Distribution of Dolichol in the Serum and Relationships between Serum Dolichol Levels and Various Laboratory Test Values

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We examined the correlations between serum dolichol levels and laboratory test parameters in patients affected by disease, as well as the distribution of dolichol in sera from patients with hyperbetalipoproteinemia and hyperalphalipoproteinemia. Serum dolichol was evaluated by a reverse-phase HPLC method. After centrifugation, the serum dolichol found in healthy controls was mainly associated with medium-sized particles of the high-density lipoprotein (HDL) fraction. For patients with hyperbetalipoproteinemia, serum dolichol was also associated with the medium HDL fractions. However, for hyperalphalipoproteinemia patients the levels of large HDL and serum dolichol were increased, and serum dolichol was mainly associated with the large HDL fraction. On laboratory tests of components, the dolichol level was not correlated with the values for markers of the liver and biliary system, with the values of renal function markers, with creatine kinase activity, amylase activity or uric acid concentration, but was correlated with total cholesterol, HDL-cholesterol and apoA-I concentrations, and with lactate dehydrogenase (LDH) activity. These results suggest that serum dolichol exclusively localized in HDL, and in subpopulation, that in normocholesterolemia or hyperbeta-cholesterolemia is associated with HDL_3, which is small sized and high density HDL, however, that in hyperalphacholesterolemia is associated with HDL_2, which is large sized and lower density HDL.

Key words dolichol; high-density lipoprotein; low-density lipoprotein; cholesterol; human

Dolichols are a group of a-saturated polyprenol lipids that generally contain 14 to 24 isoprene units.[1,2] The biosynthetic pathways of cholesterol, dolichol, and ubiquinone are the same up to farnesyl pyrophosphate (FPP), at which point the pathways diverge.[3,4] Dolichol is found in a variety of eucaryotes as free alcohols, phosphomonoesters and esters of fatty acids,[2] and its level in human serum is lower than that in other tissues.[5]

The serum dolichol level is unchanged under many clinical conditions that alter serum cholesterol, such as diabetes mellitus, cardiovascular diseases, hypothyrosis, thyrotoxicosis and pheochromocytoma,[6] but is elevated in aspartylglucosaminuria, mannosidosis, primary biliary cirrhosis, primary sclerosing cholangitis and chronic active hepatitis.[7,8] However, the serum dolichol is reported to be decreased in neuronal ceroid lipofuscinosis.[9]

Several relationships between the levels of serum dolichol and serum components have been reported. The serum dolichol level is not correlated with the level of low-density lipoprotein (LDL) cholesterol,[5,10] but is positively correlated with the level of high-density lipoprotein (HDL) cholesterol.[5,10] In addition, serum dolichol is inversely correlated with the level of triglyceride.[5,10] Serum dolichol is mainly localized in the HDL fraction in healthy people,[5,11] and therefore is a cause of the positive correlation between dolichol and HDL-cholesterol concentration. However, the relationship between dolichol and apolipoprotein levels has not been reported.

It was reported that the distribution of serum dolichol, which is mainly associated with the HDL fraction, is altered in rats under specific physical conditions. Marino *et al.* reported that serum dolichol is mainly localized in the LDL fraction (57%) in partially hepatectomized rats 24 h after surgery,[12] while Narayan *et al.* noted an increase in the LDL-cholesterol level and decrease in the HDL-cholesterol level.[13] Tijburg *et al.* also reported that the LDL-cholesterol level was increased and the HDL-cholesterol level was decreased in partially hepatectomized rats 22 h after surgery.[14] Thus, it is expected that the distribution of serum dolichol in humans would be affected by the increased level of LDL in hyperbetalipoproteinemia (a metabolic disorder of LDL). This speculation as well as the distribution of serum dolichol in hyperalphalipoproteinemia (a metabolic disorder of HDL) remain to be examined.

Biliary excretion of dolichol may be the major excretion pathway of dolichol from the body.[15,16] Furthermore, the concentration of serum dolichol is increased in chronic cholestatic liver diseases.[8] However, it is hardly known whether serum dolichol levels are correlated with laboratory test values for the liver and biliary system function. A previous report noted that serum dolichol is positively correlated with serum alkaline phosphatase (ALP) in patients with early and intermediate chronic cholestatic liver.[8]

The relationships between the serum dolichol level and laboratory test values of serum components other than lipids are also unclear. To clarify the relationship between the dolichol level and several diseases, and to elucidate the mechanism that regulates the serum dolichol level, it is important to study the relationships between the serum dolichol level and various laboratory test parameters.

Therefore, we examined the distribution of dolichol in hyperbetalipoproteinemia and hyperalphalipoproteinemia pa-

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tients, and the correlation between the level of serum dolichol and the parameters of several laboratory tests including apolipoproteins.

MATERIALS AND METHODS

Materials Dolichol-17, -18, -19, -21 and tetracosaprenol were obtained from Larodan Fine Chemicals AB (Malmo, Sweden), and dolichol-20 was purchased from Sigma (MO, U.S.A.). 9-Anthracen carboxylic acid was obtained from Aldrich (MI, U.S.A.), and azodicarboxlic acid diethyl ester was obtained from Tokyo Chemical Industry (Tokyo, Japan). All other reagents were of analytical grade and were acquired from Wako Pure Chemicals (Osaka, Japan).

Subjects and Human Serum The study population consisted of 73 patients affected by various diseases (Japanese, 60±10 years of age, 46 men and 27 women). Venous blood was collected from all subjects. The blood was centrifuged at 3500 rpm for 5 min, and the serum obtained was used for analysis of free and acyl ester-type dolichol in various laboratory tests, as well as for the isolation and identification of lipoprotein. In these 73 patients, various laboratory test parameters were determined (Table 1). The study protocol was approved by the Ethical Committee of Tokyo Teishin Hospital.

Isolation of Serum Lipoproteins Serum lipoproteins was fractionated by density gradient centrifugation. The nonprotein solvent density of the serum samples was first increased to 1.210 g/ml by the addition of 0.325 g/ml serum KBr. Two milliliters of 1.240 g/ml NaCl–KBr solution was placed onto the bottom of a Hitachi 13PA tube (1.5×9.6 cm), and 3 ml of the adjusted serum was then layered over it. The following solutions were successively layered onto the serum: 2 ml of 1.063 g/ml NaCl–KBr solution, 2.5 ml of 1.019 g/ml NaCl–KBr solution, and 1.5 ml of 1.006 g/ml NaCl solution.[17] All salt solutions contained 0.01% EDTA2Na. The tubes were then centrifuged at 36000×g for 48 h in a Hitachi model SCP70H ultracentrifuge (Hitachi Koki, Co., Ltd., Tokyo, Japan) with a Hitachi RPS40T swing rotor. After centrifugation, the tubes were divided into 9 fractions, and aliquots of the fractions were immediately used for the identification of lipoproteins by electrophoresis. The residuals of the fractions were stored at −20 °C prior to further analysis.

Agarose Electrophoresis Lipoproteins in the gradient fractions (1 μl) were analyzed by electrophoresis on agarose gel sheets (TITAN GEL Lipoprotein kit, Helena Laboratories, Saitama, Japan) in a horizontal electrophoresis apparatus (Mupid-2, Cosmo Bio Co., Ltd., Tokyo, Japan) at room temperature and 100 V for 25 min. Barbital buffer (pH 8.6 to 9.0) from the TITAN GEL Lipoprotein kit was used as the running buffer. After electrophoresis, the gels were dried and stained with Fat Red 7B solution for 3 min, which was prepared as follows. Approximately 0.12 g of Fat Red 7B was dissolved in 200 ml of methanol, and after mixing for 3 h, 0.5 ml of Triton X-100 was added and the mixture was incubated at 37 °C for 3 d. The mixture was then passed through a glass filter, and subsequently diluted 1.2-fold with distilled water. Thereafter, it was used as the gel stain reagent. The gels were destained two times, 1 min each time in 70% methanol.

Table 1. Numbers and Ages of Patients

Laboratory test	n			Age (years)
	Total	Male	Female	
Total cholesterol	73	46	27	60.1±10.0
Triglyceride	72	46	26	60.1±10.0
HDL-cholesterol	67	42	25	59.7±9.8
LDL-cholesterol	59	39	20	59.9±9.7
ALT	72	45	27	60.3±9.9
AST	72	45	27	60.3±9.9
γ-GTP	70	45	25	60.4±9.9
LAP	56	35	21	60.3±9.7
ALP	72	45	27	60.3±9.9
BUN	72	45	27	60.3±9.9
Creatinine	72	45	27	60.3±9.9
LDH	72	45	27	60.3±9.9
CK	49	29	20	60.8±10.0
AMY	24	14	10	60.1±10.7
UA	70	44	26	60.2±9.9
Apo A-I	15	10	5	60.2±8.6
Apo B	15	10	5	60.2±8.6
Apo C-II	14	10	4	59.5±8.5
Apo E	15	10	5	60.2±8.6

ALT, AST, γ-GTP, LAP, ALP, BUN, LDH, CK, AMY, UA, Apo A-I, apo B, apo C-II and apo E indicate alanine aminotransferase, aspartate aminotransferase, γ-glutamyltranspeptidase, leucine aminopeptidase, alkaline phosphatase, blood urea nitrogen, lactate dehydrogenase, creatine kinase, amylase uric acid, apolipoprotein A-1, apolipoprotein B, apolipoprotein C-II and apolipoprotein E, respectively.

Determination of the Dolichol Level The levels of free and acyl-ester type dolichol were measured using a modification of the method proposed by Yamada *et al.*[18] The concentration of dolichol was determined as the amount of free plus acyl-ester type components. Serum (0.5 ml) was mixed with 0.5 ml of 10 M KOH and 1.5 ml of methanol, and the lipoprotein fraction after centrifugation was mixed with 10 M KOH and methanol at a fraction/KOH/methanol ratio of 1 : 1 : 3. The mixtures were then heated at 100 °C for 1 h under nitrogen gas, and after cooling to room temperature 10 ng of an internal standard, tetracosaprenol was added. Saponified lipids were extracted twice with 1.5 ml of hexane. The combined hexane extracts were evaporated to dryness at 40 °C under a stream of nitrogen. Next, 100 μl of tetrahydrofuran containing 100 mM anthracene-9-carboxylic acid, 100 mM triphenylphosphine and 100 mM azodicarboxylic acid diethyl ester was added to the dried lipid. The mixture was kept at room temperature for 1 h in the dark to combine the 9-anthroyl dolichol. It was then evaporated to dryness under a stream of nitrogen, and the residue was dissolved in 0.5 ml of 57 : 43 : 6 ethyl acetate/acetonitrile/water. This solution was applied to a C_{18} Sep-Pak cartridge equilibrated with 5 ml of 57 : 43 : 6 ethyl acetate/acetonitrile/water and washed with 20 ml of the same solvent mixture. The 9-anthroyl dolichol was subsequently eluted with 4 ml hexane and the eluate was then evaporated. Finally, the residue was dissolved in 150 μl ethyl 2 : 1 acetate/acetonitrile, and this solution was injected into the HPLC column, a Shimadzu Series 6A HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a reversed-phase Luna column (4.6×150 mm, 3 μm particles) (Phenomenex, CA, U.S.A.). The elution solvent used was 67 : 33 : 2 ethyl acetate/acetonitrile/water.

The mobile phase was pumped through the column at 0.7 ml/min at 40 °C. The fluorescent intensity of the eluate was monitored with a Shimadzu fluorescence HPLC detector

RF-535 at excitation and emission wavelengths of 360 and 460 nm, respectively. The retention times of the 9-anthroyl derivatives of the dolichols, the number of isoprene units being 17, 18, 19, 20 and 21, were 13, 15, 17, 20 and 23 min, respectively. Recovery was higher than 95% as determined by the internal standard.

Laboratory Tests Various laboratory tests except apolipoprotein concentrations were carried out using a HITACHI 7350 automatic clinical analyzer (Hitachi Instruments Service Co., Ltd., Tokyo, Japan) and measurement kits. The levels of total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol and amylase (AMY) activity were determined using Determiner® L TC II (Kyowa Medex Co., Ltd., Tokyo, Japan), Pureaauto® S TG-N, Choletest HDL, Choletest LDL and Pureaauto® S AMY (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) assay kits, respectively. Merck Liquid GOT and Merck Liquid GPT (Kanto Kagaku, Tokyo, Japan) were used for the determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, respectively, and alkaline phosphatase, γ-glutamyltranspeptidase (γ-GTP) and lactate dehydrogenase (LDH) activity were determined using Quick Auto Neo ALP_{JS}, Quick Auto Neo γ-GT_{JS} and Quick Auto Neo LD_{JS} (Shino-Test, Tokyo, Japan), respectively. The levels of creatinine, blood urea nitrogen (BUN), total bilirubin (T-Bil) and uric acid (UA), and the activities of leucine aminopeptidase (LAP) and creatine kinase (CK) were measured using CRE-L Regent Kainos (Kainos Laboratories Inc., Tokyo, Japan), BUN Regent D Kokusai (International Reagent Co., Hyogo, Japan), Total Bilirubin E-HR (Wako Pure Chemical Ind. Ltd., Osaka, Japan), Serotec UA-L (Serotec Co., Ltd., Hokkaido, Japan), Santest® L-LAP (Sanko Junyaku Co., Ltd., Tokyo, Japan) and N-Assay CPK-L-Nittobo (Nitto Boseki Co., Ltd., Tokyo, Japan), respectively.

Serum levels of apolipoprotein were measured by immunoturbidimetric assay with a fully automated COBAS INTEGRA 700 analyzer (Mannheim, Germany) using reagents obtained from Daiichi Pure Chemicals. The reagents used for the apolipoprotein (apo) A-I, apo B, apo C-II and apo E assays were APO A-I AUTO·N DAIICHI, APO B AUTO·N DAIICHI, APO C-II AUTO·N DAIICHI and APO E AUTO·N DAIICHI, respectively.

Statistical Methods The serum levels of dolichol, total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, apo A-I, apo B, apo C-II, apo E, BUN, creatinine and UA, the activities of ALT, AST, γ-GTP, LAP, ALP, LDH, CK and AMY, and each patient's age were recorded. Multiple linear regression analysis was employed to characterize the relationships between serum dolichol and other variables, and significance was recognized at $p<0.05$.

RESULTS

Concentration of Serum Dolichol We measured dolichol levels and composition in patient sera (Table 2). The average free plus acyl-ester type dolichol level in patients was 145±49 ng/ml (means±S.D.). The level of free plus acyl-ester type dolichol in patients was similar to that in healthy people as reported by Elmberger *et al.*[5] The serum dolichols detected in patients consisted of dolichol-17, 18, 19, 20 and 21. The major dolichol was dolichol-19, comprising 50% of the total. The dolichol composition in patients was similar to that of the normal controls in a previous report.[19]

Table 2. Dolichol Level and Composition in Patient Serum

	Content (ng/ml)	% of total
Total dolichol	145±49	
Dol-17	2.9±1.4	2.0±0.5
Dol-18	43.7±17.0	29.7±2.5
Dol-19	73.7±24.0	50.9±1.1
Dol-20	22.1±5.6	15.7±2.3
Dol-21	2.4±0.8	1.8±0.6

Dol-17, Dol-18, Dol-19, Dol-20 and Dol-21 indicate dolichol-17, dolichol-18, dolichol-19, dolichol-20 and dolichol-21 (the numbers are those of the isoprene units), respectivery. The data are means±S.D. $n=73$.

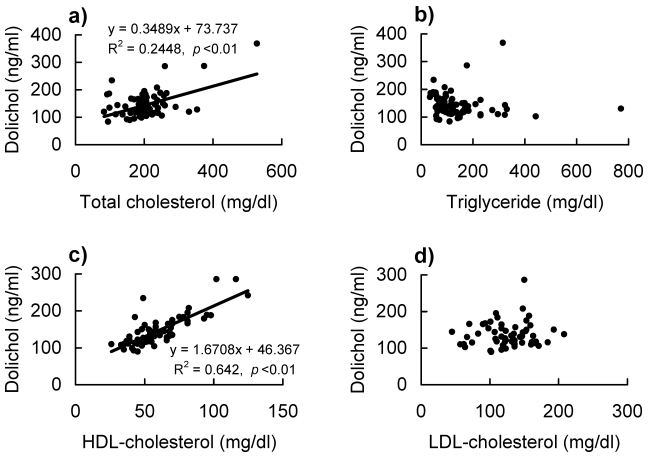

Fig. 1. The Relationship between the Dolichol Level and Serum Lipid Concentration in Patient Serum

Serum was saponified by alkaline treatment, and dolichols were extracted with hexane and converted into 9-anthroyl derivatives. The 9-anthroyl dolichols were then analyzed using an HPLC system equipped with a fluorescence detector. The concentrations of total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol were determined using a HITACHI 7350 automatic clinical analyzer and measurement kits. The correlations between dolichol and other lipids were then evaluated using a multiple correlation coefficient. (a) Total cholesterol, (b) triglyceride, (c) HDL-cholesterol, (d) LDL-cholesterol.

Relationship between the Dolichol Level and Other Lipid Levels in Human Serum The serum level of dolichol was compared with the serum levels of total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol. The level of dolichol was not related to the triglyceride level, but was positively correlated with the total cholesterol level (Figs. 1a, b). Forthermore, the level of dolichol was not related to the LDL-cholesterol level, but was positively correlated with the HDL-cholesterol level (Figs. 1c, d). There was no correlation between the HDL-cholesterol and triglyceride levels (data not shown).

Relationships between the Dolichol and Apolipoprotein Levels The dolichol level was positively correlated with the HDL-cholesterol level,[5,10] but whether it is correlated with the apo A-I level has remained. In addition, the relationships between the dolichol level and other apolipoprotein (apo B, C-II and E) levels has not been reported. As a result, dolichol was positively correlated with the apo A-I, main apolipoprotein of HDL (Fig. 2a). However, dolichol was not related to apo B, which is a apolipoprotein found in the chylomicrons, VLDL, IDL and LDL (Fig. 2b). The dolichol was also unre-

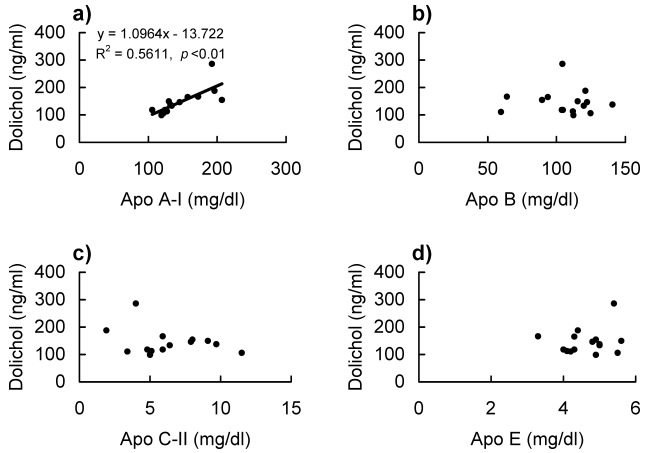

Fig. 2. The Relationships between Dolichol Level and Apolipoprotein Concentrations

The correlations between dolichol and apolipoproteins were evaluated using a multiple correlation coefficient. (a) apo A-I, (b) apo B, (c) apo C-II, (d) apo E. Apo A-I, apo B, apo C-II and apo E indicate apolipoprotein A-1, apolipoprotein B, apolipoprotein C-II and apolipoprotein E, respectively.

lated to apo C-II, a constituent of VLDL and HDL (Fig. 2c), and unrelated to apo E, a constituent of VLDL, IDL and HDL (Fig. 2d).

Distribution of Serum Dolichol in the Lipoprotein Fraction of Patients with Hyperbetalipoproteinemia and Hyperalphalipoproteinemia The localizations of dolichol were investigated in sera from healthy controls as well as from hyperbetalipoproteinemia and hyperalphalipoproteinemia patients by KBr gradient centrifugation. The levels of total cholesterol, LDL-cholesterol and HDL-cholesterol in healthy controls (normal volunteers of Japanese, 26.4±4.7 years of age, 4 men) were 171.7±31.7, 95.6±24.2 and 59.6±8.2 mg/dl, respectively. These data are normal values found on routine physical checkup. Hyperbetalipoproteinemia patients (Japanese, 62.3±15.6 years of age, 3 men and 3 women) and hyperalphalipoproteinemia patients (Japanese, 58.7±19.3 years of age, 1 man and 5 women) were the other additional patients excluding the 73 patients in Table 2. Total cholesterol and LDL-cholesterol levels of the hyperbetalipoproteinemia patients were 261.3±19.0 mg/dl and 190.7±14.3 mg/dl, respectively. In the hyperalphalipoproteinemia patients, total cholesterol and HDL-cholesterol levels were 250.2±46.2 and 117.0±10.6 mg/dl.

We could not obtain a sufficient volume of serum from these patients for KBr gradient centrigfugation. Therefore, in the hyperbetalipoproteinemia and the hyperalphalipoproteinemia patients, sera (1 ml/a patient) were pooled from 3 patients, respectively. However, since the serum volume from each healthy control was sufficient, the serum was not pooled.

Figure 3 shows typical images of agarose electrophoresis of the lipoproteins, and the typical distribution patterns of serum dolichol after the gradient centrifugation. In serum from a healthy control (25 years of age), the concentrations of total cholesterol, LDL-cholesterol and HDL-cholesterol were 159.2, 92.2 and 52.1 mg/dl, respectively, while in the pooled serum of hyperbetalipoproteinemia patients, the total cholesterol and LDL-cholesterol levels were 246.7 and 181 mg/dl, respectively. In hyperalphalipoproteinemia patients, total cholesterol and HDL-cholesterol concentrations

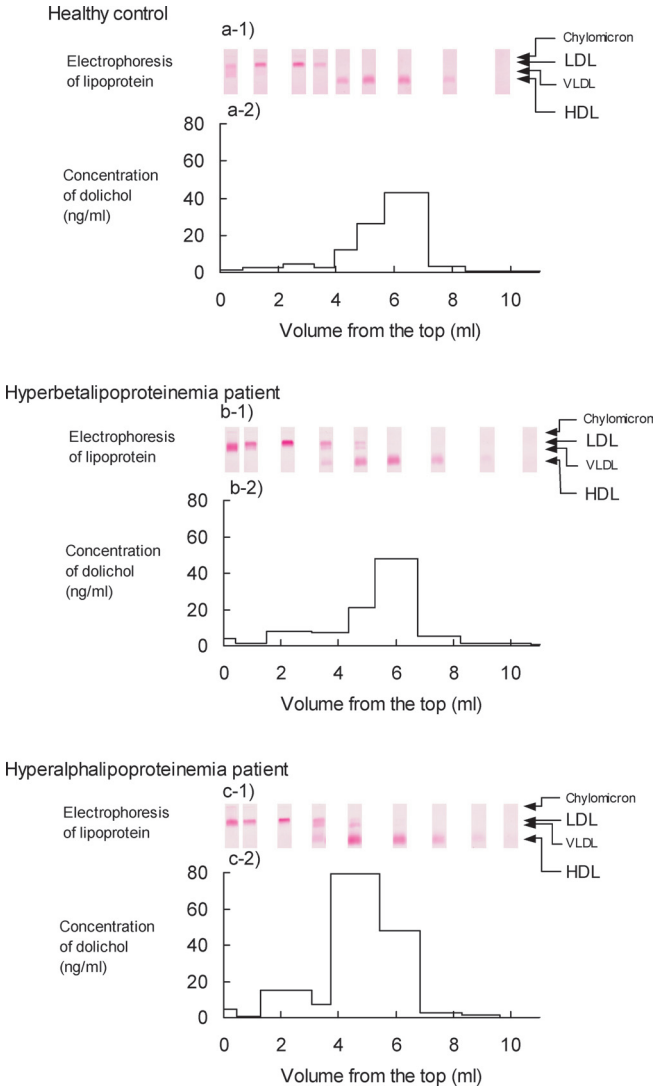

Fig. 3. Distribution Patterns of Dolichol in the Human Serum

Human serum was separated by NaCl–KBr gradient centrifugation as described in the text. After centrifugation, the fractions containing lipoproteins were collected. One microliter aliquots of a each fraction were separated on a TITAN GEL Lipoprotein agarose plate, and the lipoproteins were stained with Fat red 7B (upper panels). The concentration of dolichol was then determined using an HPLC system equipped with a fluorescence detector (lower panels). Agarose electrophoresis of lipoprotein in fractions separated by NaCl–KBr gradient centrifugation from the respective sera was carried out ((a-1), (b-1), (c-1)), and dolichol concentrations of the fractions were also determined ((a-2), (b-2), (c-2)). (a-1), (a-2) Healthy control; (b-1), (b-2) hyperbetalipoproteinemia; (c-1), (c-2) hyperalphalipoproteinemia.

of pooled serum were 247.0 and 125 mg/dl, respectively.

As the result of electrophoresis of sera from healthy controls, the HDL fractions contained few VLDL, LDL and chylomicrons. Thus, the major classes of the serum lipoproteins were well-separated by the density gradient centrifugation (Fig. 3a-1). In the healthy controls, 89% of serum dolichol was associated with the HDL fraction for a volume 4—8 ml from the top of the tube. The dolichol concentration in a fraction of 6—7 ml volume was the highest of all fractions at 42.6 ng/ml (Fig. 3a-2).

For the hyperbetalipoproteinemia patients, the LDL band (volume of 1—3 ml) after electrophoresis was more strongly stained than that of healthy controls (Figs. 3a-1, b-1). However, 80.7% of serum dolichol was associated with the HDL fraction. The dolichol concentration of 48.2 ng/ml in the

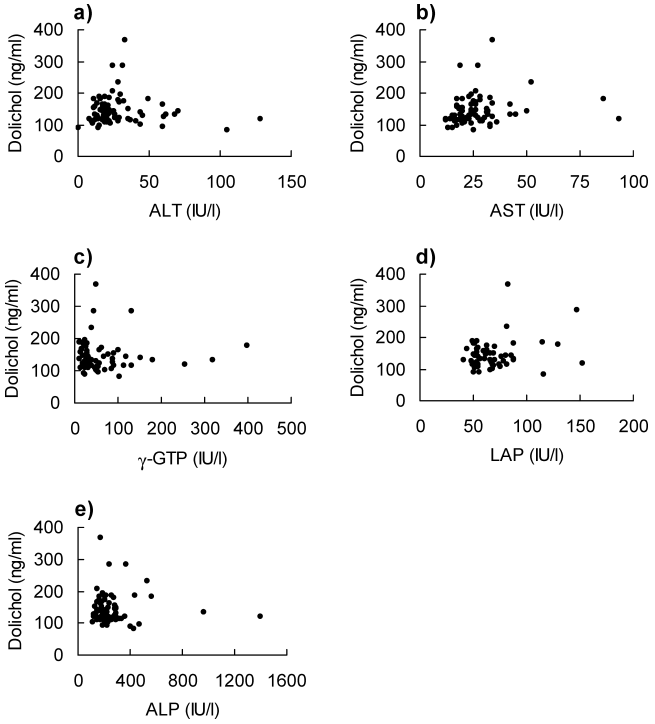

Fig. 4. The Relationship between the Dolichol Level and Test Parameters for Liver-Bile Duct Function

The concentration of dolichol was determined using an HPLC system equipped with a fluorescence detector and laboratory tests were carried out using a HITACHI 7350, automatic clinical analyzer, and measurement kits. The correlations between dolichol and other tests were evaluated by multiple correlation coefficients. (a) ALT, (b) AST, (c) γ-GTP, (d) LAP, (e) ALP. ALT, AST, γ-GTP, LAP and ALP indicate alanine aminotransferase, aspartate aminotransferase, γ-glutamyltranspeptidase, leucine aminopeptidase, and alkaline phosphatase, respectively.

highest fraction of the hyperbetalipoproteinemia patients sera was equal to that of the healthy controls (Figs. 3a-2, b-2). This indicates that the distribution pattern of dolichol in the serum of hyperbetalipoproteinemia patients was similar to that of the healthy controls.

The diameter of lower density HDL particles is larger than that of higher density HDL particles. For hyperalphalipoproteinemia patients, the band corresponding to the large HDL (volume 4—5 ml) was more strongly stained than that of the healthy controls (Figs. 3a-1, c-1), and was exclusively (85.9%) associated with the HDL fraction. The dolichol concentration in the 4—5 ml volume fraction from the top of the tube was the highest of all the fractions at 79.2 ng/ml, and was two-fold higher than that of the 6—7 ml volume from healthy controls (Figs. 3a-2, c-2).

Relationship between the Dolichol Level and Laboratory Test Parameters of Serum Components Except Lipids It is hardly known whether serum dolichol levels are correlated with laboratory test values for liver and biliary system function. As shown in Fig. 4, the dolichol level was not correlated with the values of liver and biliary system function markers AST, ALT, ALP, γ-GTP and LAP (Fig. 4).

The dolichol level was not correlated with the values of the renal function markers BUN and creatinine either (Fig. 5). Furthermore, it was not related to CK activity, AMY activity or UA content (Figs. 6b—d), although it was correlated with LDH activity (Fig. 6a). The dolichol level was not correlated with age (Fig. 7).

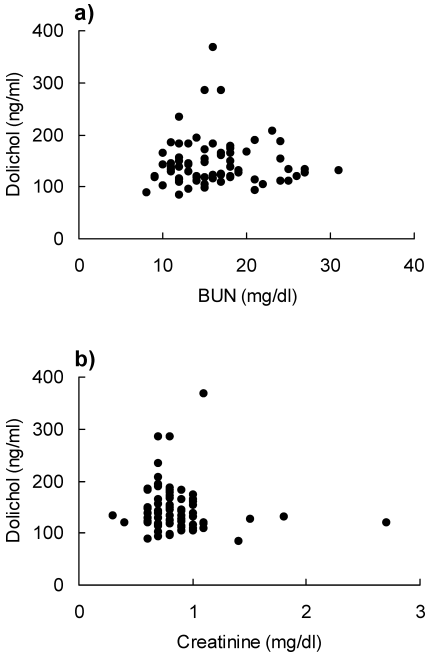

Fig. 5. The Relationships between the Dolichol Level and Renal Function Test Parameters

The concentration of dolichol was determined using an HPLC system equipped with a fluorescence detector, and laboratory tests were carried out using a automatic clinical analyzer and measurement kits. The correlations between the dolichol level and renal function test parameters were evaluated using multiple correlation coefficients. (a) BUN, (b) creatinine. BUN indicates blood urea nitrogen.

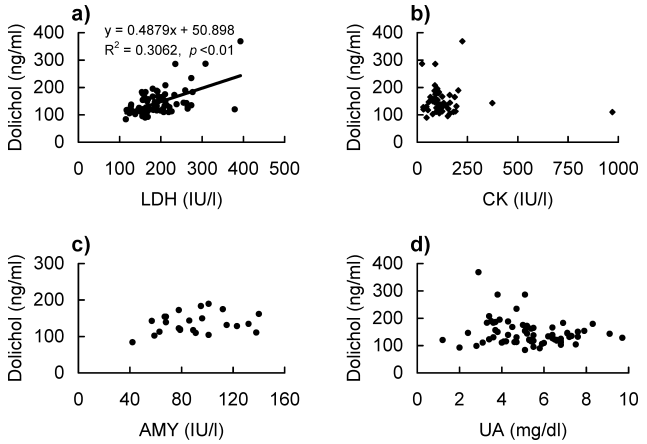

Fig. 6. The Relationships between the Dolichol Level and Other Laboratory Test Parameters

The correlations between dolichol and other factors were evaluated using multiple correlation coefficients. (a) LDH, (b) CK, (c) AMY, (d) UA. LDH, CK, AMY, and UA indicate lactate dehydrogenase, creatine kinase, amylase and uric acid, respectively.

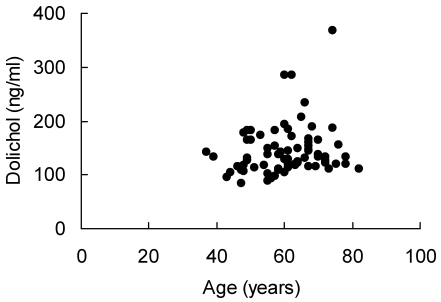

Fig. 7. The Relationship between Dolichol Level and Age

The correlation between dolichol and age was evaluated using a multiple correlation coefficient.

DISCUSSION

We examined the distribution of serum dolichol in hyperbetalipoproteinemia and hyperalphalipoproteinemia patients, and investigated the relationships between the serum dolichol level and laboratory test parameters.

We found no correlation between serum dolichol and LDL-cholesterol, but did observe a positive correlation between dolichol and HDL-cholesterol (Fig. 1c). This finding is in agreement with the data of Humaloja *et al.*[6] and Yasugi and Oshima.[10]

Humaloja *et al.*[6] and Yasugi and Oshima[10] did not observe a positive correlation between serum dolichol and total cholesterol, but we did observe a positive correlation. The calculated *r*-value for this by multiple linear regression analysis in the report of Humaloja *et al.* was 0.282 ($p<0.05$). Meanwhile, the R^2 value, calculated by single linear regression analysis, was 0.152 ($p<0.005$) for the data of Yasugi and Oshima. The correlation ($R^2=0.642$) in our study was higher than that in their reports, and so this might be the cause of our positive correlation between dolichol and total cholesterol.

Elmberger *et al.*[5] and Yasugi and Oshima[10] reported that the serum dolichol level is inversely correlated to the serum triglyceride concentration, but we did not find an inverse correlation (Fig. 1b). In their reports, the HDL concentration in plasma was inversely correlated to triglyceride concentration,[20,21] and so they considered that this inverse correlation of HDL and triglyceride concentrations might have caused inverse correlation of dolichol and triglyceride.[5,10] However, in the present study, we could not find a correlation between the HDL-cholesterol level and triglyceride level (data not shown), resulting in an absence of any correlation between serum dolichol and triglyceride (Fig. 1b). The difference between our data and those of other investigators may depend on the subject populations. Elmberger *et al.* used healthy males in their study of the *R* value (-0.61, multiple regression analysis) for the correlation between dolichol and triglyceride.[5] Yasugi and Oshima studied the correlation in workers who participated in a 2-d hospitalized annual checkup, and their R^2 value was 0.194 (single linear regression analysis).[10] Furthermore, the correlation value calculated by Elmberger *et al.* was higher than that of Yasugi and Oshima. However, we investigated the correlation in patients who may have been affected by various diseases.

In the present study of apolipoproteins, we found that the dolichol level was positively correlated with the apo A-I level only (Fig. 2a). This may correspond to the findings that the serum dolichol level was not correlated with the LDL-cholesterol level, but with the HDL-cholesterol level, because apo A-I is mainly localized in HDL.

Serum dolichol is mainly associated with HDL in humans.[5] Using electrophoresis and gradient centrifugation, we studied whether the concentration and distribution of dolichol in lipoproteins are affected by changes in LDL-cholesterol. It is reported that the LDL-cholesterol level was increased, and the HDL-cholesterol level was decreased in the partial hepatectomized rat at 22 or 24 h after surgery.[13,14] In addition, the distribution of serum dolichol is reported to shift from the HDL fraction to the LDL fraction 24 h after surgery in the partially hepatectomized rat.[12] Therefore, we expected that the distribution of serum dolichol in humans is also affected by the increase in LDL-cholesterol. We examined the distribution of serum dolichol in hyperbetalipoproteinemia patients (the increased level of LDL). As a result, we found that the serum dolichol was almost completely retained in the fraction containing medium-sized HDL (Fig. 3b). This indicates that the distribution of serum dolichol is hardly affected by the increase in LDL-cholesterol. We are interested in whether partial hepatectomy changes the distribution of plasma dolichol in humans as well as in rats. To better understand this issue, further study is required.

After separation of HDL from other components in serum by electrophoresis, we compared HDL concentrations between healthy control, hyperbetalipoproteinemia patients and hyperalphalipoproteinemia patients. In the serum of hyperbetalipoproteinemia patients, the HDL concentration was highest in the fraction containing medium HDL (volume 6 ml) (Fig. 3b-1). In hyperalphalipoproteinemia patients, the HDL content was highest in the fraction of the large HDL (volume 4—5 ml) (Fig. 3c-1). We confirmed that the HDL concentration in hyperalphalipoproteinemia patients was higher than that in the healthy controls and hyperbetalipoproteinemia patients.

After gradient centrifugation, we compared dolichol and HDL concentrations. In the hyperbetalipoproteinemia and hyperalphalipoproteinemia patients, the highest fractions of dolichol level were proportional to the highest fraction of HDL, respectively (Figs. 3b, c). A similar tendency was also seen in healthy controls (Fig. 3a). These findings therefore suggest that the dolichol concentration depends on the HDL-cholesterol concentration.

Next, we discussed the possible role of HDL on dolichol excretion. Hyperalphalipoproteinemia may be caused by a variety of genetic and environmental factors, such as a disorder in the reverse transport of cholesterol.[22—24] Cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl ester from HDL to apo B-containing lipoproteins,[25,26] and CETP deficiency increases the level of HDL-cholesterol which is mainly localized in the large HDL (HDL_2) fraction.[27,28] Therefore, CETP is a regulator of the HDL-cholesterol level and HDL particle size, and may be a key protein in reverse cholesterol transport.[24,29] Genetic CETP deficiency is an important factor as a frequent cause of hyperalphalipoproteinemia in Japanese people.[24,29—31] Nagano *et al.* reported that CETP deficiency contributes to 61.7% of marked hyperalphalipoproteinemia cases (HDL$\geqq$100 mg/dl) among the Japanese,[32] and Murayama *et al.* reported that 64.1% of patients with marked hyperalphalipoproteinemia demonstrate low CETP activity ($<$75% of normal controls).[33] In the present study, the observed patients had marked hyperalphalipoproteinemia with a HDL-cholesterol level higher than 100 mg/dl. In these patients, the concentration of large HDL particles was increased (Fig. 3c-1), similar to the increase in large HDL particles in patients with CETP deficiency. The serum dolichol level was also increased in hyperalphalipoproteinemia patients, which may have been caused by the increased dolichol in the fraction containing large HDL particles (volume 4—5 ml) (Fig. 3c). Therefore, the increase in serum dolichol may be caused by a disorder in HDL metabolism, which is a part of the reverse cholesterol transport system. HDL may play a role in the

transport of dolichol from the blood stream to the liver. The role of HDL in dolichol excretion remains hypothetical, and further studies are required.

The liver participates in the supply of dolichol to the serum and the excretion of dolichol from the body.[11,15,16] It is thus expected that the serum dolichol level is correlated with laboratory test parameters for liver and biliary system function, such as AST, ALT, ALP, γ-GTP and LAP activities. Therefore, we investigated the relationships between the serum dolichol level and these enzyme activities. The dolichol level was not correlated with enzyme activities (Fig. 4), indicating that at least a simple correlation does not exist. However, Humaloja *et al.* found a low positive correlation ($r=0.28$) between the serum dolichol level and ALP activity[8] by studying sera from patients with chronic cholestatic liver diseases. In the present study, we investigated sera from patients who may have been affected by various illnesses. Thus, the distribution of patient types was more limited in their study than in ours. Since the number of patients with liver and bile duct disease was limited in this study, we might not have been able to detect a low correlation between the dolichol level and ALP activity. However, if we had limited the patient type to liver and bile duct disease, such a correlation might have been observable.

We found no correlation between the serum dolichol level and values of the renal function markers BUN and creatinine (Fig. 5), and thus the kidney may not be related to the regulation of serum dolichol. These observations support a report that blood and urinary dolichol levels are independently regulated.[6]

This is the first report to show that the serum dolichol level is positively correlated with serum LDH activity (Fig. 6). When tissues are injured, cells release LDH into the serum. Dolichols are present in almost all human tissues, and the concentraions in the tissues are higher than in the serum. Therefore, when LDH is released from cells into blood in some disease states, dolichol might also be released.

Elmberger *et al.* reported that the plasma dolichol concentration linearly increases with increasing age in healthy men.[5,34] However, Yamada *et al.* reported the opposite.[18] Whereas, Sakakihara reported that it is gradually decreased with aging in normal volunteers and patients who undergo cardiac catheterization,[35] and Yasugi and Oshima did not observe any relationship between serum dolichol levels and age in workers who participated in a 2-d hospitalized annual medical check-up.[10] Humaloja *et al.* did not find any relationship between the serum dolichol level and age during normal pregnancy and patients affected by viral infections, bacterial infections, malignant diseases, cardiovascular diseases, gastroenterological diseases, endocrinological diseases, and rheumatic diseases.[6] In the present study, there was no apparent relationship between serum dolichol levels and patient age (Fig. 7), but the reason for this difference between our report and others is currently unclear.

With the data obtained, we evaluated sex differences of serum dolichol and HDL-cholesterol. Both levels of HDL-cholesterol (66.86 ± 15.38 mg/dl) and dolichol (167.5 ± 34.3 ng/ml) were higher in female than male (52.36 ± 10.43 mg/dl and 148.3 ± 23.1 ng/ml, respectively). As shown in above data and Fig. 1, serum level of dolichol almost proportionally behaves as that of HDL-cholesterol. However, the exceptional case may also exist in Reye's syndrome, which is a fatal children disease with aspirin consumption.[36]

This is the first report to elucidate that serum dolichol is associated with total cholesterol, apo A-1 and LDH. Consequently, we clarified that serum dolichol is only correlated a few parameters (HDL, total cholesterol, apo A-I and LDH) of serum, and that the correlation between dolichol and HDL-cholesterol is strongest (Figs. 1, 2, 6). This indicates that serum dolichol is strongly associated with HDL. This is the first report to note that the serum dolichol level is not correlated with lipoprotein parameters (apo B, apo C-II and apo E) except for apo A-I, markers (AST, ALT, γ-GTP and LAP) of the liver and biliary systems, renal function markers (BUN and creatinine), and other markers such as CK, AMY and UA. Based on these results, we consider that the localization and/or function of serum dolichol may be limited to HDL.

This is the first study to show that serum dolichols are mainly localized in the HDL fraction of hyperbetalipoproteinemia patients' sera as well as that of healthy people. Cholesterol and dolichol are converted into FPP by the same synthesis pathway, the mevalonate pathway. Cholesterol is dissolved in the blood by associating with VLDL, LDL, and HDL lipoproteins. Therefore, the cholesterol level is affected by disorders of lipoprotein metabolism. Dolichol is also dissolved in the blood by associating with lipoproteins. However, dolichol was exclusively distributed in HDL, and serum dolichol concentration was affected by alteration of the HDL concentration only. These findings suggest that the dolichol and cholesterol contents are not necessarily regulated by the same mechanism. It is unknown why dolichol is especially associated with HDL. The present clarification of the distribution of dolichol in the serum of patients with lipoprotein metabolic disorders (hyperbetalipoproteinemia and hyperalphalipoproteinemia) may be useful in elucidating the mechanism that regulates dolichol metabolism.

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