Bull. Tokyo Med. Dent. Univ., 38: 63–71, 1991

ABNORMAL PARTICLE SIZE OF LIPOPROTEIN IN NON-INSULIN-DEPENDENT DIABETICS AND NONDIABETICS WITH AND WITHOUT HYPERLIPIDEMIA

BY

Yaeko NAKAJO, Akira TANAKA and Isao UCHIMURA

ABSTRACT

To clarify the mechanism of the high incidence of ischemic heart disease in the diabetics, we studied the particle size of the lipoprotein with particular attention to the structural abnormality. Using high performance liquid chromatography, the particle size of the lipoprotein was determined with elution volume, which was precisely correlated with the particle size. The particle size of low density lipoprotein (LDL) from the diabetics with normolipidemia is larger than that from the nondiabetics ($p<0.001$) in the elution pattern of triglyceride. The examination of the elution pattern of the triglyceride and cholesterol revealed that this large LDL was composed of a large amount of triglyceride. These characteristics of the large LDL resembled that of the atherogenic intermediate density lipoprotein. The particle size of high density lipoprotein (HDL) from the diabetics with normolipidemia was larger than that from the nondiabetic controls ($p<0.001$), detected by the elution pattern of triglyceride. The comparison of the triglyceride and cholesterol elution pattern indicated that also the large HDL in the diabetics with normolipidemia was rich with triglyceride, which was reported to inhibit the activity of the lipid transfer protein.

These facts revealed that the disorder of the lipid metabolism not accompanied with hyperlipidemia produced the large LDL and HDL, which might lead to the excess mortality rate of the ischemic heart disease in the diabetics even with normolipidemia.

Key words: Lipoprotein, Particle size of lipoprotein, LDL, HDL, Diabetes mellitus, HPLC

INTRODUCTION

The Framingham Study and other epidemiologic studies showed that many risk factors such as hypertension, cigarette smoking, obesity and hyperlipidemia had important roles in the pathogenesis of the ischemic heart disease (IHD) (1–3). Among these risk factors, the hypercholesterolemia was considered to be the most important factor. Controlled clinical trials demonstrated that lowering the serum cholesterol level can prevent IHD (4–8). Many investigators reported the excess mortality rate of the cardiovascular disease in the diabetics (9–12). Hyperlipidemia is also expected to be the important risk factor of IHD in the diabetics. In fact the incidence of hyperlipidemia in the diabetics is higher than that in the nondiabetics. However, Morrish et al. failed to reveal the correlation between the incidence of cardiovascular mortality and the serum cholesterol level in the diabetics (10). Though normoglycemic and normolipidemic diabetics may suffer from IHD,

中條やえ子, 田中　明, 内村　功：The Third Department of Internal Medicine (Chief: Prof. H. MAEZAWA), Faculty of Medicine, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku)
Received for publication, August 13, 1991.

the risk factors that cause the IHD in the nondiabetics play only a part of the pathogenesis of this excess mortality rate of the IHD in the diabetics. The cause of the high incidence of IHD in the normolipidemic diabetics has not been clarified. In addition to the lipid level, the structural changes of the lipoprotein which alter its particle size may contribute to the atherogenesity in the IHD. Accordingly, the structural changes must be studied in the diabetes. Ultracentrifugation is commnly used to separate the lipoprotein. While the force of ultracentrifugation can tear off some parts from the lipoprotein particle during the several days of separation, high performance liquid chromatography (HPLC) can separate the lipoprotein with little damage in a short time and show directly the distribution of the lipoprotein particle size.

We investigated the particle size of the lipoprotein in the nondiabetics and diabetics matching the serum level of the lipids between them, using the technique of HPLC.

Subjects and Methods

Eighty healthy nondiabetic controls (all males) and eighty-four (40 males and 44 females) non-insulin-dependant diabetes mellitus (NIDDM) patients as defined by the criteria of the WHO Study Group (13) were admitted to the study. The diabetic patients were outpatients of Tokyo Medical and Dental University Hospital. They had no hepatic or renal disease, and they did not take any medication that had effects on the lipid metabolism. Nondiabetics and diabetics were divided into three groups by their serum lipid level: normolipidemia (serum cholesterol $\leq$220 mg/dl and triglyceride $\leq$150 mg/dl), hypercholesterolemia (serum cholesterol $\geq$220 mg/dl and triglyceride $\leq$150 mg/dl) and hypertriglyceridemia (serum cholesterol $\leq$220 mg/dl and triglyceride $\geq$150 mg/dl).

(NN: nondiabetics with normolipidemia, DMN: diabetics with normolipidemia, NTG: nondiabetics with hypertriglyceridemia, DMTG: diabetics with hypertriglyceridemia, NCH: nondiabetics with hypercholesterolemia, DMCH: diabetics with hypercholesterolemia)

After an overnight fast, venous samples were collected without the anticoagulant. The serum was obtained by centrifuging the blood at 3000 r.p.m. for fifteen minutes after the blood had clotted at room temperature. Blood glucose was determined by the glucose oxidase method (14). Hemoglobin A_1 (HbA_1) was measured by the HPLC method (CMA-812, Toso, Ltd., Tokyo) (15). Serum total cholesterol and triglyceride were determined by the enzymatic method (HITACHI-736, Hitachi Co., Ltd., Tokyo). High density lipoprotein cholesterol (HDLC) was measured by the dextran sulphate-MgCl precipitant method (16). We stocked the serum at -80°C until it was used in the HPLC experiments.

We separated the lipoprotein by the method of Hara et al. (17) with some modification. HPLC was carried out using the computer-controlled dual pump (CCDP Toso, Ltd., Tokyo) with aqueous gel permeation columns (TSK GEL G5000PW 7.5 mm I.D. $\times$600 mm Toso, Ltd., Tokyo). We applied 10–80 μl of the serum to the HPLC system per one injection, according to the serum cholesterol or triglyceride level, using 0.15 M NaCl (pH 7.4) for the eluent with a flow rate of 0.4 ml/min.

After separation, the quantitation of triglyceride and cholesterol was performed by the enzymatic reaction. The effluent was mixed and incubated with enzymatic agents of triglyceride or cholesterol (Determiner TG or Determiner TC555, Kyowa medex Co., Ltd., Tokyo)

at the flow rate of 0.3 ml/min at a temperature of 37°C for thirty minutes in the water bath. Then the postcolumn effluent was measured at the absorbance of 550 nm. This absorbance curve shows the distribution of the triglyceride or cholesterol level of the lipoprotein (triglyceride monitoring and cholesterol monitoring). The HPLC separated the sample according to its particle size, so we represented the particle size of the lipoprotein at the site of the peaks of each lipoprotein with the elution volume, which is reversely correlated with the particle size (17).

Results

I. Particle size of lipoprotein from normolipidemic controls and diabetics by triglyceride monitoring

An elution pattern of the serum lipoprotein by the HPLC is shown in Figure 1. The serum lipoprotein was separated into four peaks of chylomicron, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). By triglyceride or cholesterol monitoring there was no difference in each lipoprotein particle size between the men and women and there was no difference between the diabetic groups (n=53) treated with diet only (n=34), oral agents (n=11) or insulin (n=8). HbA_1 and fasting blood glucose (FBG) had also no significant effects on the particle size of the lipoprotein.

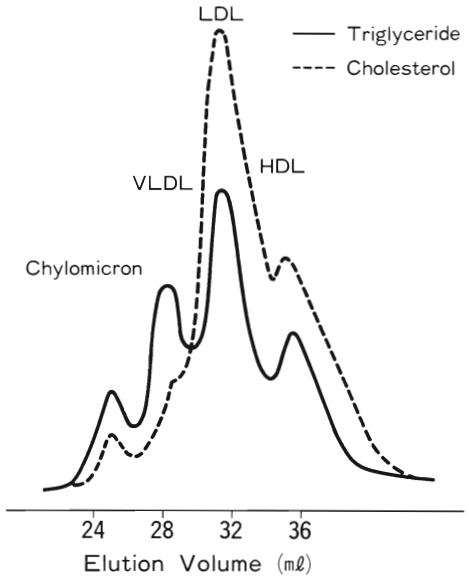

Fig. 1. Elution Pattern of Serum Lipoprotein by HPLC
The triglyceride (—) and cholesterol (- -) are measured by the absorbance of 550 nm after the enzymatic reaction. The X-axis represents the elution volume and the Y-axis represents the absorbance of 550 nm. Chylomicron, VLDL, LDL and HDL are detected as peaks from the left side. The elution volume has a reverse correlation with the particle size of the material passing through the column of HPLC.

The serum lipid level and other clinical features are shown in Table 1. The serum

Table 1. Characteristics of Nondiabetic and Diabetic Subjects with Normolipidemia
The age, level of serum cholesterol and triglyceride were matched between the nondiabetic and diabetic groups but the serum HDLC level from the diabetics is lower than from the nondiabetics.

Subjects	Age (years)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDLC (mg/dl)		FBG (mg/dl)		HbA_1 (%)
Normal n=55	49±4	174±23	84±26	55±11	*	90±8	*	
Diabetes n=53	52±13	183±23	90±22	49±11		161±67		10.2±2.6

Mean±SD *<0.005

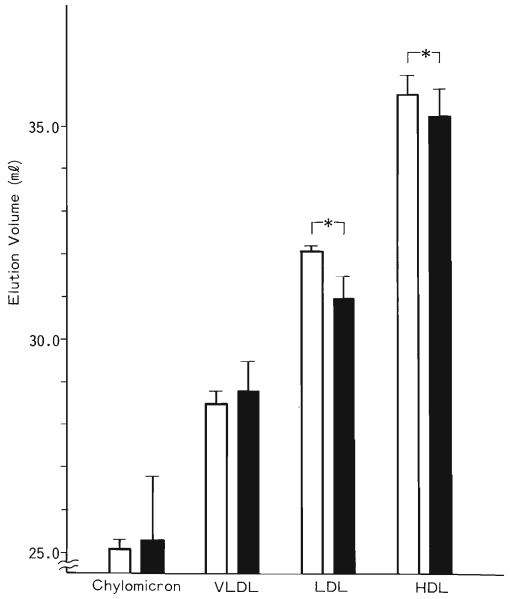

Fig. 2. Particle Size of Lipoprotein in Nondiabetic and Diabetic Patients With Normolipidemia by HPLC Triglyceride Monitoring
The open bar represents the nondiabetics and the solid bar represents the diabetics. The Particle size of LDL and HDL is larger in the diabetics than in the nondiabetics *: $p<0.001$.

HDLC was significantly lower in the DMN than that in the NN. As shown in Figure 2, the particle size of LDL from the DMN (30.96±0.53 ml) was significantly greater ($p=0.0006$) than that from the NN (31.24±0.13 ml). The particle size of LDL from the DMN showed a significant correlation with the serum triglyceride level ($r=0.3673$, $p=0.006$). The correlation between the particle size of LDL from the DMN and the serum cholesterol level, HDLC, FBG or HbA1 was not observed.

The particle size of HDL from the NN and DMN was 35.84±0.43 ml and 35.25±0.65 ml, respectively. The latter was significantly larger than the former ($p=0.0001$). There was a weak significant correlation between the HDLC level and particle size of HDL in the DMN ($r=0.264$, $p=0.0348$), but not in the NN.

The particle size of chylomicron and VLDL between the NN and DMN showed no significant difference.

II. Particle size of lipoprotein from nondiabetics with hyperlipidemia by triglyceride monitoring

As shown in Table 2, we matched the age, serum cholesterol and HDLC level between the NN and NTG. The serum triglyceride level of the NN and NTG was 89±22 mg/dl and 283±19 ml/dl, respec-

Table 2. Serum Lipid Level and Particle Size of Lipoprotein in Nondiabetic Subjects With Normolipidemia, Hypertriglycemia and Hypercholesteremia by HPLC Triglyceride Monitoring
The hypertriglycemic patients have larger VLDl and smaller LDL particle than the normolipidemic subjects.

	Nondiabetic subjects			p Value	
	Normolipidemia n=36	Hypertriglycemia n=13	Hypercholesteremia n=13	Normolipidemia vs. Hypertriglycemia	Normolipidemia vs. Hypercholesteremia
Serum					
Cholesterol (mg/dl)	182±21	189±19	243±12	NS	0.0001
Triglyceride	89±22	283±19	112±45	0.0001	NS
HDLC	48±8	43±7	49±9	NS	NS
Particle size (ml)					
Chylomicron	—	24.35±0.44	24.25±0.06	—	—
VLDL	28.64±0.40	28.14±0.33	28.81±0.15	0.0013	NS
LDL	31.23±0.12	31.54±0.22	31.38±0.31	0.0071	NS
HDL	35.93±0.49	36.05±0.61	35.95±0.70	NA	NS

Mean±SD

tively.

The particle size of VLDL from the NTG was significantly larger than that from the NN ($p=0.0013$).

The particle size of LDL from the NTG was smaller than that from the NN (31.54 ± 0.22 ml vs. 31.23 ± 0.12 ml, $p=0.0071$). In the group of all nondiabetics with and without hypertriglyceridemia, the particle size of LDL had a reverse correlation with the serum triglyceride level ($r=0.876$, $p=0.0001$).

We could not find any difference between the particle size of HDL in the NN and NTG.

After the adjustment of the age, serum triglyceride and HDLC level, the serum cholesterol level from the NN and from the NCH was 182 ± 21 mg/dl and 243 ± 12 mg/dl, respectively. There was no significant difference in the particle size of VLDL, LDL or HDL from the NN and NCH.

III. Particle size of lipoprotein from nondiabetics and diabetics with hyperlipidemia by triglyceride monitoring

To study the effect of the hyperlipidemic state on the particle size of the lipoprotein, the age, level of serum cholesterol, triglyceride, and HDLC between the NTG and DMTG or NCH and DMCH were matched. The particle size of the lipoprotein was measured by triglyceride monitoring.

As shown in Table 3A, the particle size of the chylomicron from the NTG showed no significant difference with that from the DMTG. The particle size of VLDL from the DMTG was smaller than that from the NTG, but that of LDL from the DMTG was significantly larger than that from the NTG (30.30 ± 0.71 ml vs. 31.54 ± 0.30 ml, $p=0.0002$). In the diabetes with hypertriglyceridemia, we could detect the same larger LDL as in the diabetes with normolipidemia. The particle size of LDL

Table 3A. Characteristics and Particle size of Lipoprotein Determined by HPLC Triglyceride Monitoring in Nondiabetics and Diabetics With Hypertriglycemia
The age and serum lipids are matched between the nondiabetics and diabetics. The diabetics with hypertriglycemia have a smaller VLDL and a larger LDL than the nondiabetics with hypertriglycemia.

	Hypertriglycemia		p Value
	Nondiabetics (n=16)	Diabetics (n=9)	
Age (years)	48±4	50±6	NS
FBG (mg/dl)	89±8	156±38	0.0003
HbA1 (%)	—	9.0±3.2	—
Serum cholesterol (mg/dl)	195±16	195±19	NS
triglyceride	249±40	271±47	NS
HDLC	44±7	42±10	NS
Particle size (ml)			
chylomicron	24.27±0.44	24.33±0.40	NS
VLDL	28.13±0.41	28.73±0.63	0.0058
LDL	31.54±0.30	30.30±0.71	0.0002
HDL	35.99±0.58	35.88±0.48	NS

Mean±SD

Table 3B. Characteristics and Particle Size of Lipoprotein Determined by HPLC Triglyceride Monitoring in Nondiabetics and Diabetics With Hypercholesteremia
The age and serum lipids are matched between the nondiabetics and diabetics. The particle size of HDL in the diabetics with hypercholesteremia is larger than that in the nondiabetics with hypercholesteremia.

	Hypertriglycemia		p Value
	Nondiabetics (n=16)	Diabetics (n=9)	
Age (years)	47±7	51±8	NS
FBG (mg/dl)	92±6	176±54	0.0001
HbA_1 (%)	—	10.6±2.6	—
Serum cholesterol (mg/dl)	247±12	252±18	NS
triglyceride	105±24	90±25	NS
HDLC	51±7	54±16	NS
Particle size (ml)			
Chylomicron	—	24.80±0.27	—
VLDL	28.78±0.11	28.80±0.30	NS
LDL	31.31±0.30	31.14±0.24	NS
HDL	35.92±0.81	35.05±0.61	0.0013

Mean±SD

from the normolipidemic diabetes was 31.02±0.40 ml (Figure 2), which was significantly smaller than LDL from the hypertriglycemic diabetes (p=0.00002).

The particle size of HDL revealed no significant difference between the NTG and DMTG, but the particle size of HDL in the DMN (35.18±0.51 ml, Figure 2) was significantly larger than in the DMTG (p=0.0002).

We also compared the particle size from the nondiabetics and diabetics with hypercholesterolemia (Table 3B). The particle size of VLDL and LDL showed no difference between two groups. The particle size of HDL in the DMCH was larger than that in the NCH (35.05±0.61 ml vs. 35.92±0.81 ml, p=0.0013). The particle size of HDL showed no significant difference between the DMCH and DMN.

IV. Particle size of lipoprotein from nondiabetic and diabetic subjects with normolipidemia by triglyceride and cholesterol monitoring

The cholesterol level of chylomicron and VLDL is very low, so we could not detect clear peaks of chylomicron and VLDL by cholesterol monitoring. We compared the data of the particle size of LDL nad HDL by triglyceride and cholesterol monitoring. As shown in Table 4, the particle size of LDL by cholesterol monitoring in the NN and DMN was 32.45±0.28 ml and 32.26±0.22 ml, respectively, with a statistical difference between them (p=0.0009), showing a larger LDL particle of diabetes like the results obtained by triglyceride monitoring. The particle size of LDL by triglyceride monitoring was larger than that by cholesterol monitoring either in the nondiabetics and diabetics (p<0.0001). In the nondiabetics and diabetics, we can searate the LDL into two parts, the larger particle of triglyceride-rich LDL and the smaller particle of cholesterol-rich LDL. The delimitation

Table 4. Particle Size of LDL and HDL Determined by Triglyceride and Cholesterol monitoring in Nondiabetics and Diabetics With Normolipidemia
The LDL from the diabetics showed a larger particle size than from the nondiabetics in triglyceride and cholesterol monitoring. A larger particle size of HDL from the diabetics was observed only in triglyceride monitoring and not in cholesterol monitoring.

	Normals (n=27)	Diabetics (n=50)	p Value
LDL particle			
Triglyceride monitoring	31.48±0.45	31.10±0.40	0.0002
Cholesterol monitoring	32.45±0.28	32.26±0.22	0.0009
HDL particle			
Triglyceride monitoring	35.50±0.25	35.26±0.53	0.0032
Cholesterol monitoring	35.30±0.17	35.40±0.36	NS

Mean±SD (ml)

between the two parts of LDL is difficult by this study.

Between the particle size of HDL by cholesterol monitoring from the NN and DMN, there was no statistical difference between them unlike the results obtained by triglyceride monitoring.

Discussion

Recently the number of diabetic patients has increased remarkably in Japan like in the western countries. In these patients, the IHD was revealed to be the common cuase of mortality (9), and the investigation about the pathogenesis of IHD has become an important topic. From the epidemiological study, the unknown risk factor might increase the incidence and mortality rate of the IHD in the diabetics. To reveal new risk factors for the IHD in the diabetics, we investigated the particle size of the lipoprotein using the HPLC.

In our study, the particle size of LDL in the DMN was significantly larger than that in the NN. Suzuki et al. reported that the NIDDM with hypercholesterolemia showed a multidisperse pattern which might be the shift to the lighter side of LDL using analytical ultracentrifugation (19). Suzuki's multidiserse part may correspond to or large LDL that was observed in the diabetics with or without hyperlipidema. Because VLDL is metabolized to LDL in the circulation, this large LDL in the diabetics may reflect the slow metabolic rate of VLDL to LDL. From the study of LDL by HPLC triglyceride monitoring and cholesterol monitoring, the large LDL is composed of a higher ratio of triglyceride and a lower ratio of cholesterol than the normal LDL. These results showed this large LDL to have the characteristics of the intermediate density lipoprotein (IDL). Krauss et al. reported that in the patients with increased IDL, the IHD progressed rapidly (21). From these facts, the large LDL in the diabetics will induce an excess progress of IHD. However, the mechan-

ism that produces the large LDL in the diabetics has not been fully understood. Over-production of the VLDL (22), stagnation of the VLDL-LDL cascade (22) and/or inhibition of the LDL-binding to the receptor by the glycation of the apoprotein B(23) may participate in the productin of the large LDL. As the disappearance of the large LDL was observed with improved glycemic control in the three diabetic patients with normolipidemia (data not shown), glycemic control can be considered to have effects on the particle size of LDL.

The particle size of LDL from the NTG was smaller than the NN, showing a reverse correlation with the serum triglyceride level. In the diabetic patients, a positive correlation between the particle size of LDL and serum triglyceride level was observed. Hypertriglyceridemia is commonly observed in the diabetics, but the large particle size of LDL in the diabetics can not be explained by the mechanism that makes the LDL particle smaller in the nondiabetics with hypertriglyceridemia.

The particle size of VLDL showed no significant difference between the diabetics and nondiabetics. Regarding the arteriosclerosis, among the apoprotein B containing lipoprotein, LDL is more important than VLDL in the diabetics with normolipidemia.

Hypercholesterolemia has no effect on the particle size of LDL and other lipoproteins. The particle size of HDL from the DMN was larger than that from the NN in triglyceride monitoring. The particle size of HDL correlated with the serum HDLC level in the nondiabetics, but this relation was not observed in the diabetics. Our study on triglyceride and cholesterol monitoring showed a larger HDL with a higher ratio of triglyceride and a lower ratio of cholesterol from the diabetics with normolipidemia than from the nondiabetics with normolipidemia. Biesbroeck et al. reported in the diabetics a higher ratio of triglyceride in the HDL composition (20), and Sparks et al. reported that the HDL with a high ratio of triglyceride inhibited the activity of the lipid trasfer protein (18). The large HDL with a high ratio of triglyceride in the diabetics may inhibit the activity of the lipid transfer protein and as a result, the accumulation of cholesterol in the peripheral tissue will occur. Therefore, the large HDL will be the risk factor of the IHD in the diabetics even with normolipidemia.

A large particle size of LDL and HDL was observed in the diabetics even in the cases of normal serum cholesterol and triglyceride level. This large LDL and HDL were produced by the disorder of the lipid metabolism not accompanied by the high level of serum lipids. This abnormal lipoprotein particle may explain the excessive arteriosclerosis in the diabetics. The development of treatment for the normalization of the large LDL and HDL is expected to decrease the rate of IHD mortality.

Acknowledgement

We wish to express our heartfelt thanks to Prof. H. Maezawa, the Third Department of Internal Medicine, Faculty of Medicine, Tokyo Medical and Dental University, for his continuous advice and suggestions on this study. We also wish to express our grateful thanks to Prof. Emeritus I. Hara and Dr. M. Okazaki, Laboratory of Chemistry, Department of General Education, Tokyo Medical and Dental University, for their valuable advice and direction of the method of HPLC.

References

1) Kanell WB, William P, Castelli WP, and Gordon T: Cholesterol in the Prediction of Atherosclerotic Disease. Ann. Int. Med., 90: 85–91, 1979.

2) Castelli WP: Epidemiology of Coronary Heart Disease: The Framingham Study, Am. J. Med., 76 (2A): 4–12, 1984.
3) Castelli WP, Garrison RJ, Wilson PWF, Abbott RD, Kalousdian S, and Kannel WB: Incidence of Coronary Heart Disease and Lipoprotein Cholesterol Levels; The Framingham Study. JAMA, 256: 2835–2838, 1986.
4) Lipid Research Clinics Program: The Lipid Research Clinics Coronary Primary Prevention Trial Results. I. Reduction in Incidence of Coronary Heart Disease. JAMA, 251: 351–364, 1984.
5) Lipid Research Clinics Program: The Lipid Research Clinics Coronary Primary Prevention Trial Results. II. The Relationship of Reduction in Incidence of Cronary Heart Disease to Cholesterol Lowering. JAMA,, 251: 365–374, 1984.
6) Blankenhorn DH, Nessim SA, and Johnson RL: Beneficial effects of combined colestipol-niacin therapy on coronary atherosclerosis and coronary venous grafts. JAMA, 257: 3233–3240, 1987.
7) Frik MH, Elo O, and Haapa K: Helsinki Heart Study: primary prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. N. Engl. J. Med., 317: 1237–1245, 1987.
8) Brensike JF, Levy RI, and Kelsey SF: Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: results of the NHLBI Type II Coronary Intervention Study. Circulation, 69: 313–324, 1984.
9) Morrish NJ, Stevens LK, Head J, Fuller JH, Jarrett RJ, and Keen H: A prospective study of mortality among middle-aged diabetic patients (The London Cohort of the WHO Multinational Study of Vascular Disease in Diabetics) I: Causes and death rates. Diabetologia, 33: 538–541, 1990.
10) Morrish NJ, Stevens LK, Head J, Fuller JH, Jarrett RJ, and Keen H: A prospective study of mortality among middle-aged diabetic patients (The London Cohort of the WHO Multinational Study of Vascular Disease in Diabetics) II: Associated risk factors. Diabetologia, 33: 542–548, 1990.
11) Kannel WB, and McGee DL: Diabetes and Cardiovascular Risk Factors: The Framingham Study. Circulation, 59: 8–13, 1979.
12) Fuller JH, Shipley MJ, Rose G, Jarrett RJ, and Keen H: Mortality from coronary heart disease and stroke in relation to degree of glycaemia: The Whitehall Study. British Medical Journal, 287: 867–870, 1983.
13) Diabetes Mellitus, Report of a WHO Study Group: Technical Report Series 727, WHO, Geneva, 1985.
14) Kadish AH, Little RL, and Seeinberg JC: A new and rapid method for the determination of glucose by measurement of rate of oxygen consumption. Clin. Chem., 14: 116–131, 1968.
15) Nathan DM, and Raskin P: Convenient automated method for high performance liquid-chromatography measurement of glycated hemoglobin. Clin. Chm. 30: 813–814, 1984.
16) Warnick GR, Benderson J, Albers JJ, Baillie EE, Sexton B, Schaefer EJ, Carlson D, Hill M, Brewer HB, Wiebe DA, Hazlehusrst J, and Cooper GR: Dextran sulfate-Mg2+ precipitation procedure for quantitation of hgh-density-lipoprotein cholesterol. Clin. Chem., 28: 1379–1388, 1982.
17) Hara I, Shiraishi K, and Okazaki M: High-performance liquid chromatography of human serum lipoproteins. Selective detetion of triglyceride by enzymatic reaction. Journal of Chromatography, 239: 549–557, 1982.
18) Sparks DL, Frohlich J, Lacko AG, and pritchard PH: Relationship between cholesteryl ester transfer activity and high density lipoprotein composition in hyperlipidemic patients. Atherosclerosis, 77: 183–191, 1989.
19) Suzuki N, Oikawa S, Hori S, Fujii Y, Sakuma E, Kotake H, Namai K, Yoshie K, and Goto Y: Appearance of multiple low density lipoprotein and altered lipoprotein composition in non-insulin-dependent diabetes with type IIa hyperlipoproteinemia. Metabolism, 38: 224–229, 1989.
20) Biesbroeck RC, Albers JJ, Wahl PW, Weinberg CR, Bassett ML, and Bierman EL: Abnormal composition of high density lipoproteins in non-insulin-dependent diabetics. Diabetes, 31: 126–131, 1982.
21) Krauss RM, Lindgren FT, and Williams PT: Intermediated-density lipoproteins and progression of coronary artery disease in hypercholesterolaemic man. Lancet, ii: 62–66, 1987.
22) Kissebah AH, Alfarsi S, Evans DJ, and Adams PW: Integrated regulation of very low density lipoprotein triglyceride and apolipoprotein-B kinetics in non-insulin-dependent diabetes mellitus. Diabetes, 31: 217–225, 1982.
23) Mamo JCL, Szeto L, and Steiner G: Glycation of very low density lipoprotein from rat plasma impairs its catabolism. Diabetologia, 37: 339–345, 1990.