Remnant-Like Lipoprotein Particles in Type 2 Diabetic Patients With Apolipoprotein E3/3 and Apolipoprotein E2 Genotypes

Mieko Saito, Masaaki Eto, and Kohei Kaku

Apolipoprotein (apo) E2 and diabetes mellitus are known to be associated with an accumulation of remnant lipoproteins in plasma. In this study, effects of type 2 diabetes mellitus and/or apo E2 genotypes on remnant-like lipoprotein particles (RLP) were assessed. Thirty-three subjects were divided into 6 groups: 7 apo E3/3 nondiabetic subjects, 6 apo E3/3 diabetic patients, 5 apo E3/2 nondiabetic subjects, 6 apo E3/2 diabetic patients, 5 apo E2/2 nondiabetic subjects, and 4 apo E2/2 diabetic patients. First, the effect of diabetes mellitus on RLP were estimated by comparing the apo E3/3 nondiabetic group with the apo E3/3 diabetic group. Plasma levels of RLP-cholesterol (chol) in the apo E3/3 diabetic group and the uptake of RLP from the apo E3/3 diabetic group by macrophages were significantly greater compared with the apo E3/3 nondiabetic group. Second, the effect of apo E2 on RLP was estimated in nondiabetic subjects. Apo E2/2 nondiabetic subjects had type III hyperlipoproteinemia (HLP). Plasma levels of RLP-chol in the apo E2/2 nondiabetic group and the uptake of RLP from the apo E2/2 nondiabetic group by macrophages were significantly greater compared with the apo E3/3 and apo E3/2 nondiabetic groups. Third, the effects of both apo E2 and diabetes on RLP were estimated. Plasma levels of RLP-chol in the apo E2 (E3/2 and E2/2) diabetic groups and the uptake of RLP from apo E2 (E3/2 and E2/2) diabetic groups by macrophages were significantly greater compared with apo E3/3 nondiabetic and diabetic groups or the apo E3/2 nondiabetic group. In diabetes, a gene dose effect of apo E2 on plasma levels of RLP-chol and uptake of RLP by macrophages was present (apo E3/3 < apo E3/2 < apo E2/2). The apo E2/2 diabetic group had type III HLP. Furthermore, uptake of RLP from the apo E2/2 diabetic group with type III HLP was significantly greater compared with the apo E2/2 nondiabetic group with type III HLP. In conclusion, type 2 diabetes was associated with increased RLP-chol in plasma and atherogenic RLP. In nondiabetes, apo E2/2 contributes to increased plasma RLP-chol and atherogenic RLP. In diabetes, additional effects of apo E2 to increase RLP-chol in plasma and to enhance the uptake of RLP by macrophages are present. RLP from apo E2/2 diabetes with type III HLP are more atherogenic than those from apo E2/2 nondiabetes with type III HLP.

DIABETES MELLITUS IS a major risk factor for cardiovascular disease. Lipid abnormalities contribute to the increased incidence of atherosclerosis in type 2 diabetes. The lipid abnormalities may be explained by reduced peripheral clearance and increased hepatic production of triglyceride (TG)-rich lipoproteins. It has been reported that diabetic dyslipidemia syndrome consists of increased plasma TG-rich lipoproteins including chyromicron remnants and very–low-density lipoprotein (VLDL) remnants, ie, intermediate-density lipoprotein (IDL).[1] Remnant lipoproteins have been recognized as a risk factor for atherosclerosis in type 2 diabetes.[1]

A reliable method for the determination of plasma remnant-like lipoprotein particles (RLP) using monoclonal antihuman apolipoprotein (apo) A-1 and antihuman apo B-100 antibodies has been developed.[2] Campos et al[3] showed that RLP isolated by this method are enriched in TG, cholesterol (chol), and apo E and represent remnant lipoproteins. These apo E-rich RLP are reported to be a major risk factor for coronary atherosclerosis.[4-7] Further, it was shown that plasma levels of RLP-chol are elevated in type 2 diabetes.[8]

From the Department of Medicine, Diabetes and Endocrine Division, Kawasaki Medical School, Kurashiki, Japan.

Submitted June 4, 2001; accepted February 6, 2002.

Supported by a Grant-in-Aid (No. 12671128) from the Ministry of Education, Science and Culture of Japan and by project grants (No. 12-502 and 13-505) from Kawasaki Medical School.

Address reprint requests to Masaaki Eto, MD, Kawasaki Medical School, Department of Medicine, Diabetes and Endocrine Division, Matsushima 577, Kurashiki 701-0192, Japan.

0026-0495/02/5108-0003$35.00/0

doi:10.1053/meta.2002.34014

Apo E is one of the main protein constituents of TG-rich lipoproteins. Apo E has an affinity for the lipoprotein receptors and plays an important role in receptor-mediated endocytosis of TG-rich lipoproteins including remnants. Apo E is genetically polymorphic and exists as 3 common isoproteins, E2, E3, and E4, which are coded by 3 apo E alleles, $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, respectively.[9-11] Apo E3 is the native form (wild type). The apo E3/3 genotype is the most common form in the population (approximately 70% in Japan), whereas the apo E2 genotypes are relatively rare (approximately 7% in Japan).[12] Apo E2 has reduced binding affinity for low-density lipoprotein (LDL) or remnant receptors because apo E2 is commonly caused by a change in residue 158 from Arg to Cys.[13] Thus, apo E2 is associated with impaired remnant metabolism, accumulation of remnant lipoproteins in plasma as type III hyperlipoproteinemia (HLP),[10] and premature atherosclerosis.

Both diabetes mellitus and apo E2 are thought to be independent factors, which increase remnant lipoproteins in plasma. We previously reported that type 2 diabetic patients with apo E2/2 genotype are susceptible to type III HLP and diabetic patients with apo E 3/2 genotype are susceptible to type IV HLP, and diabetic patients with apo E2 genotypes (apo E2/2 and E3/2) are characterized by increased remnant lipoproteins in plasma.[14,15] However, as far as we are aware, there has been no report concerning RLP in diabetic patients with apo E2. In a previous report,[8] the effect of apo E genetic polymorphism on RLP in type 2 diabetes was not taken into consideration. First, we compared plasma levels of RLP and uptake of RLP by macrophages between nondiabetic subjects with apo E3/3 genotype and type 2 diabetic patients with apo E3/3 genotype to show the effect of diabetes on RLP. Second, we compared these among nondiabetic subjects with the apo E3/3 genotype and with the apo E2 genotypes to investigate the effect of apo

E2 on RLP. Third, we compared these among diabetic patients with apo E3/3 genotype and with apo E2 genotypes to assess the effect of apo E2 on PLP in diabetes.

SUBJECTS AND METHODS

Subjects

Thirty-three subjects were recruited from our affiliated hospitals and were divided into 6 groups: 7 apo E3/3 nondiabetic subjects, 6 apo E3/3 diabetic patients, 5 apo E3/2 nondiabetic subjects, 6 apo E3/2 diabetic patients, 5 apo E2/2 nondiabetic subjects, and 4 apo E2/2 diabetic patients. The apo E2/2 genotype is extremely rare in Japanese (0.14%)[12] compared with Caucasians (0.72%) and, therefore, only 4 apo E2/2 diabetic patients were found for the present study. All diabetic patients were diagnosed as having type 2 diabetes because they had no history of ketoacidosis and had a 24-hour urinary C-peptide excretion rate of more than 20 μg. One of the 6 apo E3/3 diabetic subjects was treated with diet alone, 2 took oral agents, and 3 were on insulin. One of the 6 apo E3/2 diabetic patients was treated with diet alone, 4 took oral agents, and 1 was on insulin. One of the 4 apo E2/2 diabetic patients was treated with diet alone, 1 took oral agents, and 2 were on insulin. None of the subjects was on lipid-lowering agents or antioxidants. None of the subjects had elevated levels of serum creatinine, abnormal liver or endocrine function. Nondiabetes was diagnosed according to a 75-g oral glucose tolerance test. All subjects were Japanese and were on a Japanese diet. Diabetic and obese patients were advised to restrict total caloric intake. The characteristics and plasma lipid levels in these groups are presented in Table 1. The study protocol was approved by the local ethical review committee, and the study was performed according to the principle of the Declaration of Helsinki. All subjects participated in the study after giving informed consent.

Measurement of Lipids, Lipoproteins, RLP-Chol, and Other Parameters

Blood samples were collected after an overnight fast. Fasting plasma glucose was measured by the glucose-oxidase method. Glycosylated hemoglobin (HbA_{1c}) was measured by high-performance liquid chromatography. Plasma TG and total chol were measured enzymatically. Plasma high-density lipoprotein (HDL)-chol was measured by the precipitation method. Plasma RLP-chol was measured by the method of Nakajima et al.[2] Polyacrylamide gel electrophoresis using plasma from the apo E2/2 subjects was performed for the diagnosis of type III HLP. The VLDL (density $<$ 1.006 g/mL) fraction was separated by ultracentrifugation.

Apo E Phenotyping and Genotyping

Using the delipidated VLDL fraction isolated as described above, apo E phenotypes were determined using our rapid flat gel isoelectric focusing method.[16] Apo E genotypes were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method reported by Hixon et al.[17] If apo E phenotypes and genotypes are not matched, this suggests that there is another mutation in apo E. However, both were matched in all cases in the present study.

Isolation of RLP

One-milliliter capacity columns of the immunoaffinity gel mixture of anti-apo B100 and anti-apo AI monoclonal antibodies coupled to Sepharose 4B (kindly provided by Japan Immunoresearch Laboratories, Gunma, Japan) were prepared and maintained at 10°C. Fresh serum samples (250 μL) were applied to the affinity column, and the unbound fraction (RLP) was eluted with 10 mL of 10 mmol/L phosphate buffer, pH 7.3. The bound lipoproteins were then eluted with 3 mol/L NaSCN containing bovine serum albumin (BSA) (1 mg/mL), pH 7.4. The unbound fraction contained an apo E-rich population of remnant-like lipoproteins containing apo B-100, as well as TG-rich lipoproteins containing apo B-48 and thus, they represent chylomicron and VLDL RLP.[2,3,18] The unbound lipoproteins (RLP) were concentrated and then used within 24 hours for a study on the ability to stimulate cholesteryl ester synthesis in macrophages.

Human Monocyte-Derived Macrophages

Human monocytes were isolated by density gradient centrifugation from blood of healthy donors by the method of Boyum.[19] Twenty-milliliter aliquots of blood (anticoagulated with 10 U/mL heparin) were layered over 15 mL of Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) and centrifuged at 500 $\times$ *g* for 30 minutes at room temperature. The mixed mononuclear cell band was removed, and the cells were washed twice in phosphate-buffered saline (PBS) buffer. Then 3×10^5 cells were seeded into each well of 24-well plates with RPMI-1640 culture medium. After a 2-hour incubation at 37°C under 5% CO_2 and 95% air, nonadherent cells were removed, and the adherent cells were incubated in RPMI-1640 containing 20% autologous serum and fed twice weekly with the same medium. Human monocyte-derived macrophages were used for experiments within 10 to 12 days of plating. Two days before the experiments, the medium was replaced by the medium containing 5 mg/mL of human lipoprotein-deficient serum.

Effects of RLP on Cholesteryl Esters Synthesis in Macrophages

The effect of RLP on cholesteryl esters synthesis in macrophages was estimated by measurement of the stimulation of ^{14}C-oleate incorporation into cholesteryl esters.[20] For each subject, RLP, which were sterilized by passage through a 0.45-μm Millipore (Bedford, MA) filter, were adjusted to 20 mg/mL chol in RPMI-1640 culture medium. Macrophages were incubated for 24 hours at 37°C with RLP in RPMI-1640 culture medium. After removing the medium and washing with RPMI-1640 culture medium, the cells were incubated for 2 hours at 37°C with ^{14}C-oleate (0.2 mmol/L, 10 mCi/mL oleate in the presence of 0.07 mmol/L BSA) and incorporation of the radiolabel into cellular ^{14}C-cholesteryl esters was measured.[20] Values are expressed as nmoles of ^{14}C-oleate incorporated into ^{14}C-cholesteryl esters/mg cell protein, which was measured by the method of Lowry et al.[21] All results are presented as the mean of triplicate determinations.

Statistical Analysis

All values are expressed as the mean $\pm$ SEM. Statistical comparisons between multiple groups were made by analysis of variance (ANOVA). Multiple comparisons were made. Analysis was performed using standard statistical software packages (SAS Institute, Cary, NC).[22] $P < .05$ was considered significant.

RESULTS

Clinical Characteristics

Characteristics of the 3 (apo E3/3, E3/2, and E2/2) nondiabetic groups and 3 (apo E3/3, E3/2, and E2/2) diabetic groups are shown in Table 1. The levels of fasting plasma glucose were significantly higher in diabetic groups compared with nondiabetic groups. The levels of fasting plasma glucose and HbA_{1c} were not significantly different among the 3 diabetic groups. The age and body mass index (BMI) were not significantly different among the 6 groups. Plasma levels of TG and total chol were not significantly different among the 5 groups except the apo E2/2 diabetic group. The apo E2/2 diabetic group had significantly higher plasma levels of TG than apo E3/3 and

Table 1. Characteristics of Nondiabetic Subjects and Type 2 Diabetic Patients

	Apo E3/3		Apo E3/2		Apo E2/2	
	Nondiabetes	Type 2 Diabetes	Nondiabetes	Type 2 Diabetes	Nondiabetes	Type 2 Diabetes
No. (M/F)	7 (5/2)	6 (3/3)	5 (4/1)	6 (3/3)	5 (4/1)	4 (3/1)
Age (yr)	50 ± 3	54 ± 3	51 ± 2	57 ± 7	52 ± 6	61 ± 5
BMI (kg/m^2)	22.2 ± 1.1	22.6 ± 2.0	23.1 ± 0.7	24.7 ± 2.0	24.6 ± 1.4	25.0 ± 1.2
FPG (mg/dL)	87 ± 2	166 ± 19*	93 ± 3	139 ± 7*	92 ± 3	169 ± 19*
HbA_{1c} (%)	—	8.2 ± 0.5	—	8.0 ± 0.4	—	8.2 ± 0.6
Total chol (mg/dL)	171 ± 3	192 ± 15	168 ± 14	175 ± 11	195 ± 20	246 ± 35†
Triglyceride (mg/dL)	113 ± 32	138 ± 18	154 ± 46	162 ± 39	212 ± 59	389 ± 83‡§
HDL-chol (mg/dL)	57 ± 5	50 ± 4	59 ± 7	47 ± 4	53 ± 3	50 ± 7

NOTE. Values are mean ± SE.
Abbreviations: BMI, body mass index; FPG, fasting plasma glucose.
*$P < .01$ *v* nondiabetes within the same apo E genotype.
†$P < .05$ *v* apo E3/3 nondiabetes, apo E3/2 nondiabetes, and apo E3/2 diabetes.
‡$P < .01$ *v* apo E3/3 nondiabetes and apo E3/3 diabetes,
§$P < .05$ *v* apo E3/2 nondiabetes and apo E3/2 diabetes.

E3/2 nondiabetic groups and apo E3/3 and E3/2 diabetic groups. Similarly, the apo E2/2 diabetic group had significantly higher plasma levels of total chol than the apo E3/3 and E3/2 nondiabetic groups and the apo E3/2 diabetic group. There was no significant difference in plasma levels of TG and total chol between the apo E2/2 nondiabetic and apo E2/2 diabetic groups. Plasma levels of HDL-chol were not significantly different among the 6 groups. All of the apo E2/2 patients had high levels of plasma TG and a broad β band pattern on polyacrylamide gel electrophoresis, indicating type III HLP. There were no significant differences in all parameters except for fasting plasma glucose between nondiabetic and diabetic groups within the same apo E genotype.

Plasma Levels of RLP-Chol

As shown in Fig 1, the apo E3/3 diabetic group (10.5 mg/dL) had significantly higher plasma levels of RLP-chol (normal range, less than 7.5 mg/dL) than the apo E3/3 nondiabetic group (5.0 mg/dL). In addition, the apo E3/2 diabetic group (20.2 mg/dL) had significantly higher plasma levels of RLP-chol than the apo E3/3 and E3/2 (9.8 mg/dL) nondiabetic groups and the apo E3/3 diabetic group. The apo E2/2 nondiabetic group (28.9 mg/dL) had significantly higher plasma levels of RLP-chol than the apo E3/3 and E3/2 nondiabetic groups and the apo E3/3 diabetic group. The apo E2/2 diabetic group (50.1 mg/dL) had significantly higher plasma levels of RLP-chol than the apo E3/3 and E3/2 nondiabetic groups and the apo E3/3 and E3/2 diabetic groups.

There were significant differences in plasma levels of RLP-chol between the nondiabetic and diabetic groups within the apo E3/3 or apo E3/2 genotype, but no significant difference between nondiabetic and diabetic groups within the apo E2/2 genotype was noted.

Effects of RLP on Cholesteryl Esters Synthesis in Macrophages

The effect of RLP from nondiabetic and diabetic subjects with the apo E3/3 or apo E2 genotypes on cholesteryl ester synthesis in macrophages was assessed. In the present study, chol content in the medium was matched to avoid the quantitative effect of RLP on its synthesis in macrophages. As shown in Fig 2, RLP from the apo E3/3 diabetic group (0.326 nmol/mg cell protein) enhanced cholesteryl ester synthesis more significantly compared with those from the apo E3/3 nondiabetic group (0.181 nmol/mg cell protein). Similarly, RLP from the apo E3/2 diabetic group (0.490 nmol/mg cell protein) enhanced cholesteryl ester synthesis in macrophages more significantly compared with those from the apo E3/3 and E3/2 (0.253 nmol/mg cell protein) nondiabetic groups or the apo E3/3 diabetic group. Likewise, RLP from the apo E2/2 nondiabetic group (0.445 nmol/mg cell protein) enhanced cholesteryl ester

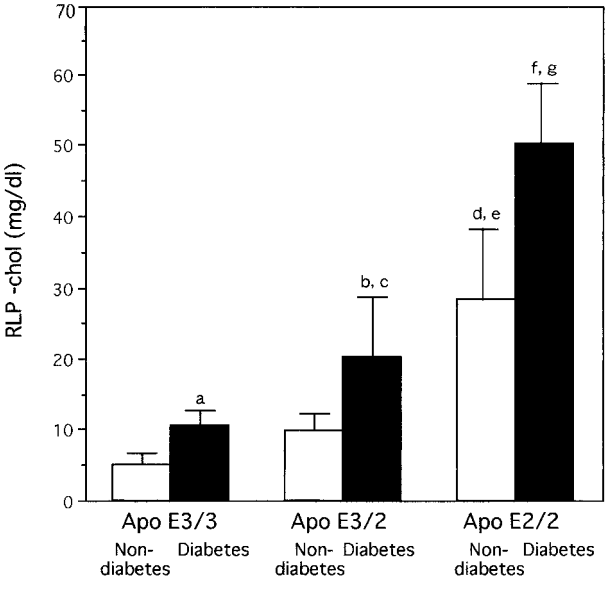

Fig 1. Plasma levels of RLP-chol in apo E3/3 nondiabetic and diabetic subjects and apo E2 nondiabetic and diabetic subjects. (a) $P < .05$ *v* apo E3/3 nondiabetes; (b) $P < .05$ *v* apo E3/3 diabetes and apo E3/2 nondiabetes; (c) $P < .01$ *v* apo E3/3 nondiabetes; (d) $P < .05$ *v* apo E3/3 diabetes and apo E3/2 nondiabetes; (e) $P < .01$ *v* apo E3/3 nondiabetes; (f) $P < .05$ *v* apo E3/2 diabetes; (g) $P < .01$ *v* apo E3/3 nondiabetes, apo E3/3 diabetes, and apo E3/2 nondiabetes.

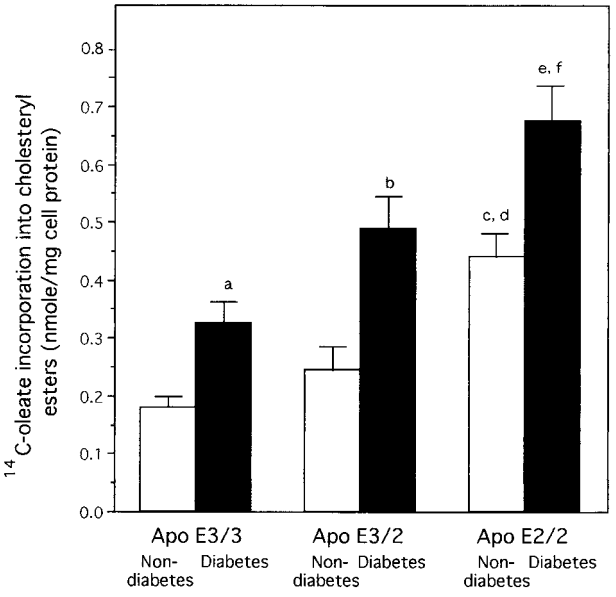

Fig 2. Effects of RLP from apo E3/3 nondiabetic and diabetic subjects and apo E2 nondiabetic and diabetic subjects on cholesteryl ester synthesis in human monocyte-derived macrophages. (a) *P* < .01 *v* apo E3/3 nondiabetes; (b) *P* < .01 *v* apo E3/3 nondiabetes, apo E3/3 diabetes, and apo E3/2 nondiabetes; (c) *P* < .05 *v* apo E3/3 diabetes; (d) *P* < .01 *v* apo E3/3 nondiabetes and apo E3/2 nondiabetes; (e) *P* < .05 *v* apo E3/2 diabetes and apo E2/2 nondiabetes; (f) *P* < .01 *v* apo E3/3 nondiabetes, apo E3/3 diabetes, and apo E3/2 nondiabetes.

synthesis more significantly compared with those from the apo E3/3 and E3/2 nondiabetic groups or the apo E3/3 diabetic group. There was no significant difference in the ability of RLP to enhance cholesteryl ester synthesis in macrophages between the apo E3/2 diabetic group and the apo E2/2 nondiabetic group. In addition, RLP from the apo E2/2 diabetic group (0.674 nmol/mg cell protein) enhanced cholesteryl ester synthesis more significantly compared with those from the other 5 groups.

There were significant differences in the ability of RLP to enhance cholesteryl ester synthesis between the nondiabetic and diabetic groups within the same apo E genotype.

DISCUSSION

Because apo E2 is a factor associated with an increase in plasma remnant lipoproteins,[23,24] we first compared the plasma levels of RLP-chol between apo E3/3 (wild type) subjects with and without diabetes mellitus to exclude the effect of apo E2. Apo E3/3 diabetic patients had significantly higher plasma levels of RLP-chol than apo E3/3 nondiabetic subjects. This finding supports the report that plasma RLP-chol is elevated in type 2 diabetes.[8] The investigators found elevation of both RLP-chol and TG in type 2 diabetes.[8] However, in the present study, no significant difference in plasma TG levels was noted between the apo E3/3 nondiabetic and diabetic subjects, indicating the possibility that an increase of RLP-chol may occur in advance of an increase of TG in diabetes.

Macrophages are precursors of arterial wall foam cells and play an important role in the formation of early events in the process leading to atherosclerosis.[25] TG-rich lipoproteins including VLDL, chylomicron remnants, and VLDL remnants,[26] as well as modified LDL, such as acetylated or oxidated LDL,[25] are taken up by receptor-mediated mechanisms, resulting in massive cholesteryl ester accumulation in macrophages. Accordingly, the ability of RLP obtained from the apo E3/3 diabetic patients to enhance cholesteryl ester synthesis in macrophages was estimated. The present study showed that RLP from apo E3/3 diabetic patients was more effective in enhancing cholesteryl ester synthesis in macrophages compared with those from the apo E3/3 nondiabetic subjects. Cholesterol content in the medium was matched in all cellular experiments and, therefore, this finding indicates that RLP from diabetic patients may be qualitatively more atherogenic than those from nondiabetic subjects. RLP have been reported to be closely associated with myocardial infarction in vasospastic angina,[4] coronary atherosclerosis in cases of sudden cardiac death,[5] coronary events in patients with coronary artery disease,[6] and cardiovascular disease in women in the Framingham Heart Study.[7] Increased plasma levels of RLP-chol in apo E3/3 diabetic patients and enhanced uptake of RLP from apo E3/3 diabetic patients by macrophages observed in the present study suggest that RLP are one of the potential atherogenic factors in type 2 diabetes.

Second, in nondiabetes, the effect of apo E2 on plasma levels of RLP-chol and on the ability of RLP to stimulate cholesteryl ester synthesis in macrophages was investigated. A close association of apo E2/2 homozygote with RLP was also observed in nondiabetes. Apo E2/2 nondiabetic subjects had significantly higher plasma levels of RLP-chol than apo E3/3 and E3/2 nondiabetic subjects, and RLP from apo E2/2 nondiabetic subjects was more effective in enhancing cholesteryl ester synthesis in macrophages compared with those from apo E3/3 and E3/2 nondiabetic subjects. Apo E2/2 nondiabetic subjects had type III HLP. This finding is in line with a recent report that plasma levels of RLP were high in type III HLP[27] and is the first demonstration that uptake of RLP from type III HLP subjects is enhanced. This agrees with clinical observations that type III HLP subjects are susceptible to atherosclerosis.

Third, the effects of apo E2, in addition to diabetes, on plasma levels of RLP-chol and on the ability of RLP to stimulate cholesteryl ester synthesis in macrophages were studied. Apo E3/2 diabetic patients had significantly higher plasma levels of RLP-chol compared with apo E3/3 and E3/2 nondiabetic subjects or apo E3/3 diabetic patients. Apo E2/2 diabetic patients had typical type III HLP and the highest levels of RLP-chol. This finding that apo E2 increases RLP in diabetes agrees well with the previous reports in which we indirectly showed increased remnant lipoproteins in diabetic patients with apo E2.[14,15,28] In addition, RLP from apo E2 (E3/2 and E2/2) diabetic patients was more effective in promoting cholesteryl ester synthesis in macrophages compared with apo E3/3 nondiabetic and diabetic patients and apo E3/2 nondiabetic patients with cholesterol content in the medium being equal. This was even greater when RLP from apo E2/2 diabetic patients was used. In diabetes, a gene dose effect of apo E2 on plasma levels

of RLP-chol and uptake of RLP by macrophages was present (apo E3/3 < apo E3/2 < apo E2/2). These findings indicate that apo E2 may predispose diabetic patients to increased remnant lipoproteins in plasma and enhanced uptake of RLP by macrophages to promote atherosclerosis. It was also found that RLP from apo E2/2 diabetic patients with type III HLP enhanced cholesteryl ester synthesis in macrophages to a greater extent compared with RLP from apo E2/2 nondiabetic subjects with type III HLP. This is also the first demonstration that RLP from type III HLP with diabetes may be qualitatively more atherogenic than those from type III HLP without diabetes.

Increased amounts of remnant lipoproteins in plasma of type 2 diabetic patients with apo E2 genotypes may be the result of overloading of TG-rich lipoproteins due to overproduction and/or decreased removal in the diabetic state,[29] and in addition, the result of a reduced binding ability of apo E2 to the LDL or remnant receptors.[13] The mechanism by which RLP from diabetic patients with apo E2 genotypes enhanced cholesteryl ester synthesis in macrophages is unknown. The present study suggests that there are qualitative differences in RLP. Remnant lipoproteins from diabetes may have greater content of apo E[1] and may undergo glycation and further oxidation,[30] and this may be enhanced by the presence of apo E2 because apo E2 may cause delayed metabolism of remnant lipoproteins. At our institute, further studies are in progress to clarify this phenomenon.

Apo E2 homozygote, E2/2, is well known to contribute to type III HLP and atherosclerosis, but the role of apo E2 heterozygote, E3/2, in atherosclerosis is not very clear.[31] In general, association of apo E2 with atherosclerosis is not thought to be as strong as apo E4 because of the lowering effect of apo E2 on LDL.[23,24] However, It seems that apo E3/2 may increase plasma remnant lipoproteins and thus be associated with atherosclerosis, particularly in Japanese if apo E3/2 subjects are exposed to additional environmental or genetic risk factors. Kameda et al[32] reported a higher frequency of apo E3/2 and increased plasma IDL in Japanese survivors of myocardial infarction. We previously reported that Japanese patients with ischemic heart disease had a higher frequency of apo E3/2 and had dyslipidemia similar to type III HLP.[33] In addition, we reported that TG-rich lipoproteins from apo E3/2 subjects with hypertriglyceridemia enhanced cholesteryl ester synthesis in macrophages.[34] In Caucasians, Haffner et al[35] reported that apo E3/2 patients with familial combined hyperlipidemia had increased susceptibility to atherosclerosis. Likewise, Andrade et al[36] and Hanon et al[37] reported that apo E3/2 was associated with carotid artery atherosclerosis. The present study indicated that if apo E3/2 subjects had diabetes mellitus, they were likely to have increased plasma remnant lipoproteins and qualitatively more atherogenic remnant lipoproteins. It is of great interest that atherogenicity of RLP from apo E3/2 patients with diabetes was almost equal to that of those from apo E2/2 type III HLP patients without diabetes. Recently, we found that the apo E3/2 genotype is closely associated with daibetic nephropathy,[38,39] which has been supported in Caucasian diabetic patients.[40] Therefore, the apo E3/2 genotype may have an important role in the pathogenesis of diabetic vascular complications. Further studies are needed to understand the effect of apo E2 on diabetic macro- and microangiopathy.

In conclusion, type 2 diabetes was associated with increased RLP-chol in plasma and atherogenic RLP. In nondiabetes, apo E2/2 contributes to increased plasma RLP-chol and atherogenic RLP. In diabetes, additional effects of apo E2 to increase RLP-chol in plasma and to enhance the uptake of RLP by macrophages are present. Apo E2/2 diabetic patients with type III HLP showed increased uptake of RLP by macrophages comapred with apo E2/2 nondiabetic patients with type III HLP. This may help explain the increased risk of cardiovascular disease seen in apo E2/2 diabetic patients with type III HLP.

ACKNOWLEDGMENT

We thank K. Nakajima, PhD and T. Nakano, PhD, Japan Immunoresearch Laboratories, for the gift of the immunoaffinity gel mixture of anti-apo B100 and anti-apo AI monoclonal antibodies. We also thank A. Saniabadi, PhD, for checking English usage in the manuscript.

REFERENCES

1. Bierman EL: Atherogenesis in diabetes. Arterioscler Thromb 12:647-656, 1992

2. Nakajima K, Saito T, Tamura A, et al: Cholesterol in remnant-like lipoproteins in human serum using anti apo B-100 and anti apo A-1 monoclonal immmunoaffinity mixed gels. Clin Chim Acta 225:53-71, 1993

3. Campos E, Nakajima K, Tanaka A, Havel RJ: Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody apolipoprotein B-100. J Lipid Res 33:369-380, 1992

4. Sakata K, Miho N, Shirotani M, et al: Remnant-like particle cholesterol is a major risk factor for myocardial infarction in vasospastic angina with nearly normal coronary artery. Atherosclerosis 136:225-231, 1998

5. Takeichi S, Yukawa N, Nakajima Y, et al: Association of plasma triglyceride-rich lipoprotein remnants with coronary atherosclerosis in cases of sudden cardiac death. Atherosclerosis 142:309-315, 1999

6. Kugiyama K, Doi H, Takazoe K, et al: Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. Circulation 99:2858-2860, 1999

7. McNamara JR, Shah PK, Nelson SM, et al: Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: Results from the Framingham Heart Study. Atherosclerosis 154:229-236, 2001

8. Watanabe N, Taniguchi T, Taketoh H, et al: Elevated remnant-like lipoprotein particles in impaired glucose tolerance and type 2 diabetic patients. Diabetes Care 22:152-156, 1999

9. Mahley RW: Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. Science 240:622-630, 1988

10. Utermann G, Hess M, Steinmetz A: Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinemia in man. Nature 269:604-607, 1977

11. Zannis VI, Breslow JL: Human very low density lipoprotein E isoprotein polymorphism is explained by genetic variation and post-translational modification. Biochemistry 20:1033-1041, 1981

12. Eto M, Watanabe K, Ishii K: A racial difference in apolipoprotein E allele frequencies between the Japanese and Caucasian populations. Clin Genet 30:422-427, 1986

13. Schneider WJ, Kovanen PT, Brown MS, et al: Familial dysbetalipoproteinemia: Abnormal binding of mutant apoprotein E to low

density lipoprotein receptors of human fibroblasts and membranes from liver and adrenal of rats, rabbits and cows. J Clin Invest 68:1975-1985, 1981

14. Eto M, Watanabe K, Sato T, et al: Apolipoprotein-E2 and hyperlipoproteinemia in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 69:1207-1212, 1989

15. Eto M, Watanabe K, Makino I, et al: Apolipoprotein E allele frequencies in non-insulin-dependent mellitus with hypertriglyceridemia (type IIb, III, IV, and V hyperlipoproteinemia. Metabolism 40: 776-780, 1991

16. Eto M, Watanabe K, Ishii K: A rapid flat gel isoelectric focusing method for the determination of apolipoprotein E phenotypes and its application. Clin Chim Acta 149:21-28, 1985

17. Hixon JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 31:545-548, 1990

18. Marcoux C, Tremblay M, Nakajima K, et al: Characterization of remnant-like particles isolated by immunoaffinity gel from the plasma of type III and type IV hyperlipoproteinemic patients. J Lipid Res 40:636-647, 1999

19. Boy A: Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Invest 21:77-99, 1968 (suppl 96-100)

20. Oram JF: Receptor-mediated transport of cholesterol between cultured cells and high density lipoproteins. Methods Enzymol 129: 645-659, 1986

21. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol regent. J Biol Chem 193:265-275, 1959

22. SAS Institute Inc: Statview Reference Manual. Cary, NC, SAS Institute, 1988

23. Davignon J, Gregg RE, Sing CF: Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8:1-21, 1988

24. Eto M, Watanabe K, Ishii K: Reciprocal effects of apolipoprotein E alleles (ϵ2 and ϵ4) on plasma lipid levels in normolipidemic subjects. Clin Genet 29:477-484, 1986

25. Brown MS, Goldstein JL, Krieger M, et al: Reversible accumulation of cholesteryl esters in macrophages incubation with acetylated lipoprotein. J Cell Biol 82:597-613, 1979

26. Goldstein JL, Ho YK, Brown MS, et al: Cholesterol ester accumulation in macrophages resulting from receptor-mediated uptake and degradation of hyper-cholesterolemic canine β-very low density lipoproteins. J Biol Chem 255:1839-1848, 1980

27. Marcoux C, Tremblay M, Nakajima K, et al: Characterization of remnant-like particles isolated by immunoaffinity gel from the plasma of type III and type IV hyperlipoproteinemic patients. J Lipid Res 40:636-647, 1999

28. Eto M, Watanabe K, Iwashima Y, et al: Apolipoprotein E polymorphism and hyperlipidemia in type II diabetics. Diabetes 35: 1374-1382, 1986

29. Howard BV: Lipid metabolism in diabetes mellitus. J Lipid Res 28:613-628, 1987

30. Sakurai T, Kimura S, Nakano M, et al: Oxidative modification on glycated low density lipoprotein in the presence of iron. Biochem Biophy Res Commun 177:433-439, 1991

31. Wilson PWF, Schaefer EJ, Larson MG, et al: Apolipoprotein E alleles and risk of coronary disease. Arterioscler Thromb Vasc Biol 16:1250-1255, 1996

32. Kameda K, Matsuzawa Y, Kubo M, et al: Increased frequency of lipoprotein disorders similar to type III hyperlipoproteinemia in survivors of myocardial infarction in Japan. Atherosclerosis 51:241-249, 1984

33. Eto M, Watanabe K, Makino I: Increased frequencies of lipoprotein ϵ2 and ϵ4 alleles in patients with ischemic heart disease. Clin Genet 36:183-188, 1989

34. Saito M, Eto M, Makino I: Triglyceride-rich lipoproteins from apolipoprotein E3/2 subjects with hypertriglyceridemia enhance cholesteryl ester synthesis in human macrophages. Atherosclerosis 129: 73-77, 1997

35. Haffner SM, Kushwaha RS, Hazzard WR: Metabolism of apolipoprotein B in members of a family with accelerated atherosclerosis: Influence of apolipoprotein E-3/E-2 pattern. Metabolism 41:241-245, 1992

36. Andrade M, Thandi I, Brown S, et al: Relationship of the apolipoprotein E polymorphism with carotid artery atherosclerosis. Am J Hum Genet 56:1379-1390, 1995

37. Hanon O, Girerd H, Luong V, et al: Association between the apolipoprotein E polymorphism and arterial wall thickness in asymptomatic adults. J Hypertens 18:431-436, 2000

38. Horita K, Eto M, Makino I: Apolipoprotein E2, renal failure and lipid abnormalities in non-insulin-dependent diabetes mellitus. Atherosclerosis 107:203-211, 1994

39. Eto M, Horita K, Morikawa A, et al: Increased frequency of apolipoprotein e2 allele in non-insulin-dependent diabetic (NIDDM) patients with nephropathy. Clin Genet 48:288-292, 1995

40. Chowdhury TA, Dyer PH, Kumar S, et al: Association of apolipoprotein e2 allele with diabetic nephropathy in Caucasian subjects with IDDM. Diabetes 47:278-280, 1998