Bull. Tokyo Med. Dent. Univ., 31: 85–91, 1984

HIGH DENSITY LIPOPROTEIN AND DIABETES MELLITUS

BY

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ABSTRACT

The serum HDL_2-C, HDL_3-C, apo AI and apo AII levels were measured in the non-insulin-dependent diabetic subjects (NIDD) and normal subjects to study the metabolism of HDL in the diabetics.

The serum HDL_2-C levels in the insulin-treated group were significantly higher than those in the normal group in which the total cholesterol (TC), triglyceride (TG), obesity index and age were matched whereas there was no difference between the serum HDL_2-C levels in the oral agent-treated group or group treated by diet only and those in the normal group. These suggest that insulin increases the HDL_2-C levels and the increase of the HDL_2-C levels is not directly related to changes in the serum TC and TG levels, obesity index and age. No significant differences in the serum apo AI and apo AII levels were found between the insulin group and normal group. From these results it is suggested that in the insulin group the cholesterol/apoprotein ratio in the HDL_2 is higher than that in the normal group.

The serum apo AI and apo AII levels were significantly lower in the diabetics with an ischemic heart disease (IHD) than those in the diabetics without the IHD. The results show that in the diabetics the apo AI and apo AII play an important role in preventing the development of IHD.

Key words: Diabetes Mellitus, HDL-cholesterol, Apoprotein A. Atherosclerosis, HDL_2-cholesterol,

INTRODUCTION

A negative correlation between the serum high density lipoprotein-cholesterol (HDL-C) level and the incidence of atherosclerotic disease has been demonstrated (Castelli *et al.* [1]; Miller *et al.* [2]; Gordon *et al.* [3]). Diabetics have a higher incidence of atherosclerotic disease than the normal subjects (Kessler [4]; Garcia *et al.* [5]; Plumbo *et al.* [6]). Therefore, the level of HDL_2-C and HDL_3-C, the major subfractions of HDL-C in the diabetics might be worthy of being measured.

Hara *et al.* succeeded in developing for the first time the method of determining HDL_2-C and HDL_3-C by high performance liquid chromatography (HPLC), using the aqueous gel permeation columns (Hara *et al.* [7]; Okazaki *et al.* [8]). With this method, the HDL_2-C and HDL_3-C can be measured directly with a small amount of whole serum (20μl) in less than 50 minutes.

In the present study, the serum HDL_2-C and HDL_3-C levels were measured in the diabetics by the HPLC method to

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Received for publication, February 29, 1984.

investigate the metabolism of the HDL subfractions in the diabetics.

Apolipoprotein AI and AII (apo AI and AII), the major protein components of HDL, were also measured.

Materials and Methods

Blood was obtained after overight fasting from 119 normal subjects (53 males, aged 42±13 yrs, M±SD; 66 females, 42±11 yrs) and 88 diabetics (42 males, 53±10 yrs; 46 females, 55±13 yrs). Normal subjects, who had no abnormalities in the serum triglyceride level (TG<160 mg/d*l*), serum total cholesterol level (TC<250 mg/d*l*), plasma glucose level (FPG<110 mg/d*l*), blood pressure (BP<150/95 mmHg), ECG findings, liver function and renal function, were obtained. All diabetics were non-insulin-dependent diabetic patients. Diabetics were divided into three groups: Those taking insulin, those on oral agents and those on diet only.

The serum HDL_2-C and HDL_3-C levels were determined by the new method using HPLC. The serum lipoprotein was resolved into VLDL, LDL, HDL_2 and HDL_3 by HPLC (HLC805A, Toyo Soda Mfg. Co., Tokyo) on the gel permeation columns (TSK-GEL, Toyo Soda Mfg. Co., Tokyo). The conditions of HPLC were as follows: Columns, G4000SW +G3000SW; eluent, 0.15mol/*l* NaCl; flow rate, 0.6m*l*/min; and temperature, ambient. The serum (20 μl) was applied to the G4000SW +G3000SW column system. Cholesterol in the final column effluent was monitored by measuring the A_{550}, with the use of the enzymatic reagent kit (Determiner TC 555, Kyowa Medix Co., Tokyo). The content of the cholesterol in each fraction was calculated from the peak area of the elution pattern of the cholesterol from the columns (Okazaki *et al.* [9]; Ohno *et al.* [10]).

The serum apo AI, apo AII and apo B levels were determined by the single radial immunodiffusion method (Daiichi Kagaku Co., Tokyo). The levels of the hemoglobin A_1 in the diabetics were measured by the mini-column method.

Results

I. Relationship between apo A and the subfractions of HDL-C in normal subjects (Table 1)

In the normal subjects, HDL_2-C showed a positive correlation with apo AI ($r=0.43$, $p<0.01$), while HDL_3-C demonstrated a positive correlation with apo AI and apo AII as well ($r=0.50$, $p<0.01$). LDL-C showed a positive correlation with apo B ($r=0.91$, $p<0.01$).

II. Comparision of HDL_2-C and HDL_3-C between each therapeutic group of diabetics and normal group

The clinical characteristics of the diabetics used in this study are shown in Table 2. Only the diabetics with normal ECG findings were used in this study. The serum TC and TG levels, obesity index and age in the diabetics were higher than those in the normal subjects. The serum TC and TG levels, obesity index and age were matched between each diabetic group and normal group to exclude the influence of these factors on the levels of HDL-C (Table 3). In both the males and females, the serum HDL_2-C levels in the insulin group were significantly higher than those in the normal group, whereas there was no difference between the serum HDL_2-C levels in the oral agent group or diet-only group and those in the normal group (Table 3, Fig.1, Fig.2). No diference was found between the serum HDL_3-C levels in each therapeutic group of diabetics and those in the normal group (Fig.1, Fig.2).

III. Comparision of apo AI and apo

Table 1. Correlation Between Apolipoproteins (Apo A, Apo AI, Apo AII and Apo B) and Lipoprotein Subfractions in Normal Subjects

n=50

	APO-A	APO-AI	APO-AII	APO-B
TG	0.11	0.10	0.13	0.54**
VLDL·C	0.09	0.06	0.16	0.48**
LDL·C	0.09	0.09	0.06	0.91**
HDL·C	0.55**	0.56**	0.32*	−0.13
HDL_2·C	0.40**	0.43**	0.14	−0.26
HDL_3·C	0.53**	0.50**	0.50**	0.23

*P<0.05 **P<0.01

Table .2 Clinical Characteristics of Diabetics Without Abnormal ECG Findings and Normal Subjects Values are mean±SD. *Significantly different from normal subjects

	Diabetics		Normal Subjects	
	M n=27	F n=31	M n=33	F n=66
Age (yrs)	55±10*	57±12*	42±13	42±11
Obesity Index (%)	101±11	111±19*	102±11	103±12
FPG (mg/dl)	159±65	139±42		
Duration (yrs)	11±6	8±5		
TC (mg/dl)	199±38*	212±36*	181±30	188±38
TG (mg/dl)	108±55*	119±91*	88±35	68±31

*Significantly different from normal subjects

Table 3. Comparison of HDL_2-C and HDL_3-C Between Each Therapeutic Group of Male Diabetics and TC, TG, Obesity Index and Age-matched Normal Group Values are mean±SD. *Significantly different from normal group. OBIX: Obesity index

	n	Age (yrs)	TC (mg/dl)	TG (mg/dl)	OBIX (%)	HDL-C (mg/dl)	HDL_2-C (mg/dl)	HDL_3-C (mg/dl)
Insulin	8	51±11	205±40	90±39	−2±9	*50±19	*40±16	25±7
Normal	12	51±8	177±23	81±39	−3±6	48±17	23±14	25±4
Oral Agent	7	60±9	198±24	121±28	8±10	34±7	15±4	19±5
Normal	8	57±6	187±26	119±43	6±11	39±9	19±10	20±4
Diet only	11	56±10	197±39	101±52	1±9	48±13	25±10	23±4
Normal	13	53±9	178±37	100±32	2±10	44±10	20±6	24±5

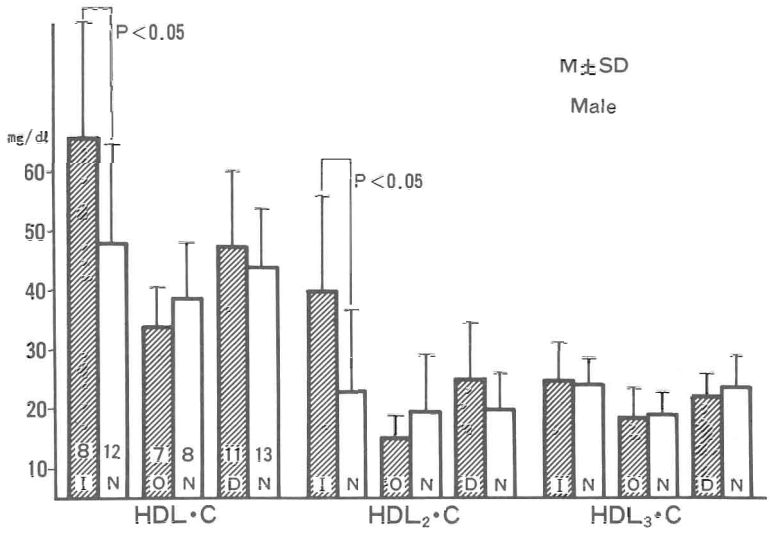

Fig.1. Comparison of HDL Subfractions Between Each Therapeutic Group of Male Diabetics and TC, TG, Obesity Index and Age-Matched Normal Group I: Insulin, O: Oral agent, D: Diet, N: Normal Each figure of the bar represents the number of subjects.

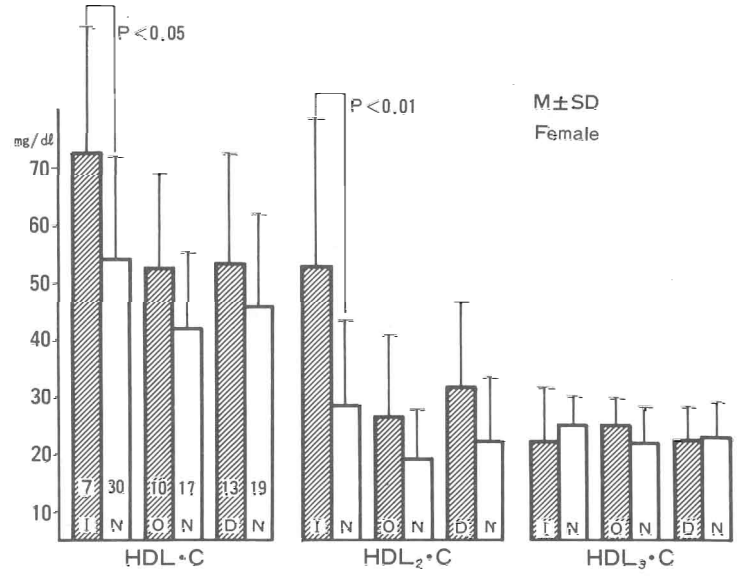

Fig.2. Comparison of HDL Subfractions Between Each Therapeutic Group of Female Diabetics and TC, TG, Obesity Index, and Age-Matched Normal Group I: Insulin, O: Oral agent, D: Diet, N: Normal
Each figure of the bar represents the number of subjects.

AII between each therapeutic group of diabetics and the normal group

The clinical characteristics of the diabetics used in this study are shown in Table 4. The serum TC and TG levels, obesity index and age were matched be-

Table 4. Clinical Characteristics of Diabetics Without Abnormal ECG Findings and Normal Subjects Values are mean±SD.

	Diabetics		Normal Subjects	
	M n=38	F n=34	M n=25	F n=25
Age (yrs)	53±10*	55±13*	44±8	43±6
Obesity index (%)	104±16	112±18	101±7	105±10
FPG (mg/dl)	148±48*	156±44*	93±7	89±7
HbA_1 (%)	10.2±2.5	10.5±2.9		
Duration(yrs)	9±7	7±6		
TC (mg/dl)	200±46	224±36*	183±32	190±26
TG (mg/dl)	108±56	113±62*	88±25	70±23

*Significantly different from normal subjects

Table 5. Comparison of Apolipoprotein AI and AII Between Each Therapeutic Group of Male Diabetics and TC, TG, Obesity Index and Age-matched Male Normal Group
Values are mean±SD.

	n	Age (yrs)	TC (mg/dl)	TG (mg/dl)	OBIX (%)	Apo A (mg/dl)	Apo A I (mg/dl)	Apo A II (mg/dl)
Insulin	8	47± 9	178±34	75±32	92±11	166±29	133±24	33± 7
Normal	9	44± 7	166±22	71±28	93± 3	156±16	124±14	32± 4
Oral Agent	9	58±14	212±46	117±58	101±15	181±21	149±16	36± 7
Normal	9	54± 4	210±31	110±20	103± 7	171±24	138±20	34± 6
Diet only	12	52± 7	188±31	108±57	105± 9	180±51	144±42	36±10
Normal	13	50± 7	197±33	105±17	105± 7	168±24	135±20	33± 5

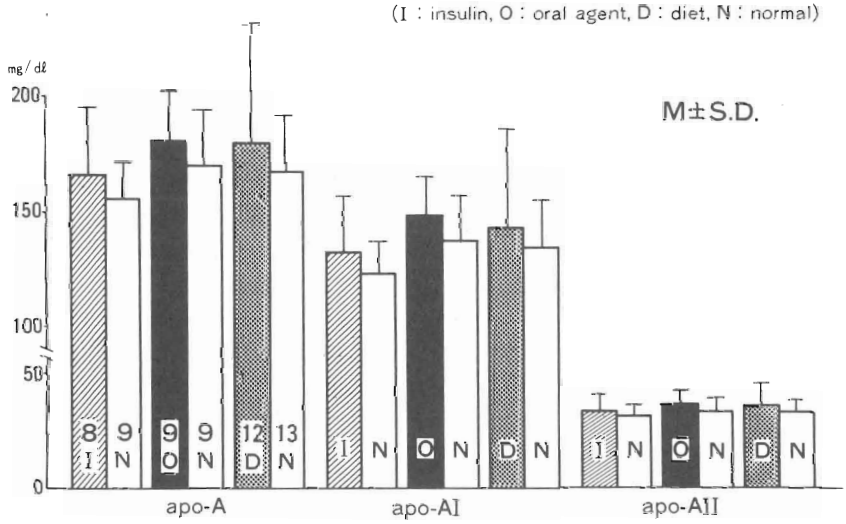

Fig.3. Comparison of Apo A, Apo AI and Apo AII Between Each Therapeutic group of Male Diabetics and TC, TG, Obesity Index and Age-matched Normal Group
Each figure of the bar represents the number of subjects.

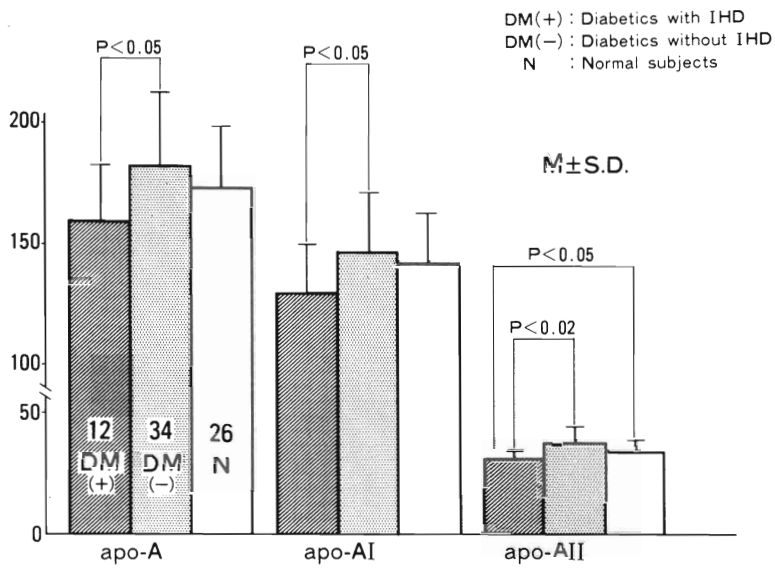

Fig.4. Comparison of Apo A, Apo AI and Apo AII Among Normal Subjects, Diabetics With and Without Ischemic Heart Disease Each figure of the bar represents the number of subjects.

tween the normal group and each diabetic group with normal ECG findings (Table 5). No significant differences in the serum apo AI and apo AII levels were found between each therapeutic group and the normal group (Table 5, Fig.3).

Then the serum apo AI and apo AII levels were compared between the diabetics with and without an ischemic heart disease (IHD). The serum apo AI and apo AII levels were significantly lower in the diabetics with IHD than those in the diabetics without IHD (Fig.4).

The levels of hemoglobin A_1 had no significant correlation with the serum apo AI or apo AII levels.

Discussion

In the present study, serum HDL_2-C, HDL_3-C, apo AI, apo AII, and ago B levels were measured in the non-insulin-dependent diabetic subjects (NIDD) and normal subjects to investigate the matabolism of HDL in the diabetics.

In the normal subjects, HDL_2-C showed a positive correlation with apo AI, while the HDL_3-C demonstrated a positive correlation with apo AI and apo AII as well (Table 1). These findings suggest that HDL_2 contains apo AI and HDL_3 contains both the apo AI and apo AII. Furthermore,the highly positive correlation between the LDL-C and apo B ($r=0.91$) suggests that apo B is a major protein component of LDL.

The serum HDL_2-C and HDL_3-C levels were compared between each therapeutic group of diabetics and the TC, TG, obesity index and age-matched normal group. In both the males and females, the serum HDL_2-C levels in the insulin group were significantly higher than those in the normal group, whereas there was no difference between the serum HDL_2-C levels in the oral agent group or diet-only group and those in the normal group (Fig.1 and Fig.2). It is suggested that insulin increases the HDL_2-C levels and that the increase of the HDL_2-C

levels is not directly related to changes in the serum TC and TG levels, obesity index and age. And no significant differences in the serum apo AI and apo AII levels were found between the insulin group and the TC, TG, obesity index and age-matched normal group (Fig.3). Apo AI and apo AII are approximately 90% of protein component of HDL. Thus, from these results, it is suggested that in the insulin group the cholesterol/apoprotein ratio in the HDL_2 is higher than that in the normal group. Further investigation is necessary to clear the meaning of the increase of the cholesterol/apoprotein ratio in the HDL_2 in the insulin group.

In the earlier paper (Tanaka [11]) it has been reported that the HDL_2-C and HDL_3-C play an important role in preventing the development of atherosclerosis. In the present study the serum apo AI and apo AII levels are significantly lower in the diabetics with IHD than those in the diabetics without IHD (Fig.4). These results show that in the diabetics the apo AI and apo AII also play an important role in preventing IHD.

ACKNOWLEDGEMENT

The authors express their deep thanks to Dr. M. Okazaki and Prof. I. Hara of the Laboratory of Chemistry, Department of General Education, Tokyo Medical and Dental University, for their useful advices and suggestions.

REFERENCES

1) Castelli, W. P., Doyle, J. T., Gordon, T., Hames, C. G., Hjortland, M. C., Hulley, S.R., Kagan, A., and Zukel, W. J.: HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation, 55: 767–772 (1977).
2) Miller, N. E., Forde, O. H., Thelle, D. S., and Mjos, O. D.: The Tromso heart study. High density lipoprotein and coronary heart disease. A prospective case-control study. Lancet, 1: 965–967 (1977).
3) Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B., and Dawber, T. R.: High density lipoprotein as a protective factor against coronary heart disease. Am. J. Med., 62: 707–714 (1977).
4) Kessler, I. I.: Mortality experience of diabetic patients. A twenty-six year follow-up study. Am. J. Med., 51: 715–724 (1971).
5) Garcia, M. J., McNamara, P. M., and Gordon, T.: Morbidity and mortality in diabetics in the Framingham population. Sixteen-year follow-up study. Diabetes, 23: 105–111 (1974).
6) Plumbo, P. J., Elveback, L. R., and Chu, C. P.: Diabetes Mellitus: incidence, prevalence, survivorship, and causes of death in Rochester, Minnesota, 1945–1970. Diabetes, 25: 556–573 (1976).
7) Hara, I., Okazaki, M., and Ohno, Y.: Rapid analysis of cholesterol of HDL and LDL in human serum by high performance liquid chromatography. J. Biochem., 87: 1863–1865 (1980).
8) Okazaki, M., and Hara, I.: Analysis of cholesterol in high density lipoprotein subfractions by high performance liquid chromatography. J. Biochem., 88: 1215–1218 (1980).
9) Okazaki, M., Shiraishi, K., Ohno, Y., and Hara, I.: High performance aqueous gel permeation chromatography of serum lipoproteins: Selective detection of cholesterol by enzymatic reaction. J. Chromatogr., 223: 285–293 (1981).
10) Ohno, Y., Okazaki, M., and Hara, I.: Fractionation of Human serum lipoproteins by high performance liquid chromatography. J. Biochem., 89: 1675–1680(1981).
11) Tanaka, A.: Role and metabolism of high density lipoprotein subfractions. Bull. Tokyo Med. Dent. Univ., 29: 131–137 (1982).