repeated measures, with all posthoc comparisons made using Fisher's PLSD test. Incremental areas under the curves were calculated geometrically using the trapezoid rule. All statistical evaluations were performed using the Statview 4 statistical package (Abacus Concepts, Berkeley, CA). A P value less than .05 was considered statistically significant.

RESULTS

Effects of High-Fructose Diet and NO-1886 on Weight Gain

The effects of the fructose diet and NO-1886 supplementation on body weight, tissue weight, and plasma parameters are shown in Table 2. Neither the fructose diet nor NO-1886 supplementation had any significant impact on body weight gain. Final liver and epididymal WAT weights were significantly increased by fructose feeding and were unaltered by NO-1886 supplementation in the fructose diet. Plasma glucose was increased by fructose feeding when compared with levels in the starch-fed group; NO-1886 supplementation abolished this increase. Fructose feeding had no significant effect on plasma insulin levels, but supplementation of the fructose diet with NO-1886 increased plasma insulin compared with the levels in starch-fed rats. Accurate measurement of food intake

Table 2. Effects of NO-1886 Supplementation and Diet on Body Weight, Final Liver and Epididymal WAT Weight (tissue taken after an overnight fast and following the IVGTT), and Postabsorptive Plasma Glucose and Insulin Concentrations (mean ± SM)

Parameter	Starch	Fructose	Fructose (+) NO-1886
No. of rats	8	7	8
Initial body weight (g)	235.5 ± 3.0	246.5 ± 5.6	240.0 ± 4.6
Final body weight (g)	399.4 ± 5.3	405.3 ± 8.8	401.4 ± 7.4
Plasma glucose (mmol/L)	8.23 ± 0.15	9.94 ± 0.45†	8.23 ± 0.15‡
Plasma insulin (pmol/L)	180.8 ± 19.7	198.5 ± 27.1	227.1 ± 25.4
Liver weight (g)	13.17 ± 0.40	15.34 ± 0.54*	15.42 ± 0.72*
Epididymal WAT (g)	11.22 ± 1.02	14.32 ± 1.46*	14.94 ± 1.08*

*$P < .05$, †$P < .01$: *v* starch-fed. ‡$P < .05$, fructose *v* fructose + NO-1886.

was not performed in this study, as food spillage could not be measured. However, gross food intake measured as the difference in the weight of the diet over 24 hours was unaltered with addition of NO-1886 to the diet (data not shown).

Effect of NO-1886 Supplementation and Diet on Plasma Triglycerides

Plasma triglyceride concentrations were significantly increased in rats fed the fructose diet from the first week of study, and this persisted for the remainder of the study period. NO-1886 supplementation the fructose diet markedly decreased plasma triglyceride concentrations, an effect that reached statistical significance in the first and fourth weeks of the study (Fig 1).

Respiratory Gas Exchange

The effects of NO-1886 supplementation and fructose diet on the 24-hour RQ are shown in Fig 2. RQ values were recorded at 1-minute intervals, and in Fig 2 each point represents the hourly mean of 60 measurements. Figure 2 demonstrates the diurnal pattern in the RQ, with all groups exhibiting a relative plateau in the RQ over the dark period of the day (6 PM to 6 AM). With the onset of the light period, the RQ decreased in all groups before returning to the nocturnal level late in the afternoon. The 24-hour mean RQ was highest in the fructose-fed group (1.06 ± 0.003). Addition of NO-1886 to the fructose diet significantly decreased the 24-hour mean RQ (1.03 ± 0.003, $P = .03$). Rats on the starch diet had a lower RQ than fructose-fed rats, which was maintained throughout the entire 24 hours (1.04 ± 0.003), although this reduction failed to reach statistical significance.

Intravenous Glucose Tolerance

Glucose and insulin responses to an intravenous glucose load in all groups of animals after overnight fasting are presented in Fig 3. Basal plasma glucose levels were higher in fructose-fed rats (9.2 ± 0.3 mmol/L) than in either the starch-fed (8.3 ± 0.3) or fructose + NO-1886 (8.5 ± 0.1) groups (both $P < .05$).

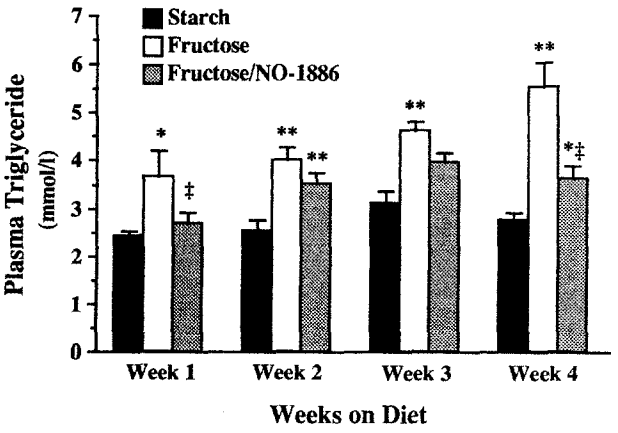

Fig 1. Weekly postabsorptive plasma triglyceride concentrations in rats fed starch or fructose with or without NO-1886 supplementation (50 mg · kg^{-1} · d^{-1}). Each bar is the mean ± SEM for 7 animals. *$P < .05$ *v* starch-fed; **$P < .01$ *v* starch-fed; ‡$P < .05$, fructose *v* fructose/NO-1886.

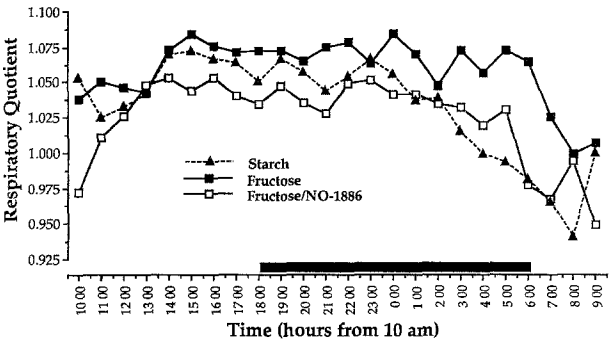

Fig 2. RQ measured over 24 hours in rats fed starch or fructose with or without NO-1886 supplementation (50 mg · kg^{-1} · d^{-1}). Solid bar represents the dark period of the day from 6 PM to 6 AM. Each point is the mean for 4 pairs of rats. All rats had ad libitum access to food and water for 24 hours. The mean 24-hour RQ was significantly lower for the fructose + NO-1886 group than for the fructose-only group.

Similarly, basal plasma insulin levels were higher in the fructose-fed group (151 ± 38 pmol/L) versus the starch-fed group (61 ± 8, $P < .02$), with the fructose + NO-1886 group being intermediate (100 ± 10). Fructose-fed rats demonstrated a higher acute elevation in plasma glucose in response to the intravenous glucose load than either the starch or fructose + NO-1886 groups. The glucose concentration of the fructose-fed group remained higher for the remaining measurements, but there was no overall difference between groups in the glucose area under the curve. Fructose-fed rats exhibited a significantly

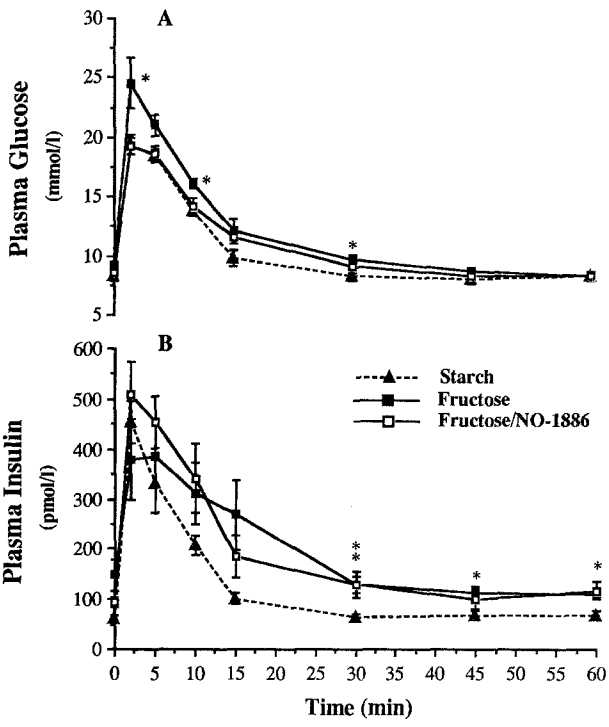

Fig 3. Plasma glucose (A) and insulin (B) concentrations in rats fed starch or fructose with or without NO-1886 supplementation (50 mg · kg^{-1} · d^{-1}) that were fasted overnight and given an intravenous glucose load of 0.5 g/kg at time 0. Each point represents the mean ± SEM for 7 animals. *$P < .05$ *v* starch-fed.

increased integrated area under the insulin curve compared with the starch-fed group ($P < .01$), but the acute insulin response was lower (change in insulin 0 to 2 minutes, 234 ± 83 pmol/L *v* 392 ± 40 and 363 ± 78 for starch and fructose + NO-1886 groups, respectively). NO-1886 supplementation made no significant difference in the integrated insulin response in fructose-fed rats compared with unsupplemented fructose- or starch-fed groups, respectively.

Tissue Triglyceride Accumulation

The triglyceride content of heart, liver, and skeletal muscle is shown in Fig 4. The liver of fructose-fed rats demonstrated a marked elevation in the level of stored triglycerides that was not significantly altered by NO-1886 supplementation when calculated either as a concentration per gram of liver wet weight or total triglycerides per liver. No excess triglyceride accumulation was present in the heart of fructose-fed rats, but supplementation of NO-1886 to the fructose diet significantly increased heart triglyceride levels. Red and white quadriceps muscle showed a tendency toward increased triglyceride content with fructose feeding; however, these increases were not significant ($P = .05$ and $P = .08$ for red and white quadriceps, respectively). There was no effect of NO-1886 on triglyceride accumulation in these muscle groups.

DISCUSSION

In the present study, we examined the actions of a triglyceride-lowering agent, NO-1886, on the plasma triglyceride concentration and intravenous glucose tolerance in fructose-fed rats, a rodent dietary model of hypertriglyceridemia and insulin resistance. Supplementation of NO-1886 in a high-fructose diet significantly ameliorated the dietary-induced hypertriglyceridemia. The decrease in plasma triglycerides with NO-1886 was not accompanied by a significantly increased triglyceride accumulation in either the liver or the skeletal muscle, but was

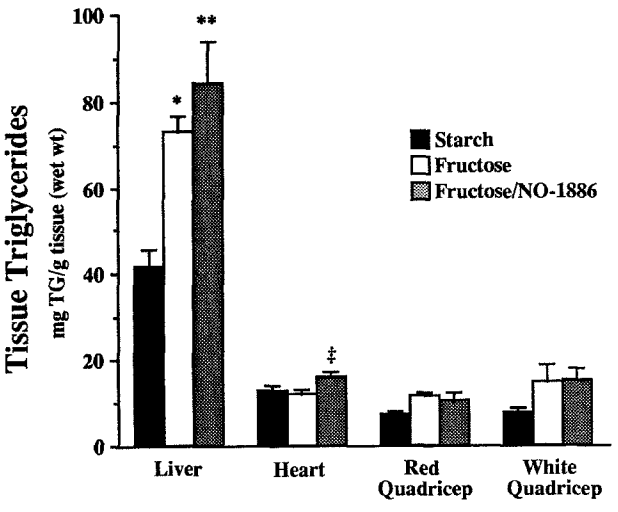

Fig 4. Tissue triglyceride accumulation in rats fed starch or fructose with or without NO-1886 supplementation (50 mg · kg^{-1} · d^{-1}) that were fasted overnight. The animals were killed by anesthetic overdose 65 minutes after administration of intravenous glucose 0.5 g/kg. Each point represents the mean ± SEM. *$P < .05$ *v* starch-fed, **$P < .01$ *v* starch fed; ‡$P < .05$, fructose *v* fructose/NO-1886.

associated with an increase in fat oxidation as indexed by a significant decrease of the mean 24-hour RQ. The increased rate of fat oxidation was not accompanied by a further impairment of whole-body insulin sensitivity as determined by the IVGTT.

Dietary-induced hypertriglyceridemia following fructose feeding is primarily due to an enhanced rate of hepatic VLDL-triglyceride synthesis and secretion. It is known that fructose stimulates de novo lipogenesis, although the mechanisms underlying this alteration are unclear.[20] Despite some data suggesting that triglyceride clearance is also impaired by fructose feeding,[21] other analyses have shown that adipose tissue LPL activity and triglyceride clearance are not altered.[13,22] Therefore, the insulin resistance and hypertension present in fructose-fed rats appear to be secondary to the elevated VLDL secretion,[13] and certainly, whole-body insulin resistance following fructose feeding is closely related to circulating triglyceride levels.[15] The insulin resistance induced by fructose feeding involves the liver,[15,23] and while there is evidence that in peripheral tissues, particularly skeletal muscle, there is also an impairment of insulin action,[15] it is not a universal conclusion.[23] Thus, in the current study, any effects on glucose tolerance are likely to reflect changes at the level of the liver, but possibly also in skeletal muscle.

It is uncertain in dietary-induced hypertriglyceridemia whether the increased lipid supply to peripheral tissues leads to increased peripheral tissue triglyceride accumulation and/or an increased rate of β-oxidation. In the current study, fructose feeding did not result in a significant elevation of skeletal muscle tissue triglyceride stores, consistent with previous reports,[24] although liver triglyceride concentrations were markedly elevated by the fructose diet. The RQ in the freely moving and feeding rats of this study showed a pronounced diurnal pattern, with a very high RQ during the dark period of the day indicating sustained lipogenesis. During the light period, the RQ decreased in all groups before increasing prior to the resumption of the dark period. Fructose-fed rats maintained a higher daily RQ compared with the other experimental groups, with the RQ for the greater part of the 24-hour period being greater than 1.0. Maintenance of this high RQ is most likely due to the fructose-induced stimulation of de novo lipid synthesis in these animals. From analysis of the reaction sequence from glucose to stearic acid, a common fatty acid, a RQ of 5.6 has been estimated by Frayn.[16] Therefore, in the fructose-fed rats, the resultant whole-body RQ is likely the sum of a complex interaction between lipogenesis, fat oxidation, and carbohydrate oxidation.

Addition of the LPL activator NO-1886 to the fructose diet decreased circulating triglyceride concentrations and resulted in a reduction in the RQ both over the entire day and during the dark period. The absence of increased tissue triglyceride storage after NO-1886 treatment would suggest that the decreased RQ is the result of increased fat oxidation rather than a reduction in the lipogenic rate. It is well established that there is substrate competition between FFAs and glucose for oxidation, with elevated rates of fat oxidation resulting in reduced glucose oxidation.[25] Because of the increased LPL activity and fat oxidation following NO-1886 supplementation, a further impairment of insulin sensitivity in NO-1886–treated fructose-fed rats might have been observed. In the present study, evaluation of

whole-body insulin sensitivity using an IVGTT did not reveal any significant additional impact of NO-1886 supplementation on glucose clearance and insulin secretion in fructose-fed rats. If anything, the lower fasting insulin and glucose levels in NO-1886 rats compared with non–drug-treated fructose-fed rats suggest a positive effect on insulin action.

In summary, NO-1886 significantly decreased the elevated postabsorptive plasma triglyceride concentrations in fructose-fed rats. This reduction in plasma triglycerides was not accompanied by increased tissue accumulation of triglyceride, suggesting that the increased triglyceride clearance may be associated with an elevated rate of fat oxidation. NO-1886 had no detrimental effect on intravenous glucose tolerance. These findings suggest that NO-1886 may provide the basis for minimizing the degree of hypertriglyceridemia in insulin resistance.

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REFERENCES

1. Wingard DL, Barrett-Connor E, Criqui MH, et al: Clustering of heart disease risk factors in diabetic compared to non-diabetic adults. Am J Epidemiol 117:19-26, 1983

2. Fontbonne A, Eschwege E, Cambien F, et al: Hypertriglyceridaemia as a risk factor of coronary heart disease mortality in subjects with impaired glucose tolerance of diabetes. Diabetologia 32:300-304, 1989

3. Patsch JR, Misesnbock G, Hopferwieser T, et al: Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 12:1336-1345, 1992

4. Eckel RH: Lipoprotein lipase: A multifunctional enzyme relevent to common metabolic diseases. N Engl J Med 320:1060-1068, 1989

5. Knudsen P, Eriksson J, Lahdenpera S, et al: Changes of lipolytic enzymes cluster with insulin resistance syndrome. Diabetologia 38:344-350, 1995

6. Coppack SW, Evans RD, Fisher RM, et al: Adipose tissue metabolism in obesity: Lipase action in vivo before and after a mixed meal. Metabolism 41:264-272, 1992

7. Pollare T, Vessby B, Lithell H: Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. Arterioscler Thromb 11:1192-1203, 1991

8. Yost TJ, Froyd KK, Dalan RJ, et al: Changes in skeletal muscle lipoprotein lipase activity in response to insulin/glucose in non–insulin-dependent diabetes mellitus. Metabolism 44:786-790, 1995

9. Randle PJ, Kerbey AL, Espinal J: Mechanisms decreasing glucose oxidation in diabetes and starvation: Role of lipid fuels and hormones. Diabetes Metab Rev 4:623-638, 1988

10. 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 ω-3 fatty acids in muscle phospholipid. Diabetes 40:280-291, 1991

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

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

13. Zavaroni I, Chen Y-DI, Reaven GM: Studies of the mechanism of fructose-induced hypertriglyceridemia in the rat. Metabolism 31:1077-1083, 1982

14. Hwang IS, Ho H, Hoffman BB, et al: Fructose-induced insulin resistance and hypertension in rats. Hypertension 10:512-516, 1987

15. Thorburn AW, Storlien LH, Jenkins AB, et al: Fructose-induced in vivo insulin resistance and elevated plasma triglyceride levels in rats. Am J Clin Nutr 49:1155-1163, 1989

16. Frayn KN: Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 55:628-634, 1983

17. Rafecas I, Esteve M, Fernandez-Lopez J-A, et al: Methodological evaluation of indirect calorimetry data in lean and obese rats. Clin Exp Pharmacol Physiol 20:731-742, 1993

18. Bryson JM, Cooney GJ, Wensley V, et al: High-fat feeding alters the response of rat PDH complex to acute changes in glucose and insulin. Am J Physiol 268:E752-E757, 1995

19. Armstrong RB, Phelps RO: Muscle fibre type composition of the rat hindlimb. Am J Anat 171:259-272, 1984

20. Park O-J, Cesar D, Faix D, et al: Mechanisms of fructose-induced hypertriglyceridaemia in the rat. Biochem J 282:753-757, 1992

21. Kazumi T, Vranic M, Steiner G: Triglyceride kinetics: Effects of dietary glucose, sucrose or fructose alone or with hyperinsulinemia. Am J Physiol 250:E325-E330, 1986

22. Yoshino G, Matsushita M, Iwai M, et al: Effect of mild diabetes and dietary fructose on very–low-density lipoprotein triglyceride turnover in rats. Metabolism 41:236-240, 1992

23. Tobey TA, Mondon CE, Zavaroni I, et al: Mechanism of insulin resistance in fructose-fed rats. Metabolism 31:608-612, 1982

24. Storlien LH, Oakes ND, Pan DA, et al: Syndromes of insulin resistance in the rat: Inducement by diet and amelioration with benfluorex. Diabetes 42:457-462, 1993

25. Ebeling P, Koivisto VA: Non-esterified fatty acids regulate lipid and glucose oxidation and glycogen synthesis in healthy men. Diabetologia 37:202-209, 1994