Lipoprotein Lipase and Atherosclerosis

Kusunoki M[1*], Tsutsumi K[2], Natsume Y[1], Tsutsui H[3], Miyata T[4], Oshida Y[1]

[1] *Research Center of Health, Physical Fitness and Sports, Nagoya University, Nagoya, Japan*

[2] *Okinaka Memorial Institute for Medical Research, Tokyo, Japan*

[3] *General Medical Education Center of Teikyo University, Tokyo, Japan*

[4] *Vascular Center, Sanno Medical Center, Tokyo, Japan*

[*] **Corresponding author:** Kusunoki M, Research Center of Health, Physical Fitness and Sports, Nagoya University, Furo-cho Chikusa-ku, Nagoya 464-8601, Japan, Tel: 81527893946; E-mail: info@tonyo.jp

Abstract

Lipoprotein lipase (LPL) plays a pivotal role in lipids and metabolism of lipoprotein, especially in breaking down plasma triglycerides (TG) of TG-rich lipoproteins, including chylomicrons and very low density lipoproteins (VLDL). Dysfunction of LPL contributes to hypertriglyceridemia and decreased high density lipoprotein cholesterol level and has been found to be associated with dyslipidemia, obesity, diabetes, and insulin resistance. Dyslipidemia, obesity, diabetes and insulin resistance are risk factor for atherosclerosis. In the studies, we hypothesized that elevating LPL activity would cause protection of atherosclerosis. To test this hypothesis, the effects of the LPL activator NO-1886 were investigated in various experimental animal models. Taken together, NO-1886 increased LPL mRNA and LPL activity in adipose tissue, myocardium and skeletal muscle, resulting in an elevation of post-heparin plasma LPL activity and LPL mass, and also decreased plasma TG concentration with a concomitant rise in plasma HDL-C, inhibited the development of atherosclerosis in rats and rabbits.

Keywords: Lipoprotein lipase, Insulin resistance, Dyslipidemia, Atherosclerosis

Introduction

Lipoprotein lipase (LPL) is a rate-limiting enzyme that hydrolyzes circulating triglyceride (TG)-rich lipoproteins such as very low-density lipoprotein (VLDL) and chyromicrons. Dysfunctions of LPL have been found to be associated with dyslipidemias, obesity, insulin resistance, and atherosclerosis.

Insulin resistance, which can in part be defined as a reduced capacity to increase glucose uptake and metabolism in target tissues such as skeletal muscle and adipose tissue, has long been associated with elevated plasma TG and non-esterified fatty acids (NEFA).[1] Therefore, understanding the factors that modulate TG and NEFA levels could provide clues for the treatment and prevention of the disease.

Activation of lipoprotein lipase (LPL) results in lowering of the plasma triglyceride (TG) levels and elevation of the plasma high-density lipoprotein cholesterol (HDL-C) levels. Reduction of the plasma TG level and elevation of the plasma HDL-C by activation of LPL may be useful for the prevention of atherosclerosis, since hypertriglyceridemia and HDL-hypocholesterolemia are well-known risk factors for arteriosclerosis.

LPL hydrolyzes the TG in circulating very low-density lipoproteins (VLDL) to yield nonesterified fatty acids (NEFA). The NEFA thus formed are taken up by the skeletal muscles or, peripheral tissues, where they are utilized as energy. In the adipose tissues, the NEFA formed from the TG in VLDL by the action of LPL is resynthesized into TG and stored in the adipose tissues. It could be inferred therefore that activation of LPL has two contrasting effects specifically, reduction of weight and gain in weight; the fate of the body weight, whether it increases or decreases, is considered to depend on the balance between the LPL activity in the skeletal muscles and also in the adipose tissues. Assuming that LPL activation is associated with a reduction of the body weight (fat weight), treatment directed at LPL activation could be expected to lead to a reduction in the risk of obesity and improvement of insulin resistance, and eventually result in suppression of arteriosclerosis.

We have put forward the hypothesis that activation of LPL could lead to a lowering of the TG level in the blood and elevation of the plasma HDL-C level, with the consequent effect of preventing atherosclerosis. With a view to proving this hypothesis, we conducted this study to clarify the relationship of LPL activation to the risk of atherosclerosis using a low-molecular-weight compound (NO-1886: [4-(4-bromo-2-cyano-phenylcarbamoyl)-benzyl]-phosphonic acid diethyl ester, CAS133208-93-2, generic name ”ibrolipim”) endowed with the effect of activating LPL, synthesized by Otsuka Pharmaceutical Factory Inc., Japan.

Background of Lipoprotein Lipase

LPL is a glycoprotein enzyme that catalyzes the hydrolysis of TG in (TG)-rich lipoproteins, and is distributed on the surface of the vascular endothelial cells. LPL occurs mainly in the adipose tissues, skeletal muscles, and myocardium. It is bound to heparan sulfate, a polysaccharide, on the vascular endothelial cells of skeletal muscles and adipose tissues.[2,3] In the assay for LPL, therefore, heparin is injected intravenously to liberate the LPL from the heparan sulfate and the amount of LPL liberated is determined. Plasma collected after the intravenous injection of heparin is called post-heparin plasma, and, usually the term blood LPL assay means quantification of LPL in post-heparin plasma.

It has been reported that elevation of the LPL activity results in lowering of the TG level in the blood, with a concurrent increase of the plasma HDL-C. High-density lipoproteins (HDL), when roughly classified, occur in two major subfractions, HDL2 and HDL3.

According to a report by Nikkilä et al., the subfraction that is best correlated with the LPL activity is HDL2.[4] Tsutsumi and colleagues also noted, in the study of the LPL-activator NO-1886, that an increase of the LPL activity was accompanied by an increase of the HDL2 subfraction.[5]

Relationship between LPL and lipid metabolism and atherosclerosis

There is no conclusive evidence yet clarifying whether activation of LPL might indeed suppress the development of atherosclerosis.

In their clinical study on human LPL mutations, Reymer et al. observed that in subjects with abnormalities of the LPL gene, the activity of LPL was low, the plasma HDL-C level was also low, and the prevalence of atherosclerosis was high.[6] Another study suggested elevation of the serum TG and reduction of the serum HDL-C, associated with a high prevalence of atherosclerosis, in patients heterozygous for LPL deficiency.[7]

An animal study reported by Fan et al. showed dramatic suppression of the development of atherosclerosis in human LPL gene-transgenic rabbits maintained on a cholesterol-rich diet.[8]

Shimada et al. generated transgenic mice overexpressing the human LPL gene in the heart, skeletal muscle and adipose tissue, and found that the transgenic mice exhibited reduced plasma TG levels and elevated plasma HDL2-C levels, reflecting an antiatherosclerotic plasma lipid profile.[9] They also generated (LPL/LDLKO) mice crossbred from LDL receptor-knockout mice (LDLRKO) and LPL-overexpressing mice, and noted that the hybrid mice displayed an antiatherosclerotic plasma lipid profile, similar to the case in the LPL-overexpressing mice, thus concluding that activation of LPL would yield an antiatherosclerotic state.[10]

Effect of LPL activator NO-1886 on LPL, lipid metabolism and atherosclerosis

Single doses of NO-1886 significantly and dose-dependently increased post-heparin plasma LPL activity in normal rats.[5] On the other hand, NO-1886 did not affect post-heparin plasma hepatic TG lipase (HTGL). NO-1886 also significantly and dose-dependently increased tissue LPL activity in normal rats. NO-1886 enhanced expression of LPL mRNA in adipose tissue and myocardium, and increased LPL protein mass and LPL activity in post-heparin plasma.[5] NO-1886 significantly and dose-dependently decreased plasma TG levels, with concomitant increase in plasma HDL-C in rats.

NO-1886 administration for 7 days also significantly decreased plasma TG concentrations and increased HDL-C in hamsters and rabbits.[11] NO-1886 resulted in increased plasma total cholesterol concentrations and concomitantly increased plasma HDL-C in rats, but this phenomenon was not observed in hamsters, rabbits or monkeys. NO-1886 caused a marked elevation of plasma HDL-C, especially HDL2-C. Previous reports have clearly demonstrated that enhanced lipolysis of TG-rich lipoproteins results in an increase in HDL2 particles, and therefore, a precursor-product relationship exists between the two.[12] The transfer of cholesterol from newly formed HDL2 particles to VLDL is mediated by cholesterol ester transfer protein (CETP).[13] However, rats, mice and dogs lack CETP.[14] Because of this, the number of HDL particles following enhanced VLDL degradation by LPL was increased and accumulated in the circulation, resulting in a marked elevation of HDL-C. The increases in plasma total cholesterol are obviously a result of increases in HDL2, as there was no change in cholesterol in the LDL fraction after NO-1886 administration. Hamster, rabbits and monkeys have CETP, and therefore plasma total cholesterol did not increase. These results indicate that NO-1886 may not increase plasma total cholesterol levels in humans because of the presence of CETP.[15]

Endothelial function is closely related to the development of atherosclerosis and is impaired before the development of initial lesions in hypercholesterolemic animals.[16] Aging is associated with a progressive development of dyslipidemia, insulin resistance and obesity, all of which are risk factors for cardiovascular disease and atherosclerosis.[17] It is known that endothelium-dependent relaxation decreases with age.[18] Hara et al. reported that NO-1886 ameliorated the aging-related deterioration of endothelium-dependent relaxation in thoracic aorta in 10-month-old male rats.[19] Kusunoki et al. also reported that NO-1886 prevented the development of impaired endothelium-dependent relaxation of rat thoracic aorta in 2-year-old male rats.[20] These research groups speculated that NO-1886 might have improved the endothelium-dependent relaxation by normalizing the lipid disorder, in particular by elevating plasma HDL-C, which possesses antioxidant effect and is very important in such old rats due to elevated plasma lipid peroxide levels caused by exercise.[20,21]

Tsutsumi et al. reported that there was a noticeable reduction in the incidence of coronary arteriosclerosis following administration of NO-1886 for 90 days in rats maintained on an atherosclerogenic rodent diet, describeing that the multivariate analysis identified elevation of the serum HDL-C as a major factor suppressing the development of arteriosclerosis.[5]

In a study reported by Chiba and coworkers, a decrease of the plasma TG level, elevation of the plasma HDL-C level, and inhibition of aortic atheromatous plaque deposition were evident following 20-week' treatment with NO-1886 in rabbits fed a high-cholesterol diet.[22]

These reports indicate that it is feasible to suppress the development of atherosclerosis by raising the activity of LPL, lowering the plasma TG level and increasing the plasma HDL-C level, even if the plasma cholesterol level is not lowered.

Relationship between LPL and HDL particle size

Plasma HDL-C is known to be a strong protector against coronary artery sclerosis and a number of studies have shown a significant inverse relationship between HDL cholesterol and coronary heart disease.[23,24] Alan Tall and Donald Small hypothesized that HDL may be produced by LPL mediated lipolysis of chylomicrons and VLDL.[25] Furthermore, LPL is reported to cause enlargement of HDL particle size in vitro. HDL particle size is regulated by enzymes and proteins, such as LPL, HTGL and CETP. LPL is involved in the conversion of small (HDL3) particles to large (HDL2) particles.[26] Cheung et al. have reported that HDL particle size in patients with coronary artery disease (CAD) tends to be shifted toward the smaller particle size when compared with the CAD-free control groups.[27] These reports indicate that non-functional HDL does not increase in size and that functional HDL is larger in size compared with non-functional HDL. We believe that not only serum HDL-C concentration but also HDL particle size is very important for prevention of CAD. Kusunoki et al. reported that NO-1886 increased post-heparin plasma LPL activity without influencing of HTGL activity, and not only increased serum HDL2-C concentration but also enlarged HDL2 particle size in rats. On the other hand, NO-1886 had no effect on HDL3-C level and particle size of HDL3.[28] Plasma HDL2-C concentration was positively correlated with the HDL2 particle size in their study. These results may indicate that an enlargement of HDL2 particle size causes an increase in cholesterol content in HDL2 particles. Therefore, it is possible that LPL may cause production of HDL2-C by catabolism of triglyceride-rich lipoproteins and enlarges HDL2 particle size in rats.

Effect of LPL activator NO-1886 on fatty liver

Fatty liver may result from raised levels of plasma free fatty acids (FFA). The quantity of TG present in the liver is significantly increased during starvation and after consumption of high-fat diet. This may be due to low insulin levels. In conditions such as uncontrolled diabetes mellitus, pregnancy toxemia in ewes, and ketosis in cattle, fatty infiltration is sufficiently severe to cause visible pallor (fatty appearance) and enlargement of the liver with dysfunction.[29]

Insulin resistance has been reported to cause fatty liver, but to mild degree only.[30,31] Kusunoki et al. attempted to create a severe fatty liver animal model.[32]

Long-term high-fat feeding in streptzotocin (STZ)-induced diabetic rats increased liver cholesterol contents and plasma TG contents, and as a result caused fatty liver (observed visually). STZ-induced diabetic rats are characterized by low plasma insulin levels and low LPL activity.[33] Therefore, low insulin levels cause high plasma levels of TG and FFA and low levels of HDL-C, and as a result high FFA levels cause fatty liver. Also,

low insulin levels suppress secretion of VLDL, which may result in fatty liver. The high-fat feeding may accelerate fatty liver in STZ-induced diabetic rats. The liver weights in these rats were greater than the liver weights in normal rats, and asparatate aminotransferase (AST) and alanine aminotransferase (ALT) values, which are indicators of abnormal liver, were elevated compared to normal rats. These results indicates that fatty liver causes liver dysfunction.

Kusunoki et al. reported that LPL activator NO-1886 increased plasma HDL-C and decreased plasma TG levels, and decreased the liver cholesterol contents and TG contents, resulting in improvement of the fatty liver (observed visually).[32] Furthermore, NO-1886 decreased the plasma AST and ALT levels. These results indicate that NO-1886 improved fatty liver and associated liver dysfunction. In this report, they observed the relationship between plasma lipid levels and liver lipid contents in STZ-induced diabetic rats fed a high-fat diet. The liver cholesterol contents were inversely correlated with plasma HDL-C levels and were positively correlated with plasma TG levels. There was no noticeable relationship between the plasma cholesterol levels, and the liver cholesterol and TG contents. These results are compatible with the understanding that a reduction in plasma TG levels and an elevation in HDL-C levels may necessary to improve fatty liver.

More recently, Ciccone et al. mentioned that hepatitis and nonalcoholic fatty liver disease could be aligned to inflammatory bowel disease in an induction of atherosclerosis disease.[34] We like to study regarding the relationship between inflammatory and LPL in the future.

Relationship between LPL and obesity and insulin resistance

Dysfunctions of LPL have been found to be associated with dyslipidemias, atherosclerosis obesity and insulin resistance. Insulin resistance, which can in part be defined as a reduced capacity to increase glucose uptake and metabolism in target tissues such as skeletal muscle and adipose tissue, has long been associated with elevation of plasma TG and NEFA.[1] Therefore, understanding the contributing factors that modulate TG and NEFA levels could provide clues for the treatment and prevention of the disease.

LPL plays a pivotal role in lipids and the metabolism of lipoprotein.1 Major functions of LPL include the hydrolysis of TG-rich lipoproteins and release of NEFA, which are taken up and used for metabolic energy in peripheral tissue such as muscle, or are re-esterified into TG and stored in adipose tissue. The balance between these competing effects could determine whether increased LPL activity will lead to a reduced rate of weight gain or to increased adiposity through increased rates of adipose tissue storage of TG. An imbalance of LPL activity may alter the partitions of plasma TG between muscle and adipose tissue, and thus influence insulin resistance and obesity. LPL activity was found to be higher in adipose tissue of obese animals and humans, which could lead to increased NEFA influx through the portal vein into the liver, and could again result in a state of insulin resistance.[35,36] These suggest that high LPL activity could be associated with, or are causes of, insulin resistance and obesity.

On the other hand, increased LPL activity has been shown to have completely opposite effects on glucose metabolism and insulin sensitivity. Patients deficient in LPL have hypertriglyceridaemia and high levels of NEFA, which is associated with insulin resistant diabetes and can be ameliorated by lowering levels of TG.[37]

It might be difficult to elucidate the multiple and complex roles of LPL in humans in terms of insulin resistance and disorders such as diabetes and obesity. One of the best experimental approaches is to use transgenic animals that overexpress LPL. Kitajima et

al. created human-LPL-overexpressing transgenic rabbits by microinjection of human LPL cDNA under the control of the chicken β-actin promoter and feeding of fed high-fat diet.[38] They showed that systematically increased LPL activity improves insulin resistance and reduces adipose accumulation in transgenic rabbits, indicating that systemic elevation of LPL may have potential benefits for the treatment of insulin resistance and obesity.

Hara et al. reported that LPL activator NO-1886 treatment in high-fructose diet induced insulin resistance rats decreases the respiratory quotient (RQ) and plasma TG.[39] Such results may indicate that NO-1886 ameliorates insulin resistance and obesity. Therefore, Kusunoki et al. hypothesized that elevating LPL activity would cause an improvement of insulin resistance, and to test this hypothesis, they studied the effects of the LPL activator NO-1886 in obese rats with insulin resistance.[40]

As has been persuasively argued, long-term imbalance between intake and expenditure of fat is a central factor in the etiology of obesity.[41] Hence the distribution of lipid between adipose tissue (largely storage) and muscle (largely oxidation) is critical. The role for tissue-specific regulation of LPL in treatment of obesity was postulated previously.[42] Rats fed a high-fat diet are a useful model of metabolic syndrome in terms of insulin resistance and obesity. Kusunoki et al. have shown that NO-1886 in a high-fat diet tends to increase muscle LPL, reduce body weight and fat accumulation and improve insulin action, all consistent with the increase in fat oxidation (a low RQ) observed.40 These results are consistent with the observation that diet-induced obesity in mice can be prevented by creating transgenics overexpressing a skeletal muscle-specific human LPL gene.[43] Taken together these results suggest that tissue-specific LPL activators have potential in the therapy for obesity and related disorders.

Yin et al. reported that LPL promoting agent NO-1886 suppresses the elevation of blood glucose in rabbits induced by feeding a high-fat/high-sucrose diet, probably through controlling lipid metabolism and improving insulin resistance.[44]

Although the LPL activator NO-1886 shows antiobese effects in high-fat induced obese animals, the mechanism remains unclear. To clarify the mechanism, Kusunokiet al. studied the effects of NO-1886 on the expression of uncoupling protein (UCP) 1, UCP2, and UCP3 in rats.[45] At the end of administration period, brown adipose tissue (BAT), mesenteric fat, and soleus muscle were collected and levels of UCP1, UCP2, and UCP3 mRNA were determined. NO-1886 suppressed the body weight increase seen in the high-fat control group after 8-week administration. NO-1886 also suppressed fat accumulation in visceral and subcutaneous tissues and increased the level of plasma HDL-C. In contrast, NO-1886 decreased the levels of plasma TG, NEFA, glucose, and insulin. NO-1886 increased LPL activity in soleus muscle. NO-1886 increased the expression of UCP3 mRNA in soleus muscle. NO-1886 did not affect the expression of UCP1 and UCP2 in BAT, mesenteric adipose tissue, and soleus muscle. In conclusion, NO-1886's antiobese effects in rats may be the enhancement of LPL activity in skeletal muscle and the accompanying increase in UCP3 expression.

Pioglitazone improves insulin resistance in diabetes but often causes body weight gain. On the other hand, NO-1886 has been shown to exert both anti-obesity and anti-insulin resistance. Therefore, Kusunokiet et al. investigated the effect of the combined administration of pioglitazone with NO-1886 in preventing body weight gain in insulin resistant, high-fat fed rats.[46] The high-fat fed control rats developed obesity and insulin resistance. After 7 weeks of drug treatment, additional administration of NO-1886 to pioglitazone prevented the body weight gain caused by pioglitazone alone and to increase glucose infusion rate during insulin clamp. These results indicate that combined

therapy with pioglitazone and NO-1886 may be beneficial for the treatment of type 2 diabetes.

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.[31] Insulin resistance causes a reduction in the rate of muscle glycogen synthesis in subjects with type 2 diabetes.[47,48] 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.

Kusunoki et al. studied that the relationship between fatty liver and glycogen contents in liver of high-fat fed rats. LPL activator NO-1886 administration resulted in glucose infusion rate (GIR) values returning to normal.[49] These results indicate that NO-1886 improved insulin resistance in this model. 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 release. Also, NO-1886 did not decrease plasma glucose in insulin-deficient streptzotocin-induced diabetic model rats.[14] 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. 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.[50] Therefore, NO-1886 and MCC-555 may have different mechanisms for the improvement of insulin resistance. However, these results may indicate that the improvement of insulin resistance causes the increase in glycogen contents in diabetes.

In conclusion, as discussed above, the results of studies suggest that LPL may increase insulin sensibility, and current studies indicate that elevation of LPL may protect against high-fat fed induced insulin resistance and obesity. Furthermore, it is suggested that LPL might be a therapeutic target for treatments of atherosclerosis as well as insulin resistance and obesity. In addition, it is anticipated that such results and data from animal experiments can be proved and confirmed clinically in the future.

References

1. Randle PJ., Garland PB., Hales CN., Newsholme EA. The glucose-fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1963; 1: 785-789.
2. Clarke AR., Luscombe M., Holbrook JJ. The effect of chain length of heparin units interaction with lipoprotein lipase. Biochim Biophys Acta 1983; 747: 130-137.
3. Pedersen ME., Cohen M., Schotz MC. Immunocytochemical localization of the functional fraction of lipoprotein lipase in the perfused heart. J Lipid Res 1983; 24: 512-521.

4. Nikkilä EA., Taskinen MR., Kekki M. Relation of plasma high-density lipoprotein cholesterol to lipoprotein-lipase activity in adipose tissue and skeletal muscle of man. Atherosclerosis 1978; 29: 497-501.

5. Tsutsumi K., Inoue Y., Shima A., Iwasaki K., Kawamura M., et al. The novel compound NO-1886 increase 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 1993; 92: 411-417.

6. Reymer PW., Gagné E., Groenemeyer BE., Zhang H., Forsyth I., et al. A lipoprotein lipase mutation (Asn291Ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis. Nat Genet 1995; 10: 28-34.

7. Gaziano JM., Hennekens CH., O'Donnell CJ., Breslow JL., Buring JE. Fasting triglycerides, high density lipoprotein, and risk of myocardial infarction. Circulation 1997; 96: 2520-2525.

8. Fan J., Unoki H., Kojima N., Sun H., Shimoyamada H., et al. Overexpression of lipoprotein lipase in transgenic rabbits inhibits diet-induced hypercholesterolemia and atherosclerosis. J Biol Chem 2001; 276: 40071-40079.

9. Shimada M., Shimano H., Gotoda T., Yamamoto K., Kawamura M., et al. Overexpression of human lipoprotein lipase in transgenic mice. Resistance to diet-induced hypertriglyceridemia and hypercholesterolemia. J Biol Chem 1993; 268: 17924-17929.

10. Shimada M., Ishibashi S., Inaba T., Yagyu H., Harada K., et al. Suppression of diet-induced atherosclerosis in low density lipoprotein receptor knockout mice overexpressing lipoprotein lipase. Proc Natl Acad Sci USA 1996; 93: 7242-7246.

11. Tsutsumi K., Inoue Y., Hagi A., Murase T. The novel compound NO-1886 elevates plasma high-density lipoprotein cholesterol levels in hamsters and rabbits by increasing lipoprotein lipase without any effect on cholesteryl ester transfer protein activity. Metabolism 1997; 46: 257-260.

12. Taskinen MR., Nikkilä EA. High density lipoprotein subfractions in relation to lipoprotein lipase activity of tissues in man – evidence for reciprocal regulation of HDL2 and HDL3 levels by lipoprotein lipase. Clin Chim Acta 1981; 112: 325-332.

13. Tall AR. Plasma lipid transfer proteins. Ann Rev Biochem 1995; 64: 235-257.

14. Tall AR. Plasma lipid transfer proteins. J Lipid Res 1986; 27: 361-367.

15. Ha YC., Barter PJ. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. Comp Biochem Physiol B 1982; 71: 265-269.

16. Shimokawa H., Vanhoutte PM. Impaired endothelium-dependent relaxation to aggregating platelets and related vasoactive substances in porcine coronary arteries in hypercholesterolemia and atherosclerosis. Circ Res 1989; 64: 900-914.

17. Lakatta EG., Yin FC. Myocardial aging: functional alterations and related cellular mechanisms. Am J Physiol 1982; 242: H927-H941.

18. Moritoki H., Tanioka A., Maeshiba Y., Iwamoto T., Ishida Y., et al. Age-associated decrease in histamine-induced vasodilation may be due to reduction of cyclic GMP formation. Br J Pharmacol 1988; 95: 1015-1022.

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

20. Kusunoki M., Tsutsumi K., Hara T., Ogawa H., Nakamura T., et al. A lipoprotein lipase activator, NO-1886 prevents impaired endothelium-dependent relaxation of aorta caused by exercise in aged rats. Exp Gerontol 2002; 37: 891-896.

21. Ohta T., Takata K., Horiuchi S., Morino Y., Matsuda I. Protective effect of lipoproteins containing apolipoprotein A-I on Cu2+ catalyzed oxidation of human low density lipoprotein. FEBS Lett 1989; 257: 435-438.

22. Chiba T., Miura S., Sawamura F., Uetsuka R., Tomita I., et al. (1997) Antiatherogenic effects of novel lipoprotein lipase-enhancing agent in cholesterol-fed New Zealand White rabbits. Arterioscler Thromb Vasc Biol 1997; 17: 2601-2608.

23. Tall AR. Plasma high density lipoproteins. Metabolism and relationship to atherogenesis. J Clin Invest 1990; 86: 379-384.

24. Jacobs DR., Mebane IL., Bangdiwala SI., Criqui MH., Tyroler HA. High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: the follow-up study of the Lipid Research Clinics Prevalence Study. Am J Epidemiol 1990; 131: 32-47.

25. Tall AR., Small DM. Plasma high-density lipoproteins. N Engl J Med 1978; 299: 1232-1236.

26. Patsch JR., Gotto AM., Olivercrona T., Eisenberg S. Formation of high density lipoprotein2-like particles during lipolysis of very low density lipoproteins in vitro. Proc Natl Acad Sci USA 1978; 75: 4519-4523.

27. Cheung MC., Brown BG., Wolf AC., Albers JJ. Altered particle size distribution of apolipoprotein A-I-containing lipoproteins in subjects with coronary artery disease.J Lipid Res 1991; 32: 383-394.

28. Kusunoki M., Tsutsumi K., Sato D., Nakamura A., Habu S., et al. Activation of lipoprotein lipase increases serum high density lipoprotein 2 cholesterol and enlarges high density lipoprotein 2 particles in rats. Eur J Pharmacol 2011; 668: 337-339.

29. Mayes PA. Lipid transport and storage and cholesterol synthesis, transport, and excretion. Harper's Biochemistry, 25th ed. New York: McGraw-Hill, 2000; 268-284.

30. Nakamura M., Yamada K. Studies on diabetic (KK) strain of the mouse. Diabetologia 1967; 3: 212-221.

31. Moller DE., Filer JS. Insulin resistance – mechanisms, syndromes, and implications. New Engl J Med 1991; 325: 938-948.

32. Kusunoki M., Tsutsumi K., Inoue Y., Hara T., Miyata T., et al. Lipoprotein lipase activator NO-1886 improves fatty liver caused by high-fat feeding in streptzotocin-induced diabetic rats. Metabolism 2004; 53: 260-263.

33. Tsutsumi K., Inoue Y., Shima A., Murase T. Correction of hypertriglyceridemia with low high-density lipoprotein cholesterol by the novel compound NO-1886, a lipoprotein lipase-promoting agent, in STZ-induced diabetes rats. Diabetes 1995; 44: 414-417.

34. Ciccone MM., Pricipi M., Ierardi E., Di Leo A., Ricci G., et al. Inflammatory bowel disease, liver diseases and endothelial function: is there a linkage? J Cardiovasc Med 2015; 16: 11-21.

35. Hotamisligil GS., Shargill NS., Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 1993; 259: 87-91.

36. Hotamisligil GS., Peraldi P., Budavari A., Ellis R., White MF., et al. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. Science 1996; 271: 665-668.

37. Mingrone G., Henriksen FL., Greco AV., Krogh LN., Capristo E., et al. Triglyceride-induced diabetes associated with familial lipoprotein lipase deficiency. Diabetes 1999; 48: 1258-1263.

38. Kitajima S., Morimoto M., Liu E., Koike T., Higaki Y., et al. Overexpression of lipoprotein lipase improves insulin resistance induced by a high-fat diet in transgenic rabbits. Diabetologia 2004; 47: 1202-1209.

39. Hara T., Cameron-Smith D., Cooney GJ., Kusunoki M., Tsutsumi K., et al. (1998) The actions of a novel lipoprotein lipase activator, NO-1886, in hypertriglyceridemic fructose-fed rats. Metabolism 1998; 47: 149-153.

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

41. Flatt JP. McCollum Award Lecture, 1995: diet, lifestyle, and weight maintenance. Am J Clin Nutr 1995; 62: 820-836.

42. Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. N Eng J Med 1989; 320: 1060-1068.

43. Jensen DR., Schlaepfer IR., Morin CL., Pennington DS., Marcell T., et al. Prevention of diet-induced obesity in transgenic mice overexpressing skeletal muscle lipoprotein lipase. Am J Physiol 1997; 273: R683-R689.

44. Yin W., Yuan Z., Tsutsumi K., Xie Y., Zhang Q., et al. A lipoprotein lipase-promoting agent, NO-1886, improves glucose and lipid metabolism in high fat, high sucrose-fed New Zealand White rabbits. Int J Exp Diabesity Res 2003; 4: 27-34.

45. Kusunoki M., Tsutsumi K., Iwata K., Yin W., Nakamura T., et al. NO-1886 (ibrolipim), a lipoprotein lipase activator, increases the expression of uncoupling protein 3 in skeletal muscle and suppresses fat accumulation in high-fat diet-induced obesity in rats. Metabolism 2005; 54: 1587-1592.

46. Kusunoki M., Tsutsumi K., Sato D., Nakamura A., Habu S., et al. Pioglitazone-induced body weight gain is prevented by combined administration with the lipoprotein lipase activator NO-1886. Eur J Pharmacol 2011; 668: 486-491.

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

48. Thorburn AW., Gumbiner B., Bulacan F., Brechtel G., Henry RR. Multiple defects in muscle glycogen synthase activity contribute to reduced glycogen synthesis in non-insulin dependent diabetes mellitus. J Clin Invest 1991; 87: 489-495.

49. Kusunoki M., Tsutsumi K., Hara T., Ogawa H., Nakamura T., et al. Correlation between lipid and glycogen contents in liver and insulin resistance in high-fat fed rats treated with the lipoprotein lipase activator NO-1886. Metabolism 2002; 51: 792-795.

50. Upton R., Widdowson PS., Ishii S., Tanaka H., Williams G. 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 1998; 125: 1708-1714.