Global Health & Medicine. 2019; 1(1):30-35. REVIEW

DOI: 10.35772/ghm.2019.01007

Diabetic dyslipidemia: evaluation and mechanism

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Abstract: Diabetes is one of the well-established independent risk factors for cardiovascular diseases. Diabetes induces dyslipidemia which is characterized by elevated fasting triglyceride (TG) and reduced high-density lipoprotein-cholesterol (HDL-C), and such diabetic dyslipidemia is a crucial determinant for atherogenesis and atherosclerotic progression in patients with diabetes. Previous measurement methods of lipoproteins have problems including time-consuming (ultracentrifugation) and inaccurate and impossible measurements of TG-rich lipoproteins such as chylomicron, intermediate-density lipoprotein (IDL) and very low-density lipoprotein (VLDL). Our developed anion-exchange high-performance liquid chromatography (AEX-HPLC) can measure all fractions of lipoproteins accurately. Our studies using AEX-HPLC showed that IDL and VLDL in type 2 diabetes were higher than non-diabetic subjects, and IDL and VLDL were higher in the order of type 2 diabetic patients with obesity, type 2 diabetic patients without obesity, and non-diabetic subjects. Here, we also describe the underlying mechanisms for development of diabetic dyslipidemia.

***Keywords*:** Diabetes, high-performance liquid chromatography, insulin resistance, lipoproteins, triglyceride

Introduction

Diabetes is one of the well-established independent risk factors for cardiovascular diseases (*1*). Diabetes induces atherosclerosis and resulting atherosclerotic diseases such as cerebral vascular diseases, ischemic heart diseases, and peripheral arterial diseases, which are major causes of death in patients with diabetes and significantly reduce their quality of life (*2-4*). Although various factors such as hyperglycemia are involved in progression of atherosclerosis in diabetes, diabetic dyslipidemia may be a crucial determinant for atherogenesis and atherosclerotic progression in patients with diabetes.

Diabetic dyslipidemia is characterized by elevated fasting triglyceride (TG), reduced high-density lipoprotein-cholesterol (HDL-C), and elevated low-density lipoprotein-cholesterol (LDL-C) and small dense LDL (*5*). Here, we show the characteristics of diabetic dyslipidemia, which have been demonstrated by our developed anion-exchange high-performance liquid chromatography (AEX-HPLC) (*6*). We also discuss the underlying mechanisms for development of diabetic dyslipidemia.

The measurement of lipoproteins in patients with diabetess

Profiles of serum lipoproteins

Profiles of serum lipoproteins are shown in Figure 1 (*7*). Lipoproteins are classified by density, which is determined mainly by content of TG. The particle sizes of chylomicron (CM) and very low-density lipoprotein (VLDL) are relatively large, however, densities of such lipoproteins are low due to high content of TG. CM, VLDL and intermediate-density lipoprotein (IDL) are called TG-rich lipoproteins, which are increased in type 2 diabetes, insulin resistance and obesity. Studies of lipoprotein/arterial wall interactions have demonstrated that the larger the lipoprotein particle, the lower the influx into intima. Therefore, CM are unlikely to induce atherosclerosis because CM do not seem to enter intima (*8*). Among TG-rich lipoproteins, IDL and VLDL are important for atherogenesis. LDL is a cholesterol-rich lipoprotein, which is a well-established atherogenic lipoprotein (*9*). HDL contains small amounts of TG and cholesterol, and plays an important role in anti-atherogenesis by reverse cholesterol transport. The incidence rates of coronary heart disease and definite myocardial infarction were three to four times higher in the lowest HDL-C quartile (< 1.24 mmol/L) than the highest quartile (≥ 1.66 mmol/L), and there was a significant dose response for definite myocardial infarction (*10*).

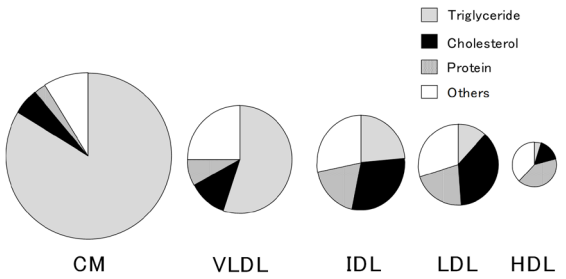

Figure 1. Profiles of serum lipoproteins. We made this figure by modification of figure, which we previously made in reference 7.

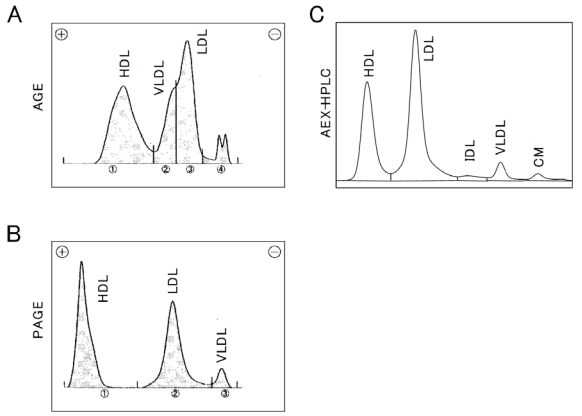

Figure 2. Results of measurement of the same sample by agarose gel electrophoresis (AGE), polyacrylamide-gel electrophoresis (PAGE) and anion-exchange high-performance liquid chromatography (AEX-HPLC). CM, chylomicron; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

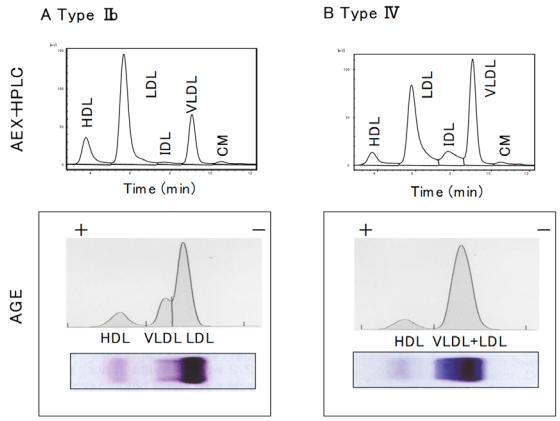

Figure 3. Results of measurement of serum of patients with hyperlipoproteinemia type IIb and type IV by agarose gel electrophoresis (AGE) and anion-exchange high-performance liquid chromatography (AEX-HPLC). CM, chylomicron; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Excellence of AEX-HPLC as compared with previous methods to measure lipoproteins

Various methods for analysis of lipoproteins by ultracentrifugation (*11-13*), electrophoresis (*14-16*), gel-permeation chromatography (*17,18*), and anion-exchange chromatography (*19*) have been reported. The cholesterol levels of all major classes of lipoproteins in serum can be measured by ultracentrifugation, but it takes a long time to complete the analysis (*11-13*). The other methods have poor ability to measure IDL -C levels (*14-19*).

Very recently, in measurements of lipoprotein fraction, AEX-HPLC became commercially available, in addition to agarose gel electrophoresis (AGE) and polyacrylamide-gel electrophoresis (PAGE), in Japan. Results of measurements of the same sample by AGE, PAGE and AEX-HPLC are shown in Figure 2. AGE and PAGE could not measure CM and IDL, and AGE could not distinguish between LDL and VLDL, however, AEX-HPLC could accurately measure all fractions of lipoproteins.

Diabetic dyslipidemia is characterized by elevated TG and LDL-C, and reduced HDL-C (*5*). Therefore, hyperlipoproteinemia type IIb and type IV (WHO classification) are commonly observed in patients with diabetes (*20*). Results of measurements of serum of patients with hyperlipoproteinemia type IIb and type IV by AGE and AEX-HPLC are shown in Figure 3. AGE could not measure CM and IDL, and could not distinguish between LDL and VLDL. However, AEX-HPLC could distinguish and measure all fractions of lipoproteins in patients with hyperlipoproteinemia type IIb and type IV.

The characteristics of lipoproteins in patients with diabetes

We compared lipoprotein profiles obtained by our previous studies using AEX-HPLC (*21*), which included studies using young lean men (*22*), subjects with low Framingham risk score (FRS) (*23,24*), type 2 diabetic patients without and with obesity (*25,26*) (Figure 4). HDL-C in type 2 diabetes, especially in type 2 diabetic patients with obesity was lower than young lean men and low FRS subjects. IDL-C in type 2 diabetes was higher than the other two groups, and IDL-C was higher in the order of type 2 diabetic patients with obesity, type 2 diabetic patients without obesity, low FRS subjects, young lean men. VLDL-C clearly showed higher values in the order of type 2 diabetic patients with obesity, type 2 diabetic patients without obesity, low FRS subjects, and young lean men. LDL-C and CM-C did not show a difference between patients with and without diabetes. According to the accumulation of our previous AEX-HPLC data (*22,24-26*), characteristics of diabetic dyslipidemia are reduced HDL-C, and increased IDL-C and VLDL-C, which further deteriorated due to complication with obesity.

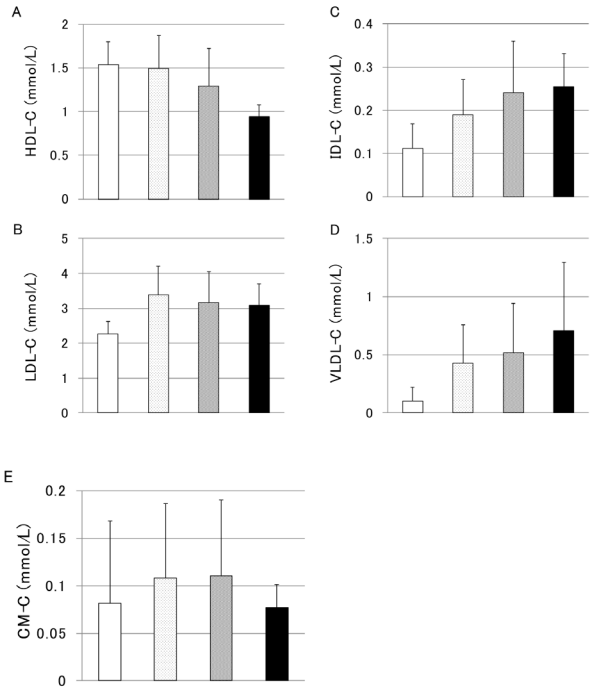

Figure 4. Serum concentration of HDL-C, LDL-C, IDL-C, VLDL-C and CM-C in young lean men (white boxes), subjects with low Framingham risk score (FRS) (dotted boxes), type 2 diabetic patients without (shaded boxes) and with obesity (black boxes).

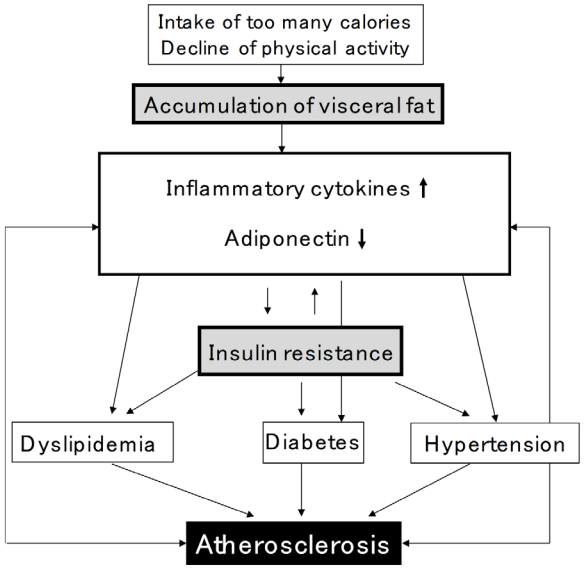

Figure 5. Pathophysiology of the metabolic syndrome. We made this figure by modification of figure in reference 27.

The mechanisms of development of diabetic dyslipidemia

A significant association of visceral obesity and insulin resistance with diabetic dyslipidemia

Pathophysiology of the metabolic syndrome is shown in Figure 5 (*27*). Accumulation of visceral fat and resulting insulin resistance may play a crucial role in inducing diabetic dyslipidemia.

A significant influence of visceral obesity on diabetic dyslipidemia

Our previous study showed a significant and positive association between serum TG levels and the ratio of visceral fat area (VFA) to subcutaneous fat area (SFA) in obese individuals (*28*). Further, our other previous studies demonstrated a significant and negative correlation between VFA and HDL-C levels and a significant and positive correlation between VFA and TG levels in patients with type 2 diabetes (*29,30*), supporting a significant influence of visceral fat on diabetic dyslipidemia.

Significance and mechanism for elevation of VLDL-C in patients with type 2 diabetes

We previously observed a significant and positive correlation between VFA and VLDL-C levels, suggesting a significant influence of visceral obesity on VLDL (*29,30*). In the accumulated analysis of AEX-HPLC studies, VLDL-C clearly showed higher values in the order of type 2 diabetic patients with obesity, type 2 diabetic patients without obesity, and non-diabetic individuals (*21*), indicating an importance of VLDL in diabetic dyslipidemia.

Our previous study showed that supervised aerobic exercise training (60 min/day, 2 to 3 times/week) markedly reduced VLDL-C at week 8 (-45%) and week 16 (-50%) with an improvement of insulin resistance, in individuals with dyslipidemia (*31*). Another study showed that the glucagon-like peptide-1 analogue (GLP-1A) used as treatment for type 2 diabetes reduced serum TG and VLDL-C with an improvement of glycemic control, in type 2 diabetic patients with obesity (*26*). In this study, reduction of TG by GLP-1A was significantly correlated with a decrease in VLDL-C (Figure 6A). Further, reduction of TG was significantly correlated with small dense LDL, which is a strong atherogenic lipoprotein (Figure 6B). Overproduction of VLDL is metabolically associated with preponderance of small dense LDL (*32*). In Japanese patients with type 2 diabetes, serum TG level was a leading predictor of coronary heart disease, comparable to LDL-C (*33*), supporting the importance of VLDL in diabetic dyslipidemia.

VLDL is the leading actor in diabetic dyslipidemia (*34*). The main mechanism for elevation of VLDL-C in patients with type 2 diabetes is shown in Figure 7. Insulin resistance increases activity and expression of hormone-sensitive lipase (HSL) in adipose tissue, which catalyzes the breakdown of TG, releasing free fatty acids (FFA) (*35*). Increased FFA entry into liver elevates hepatic production of VLDL. Insulin resistance also decreases the activity of lipoprotein lipase (LPL), the rate-limiting

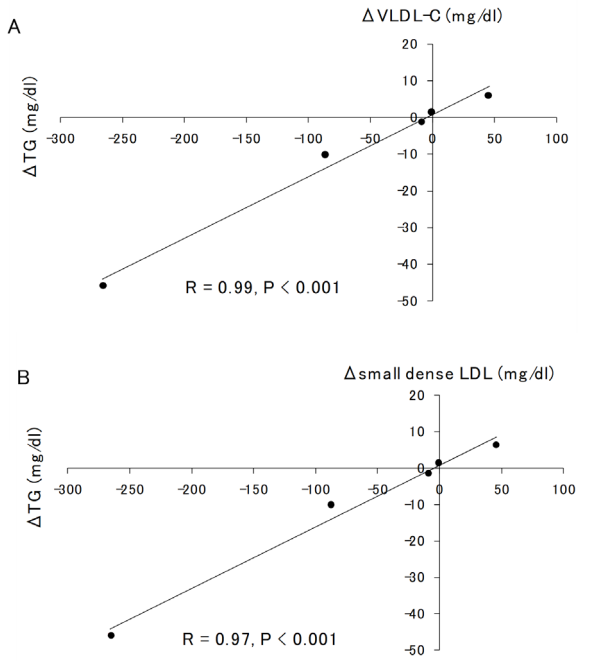

Figure 6. Correlation of changes in serum triglyceride with changes in VLDL-C and small dense LDL by glucagon-like peptide-1 analogue in patients with type 2 diabetes. Correlation coefficient was analyzed by Spearman's rank correlation.

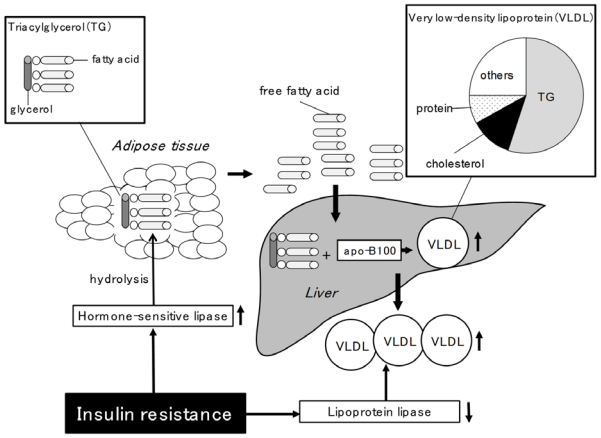

Figure 7. The main mechanism for elevation of VLDL in patients with type 2 diabetes. We made this figure by modification of figure in reference 34.

enzyme of catabolism of VLDL (*36*). Therefore, reduced LPL activity increases VLDL in diabetes.

Molecular mechanisms for development of diabetic dyslipidemia

Molecular mechanisms for development of diabetic dyslipidemia are shown in Figure 8 (*21*). Insulin resistance increases activity and expression of HSL in adipose tissue, which catalyzes the breakdown of TG, releasing FFA (*35*). Insulin promotes apoB100 degradation, and hepatic insulin resistance reduces apoB100 degradation (*37*). Insulin resistance also

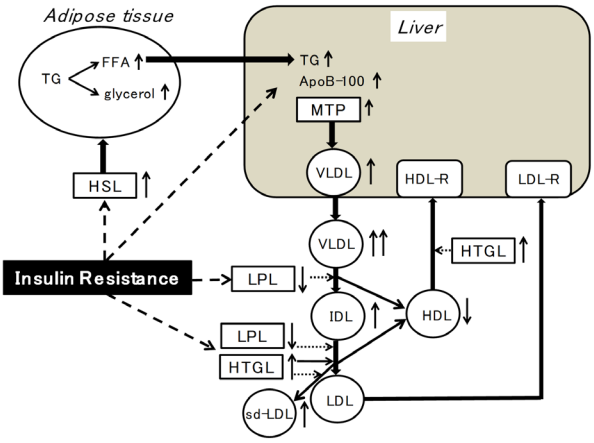

Figure 8. Molecular mechanisms for development of diabetic dyslipidemia. We made this figure by modification of figure in reference 21. FFA, free fatty acids; HDL, high-density lipoprotein; HSL, hormone-sensitive lipase; HTGL, hepatic triglyceride lipase; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LPL, lipoprotein lipase; MTP, microsomal triglyceride transfer protein; sd-LDL, small dense LDL; TG, triglyceride; VLDL, very low-density lipoprotein.

enhances expression of microsomal TG transfer protein (MTP), a key enzyme involved in VLDL assembly (*38*). In type 2 diabetes, increased FFA entry to liver, reduced degradation of apoB100, and enhanced expression of MTP may elevate hepatic production of VLDL. Insulin resistance also decreases the activity of LPL, the rate-limiting enzyme of the catabolism of TG-rich lipoproteins such as CM, VLDL and IDL (*36*). The formation of HDL is related to the catabolism of TG-rich lipoproteins by LPL (*39*). Therefore, reduced LPL activity increases IDL and VLDL, and reduces HDL. The activity of hepatic TG lipase (HTGL), the enzyme that facilitates the catabolism of HDL, is correlated with insulin resistance (*40*). In type 2 diabetes, low serum HDL-C may be partially due to an increased rate of clearance by HTGL (*40*). LDL size and buoyancy are inversely proportional to HTGL activity (*41*), and patients with high HTGL have smaller, denser LDL particles, as compared to subjects with low HTGL activity (*42*). Increased HTGL activity due to insulin resistance/relative insulin deficiency may increase super-atherogenic lipoprotein, small dense LDL, in type 2 diabetes. Further, overproduction of hepatic VLDL is metabolically associated with a preponderance of small dense LDL and reduced large cholesterol-rich HDL (*32*).

Acknowledgements

We thank the staff (Yukie Kawamura, Keiko Nakamura, Harue Aoki and Ayano Sakakibara) of the Division of Research Support, National Center for Global Health and Medicine Kohnodai Hospital, for technical help.

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Received July 29, 2019; Revised September 20, 2019; Accepted September 30, 2019.

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