Figure 1. Multicompartmental model for apolipo-protein B–containing lipoproteins. Compartment 1 represents the intracellular amino acid pool and compartment 2 represents a delay for synthesis of lipoproteins. Very-low-density lipoprotein (VLDL) comprises 3 compartments: VLDL1, VLDL2, and VLDL remnant. Two compartments were allocated for intermediate-density lipoprotein (IDL): IDL and IDL remnant. LDL had intravascular and extravascular pools.

and a deficiency in this protein results in the decline of LDL-C catabolism, as seen with homozygous FH.[8] However, ARH differs from homozygous FH in the severity of the clinical phenotype and response to statins, the cause of which still remains unclear.[5]

One of the possible mechanisms of great responsiveness to statins was elucidated by a metabolic study using LDLRAP1 knockout mice that showed preserved ability for LDLR-dependent VLDL clearance.[9] However, few data exist regarding the metabolic basis of LDLRAP1 in clinical settings, especially, the metabolism of remnant lipoprotein fractions. Therefore, we examined lipoprotein kinetics in the homozygous ARH patient, using a stable isotope methodology with kinetic modeling including several remnant lipoprotein fractions, before and after atorvastatin therapy.

Methods

Study Subjects
This study was approved by the Ethics Committee of Kanazawa University, Suzu General Hospital, for the ARH patient and Jikei University School of Medicine for the control subjects. All study subjects gave their written informed consent to participate. We examined 8 subjects including 1 patient with suspected ARH without any evidence of chronic disease or malignancy and 7 normal control subjects (all men; age, 41 ± 8 years). All lipid-lowering therapy had been strictly suspended for 3 months until the baseline study. We checked the lipid level of the patient suspected ARH 1 month before the baseline study as well as 1 week before the baseline study to confirm that his cholesterol level was appropriately elevated and reached plateau. Next, we reexamined ARH patient after treatment with atorvastatin of 20 mg/d for 3 months.

Genetic Studies
Genomic DNA was isolated from peripheral blood white blood cells according to standard procedures and was used for PCR. We analyzed the coding regions of LDLR, PCSK9, and LDLRAP1 genes. Primers for the study were as used previously.[10,11] PCR products were purified by Microcon (Millipore Corp, Bedford, MA) and used as templates for direct sequencing. DNA sequencing was carried out according to the manufacturer's instructions, using a dye

terminator method (ABI PRISM 310 Genetic Analyzer (PerkinElmer Biosystems, Waltham, MA).

Biochemical Analysis and LDLR Activity
Serum concentrations of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were determined enzymatically. LDL-C concentrations were derived by means of the Friedewald formula. Apolipoprotein E (apoE) phenotype was separated by isoelectric focusing and detected by Western blot with apoE polyclonal antibody (phenotyping apoE IEF system, JOKOH, Tokyo, Japan). Lipoprotein lipase (LPL) mass in postheparin plasma was measured according to the method we previously reported.[12]

LDLR activity was measured by 2 methods, both of which used peripheral lymphocytes; The first was commercially available binding assay and the second was our original assay, which was described in detail elsewhere.[13] Briefly, we could measure accurate LDLR activity by using heparin to exclude the overestimation signals only bound at the surfaces of lymphocytes, even in the case with internalization defective type of disease.

Lipoprotein Kinetic Study
After an overnight fast, the study subjects were given a bolus injection (10 mg/kg) of [^{2}H$_3$]-leucine (Cambridge Isotope Laboratories, Woburn, MA). Blood samples were drawn periodically for 48 hours after the bolus injection.

Determination of Isotopic Enrichment
Samples were prepared for GC-MS analysis as reported previously.[14,15] For detailed determination of isotopic enrichment, please see online-only Data Supplement Method I.

Kinetic Modeling
Figure 1 shows the multicompartmental model used in this study, which was built using an interactive computer program (SAAM II, version 1.1; SAAM Institute Inc) to determine apoB kinetic parameters.[16,17] For detailed kinetic modeling, please see online-only Data Supplement Method 2.

Changes in Lipoprotein Subfractions
Lipoproteins of ARH were separated by the method based on those sizes using HPLC (LipoSEARCH, Skylight Biotech, Akita, Japan).[18] Changes in cholesterol, triglyceride, free cholesterol, and phospholipids in each lipoprotein subfraction was assessed by HPLC.
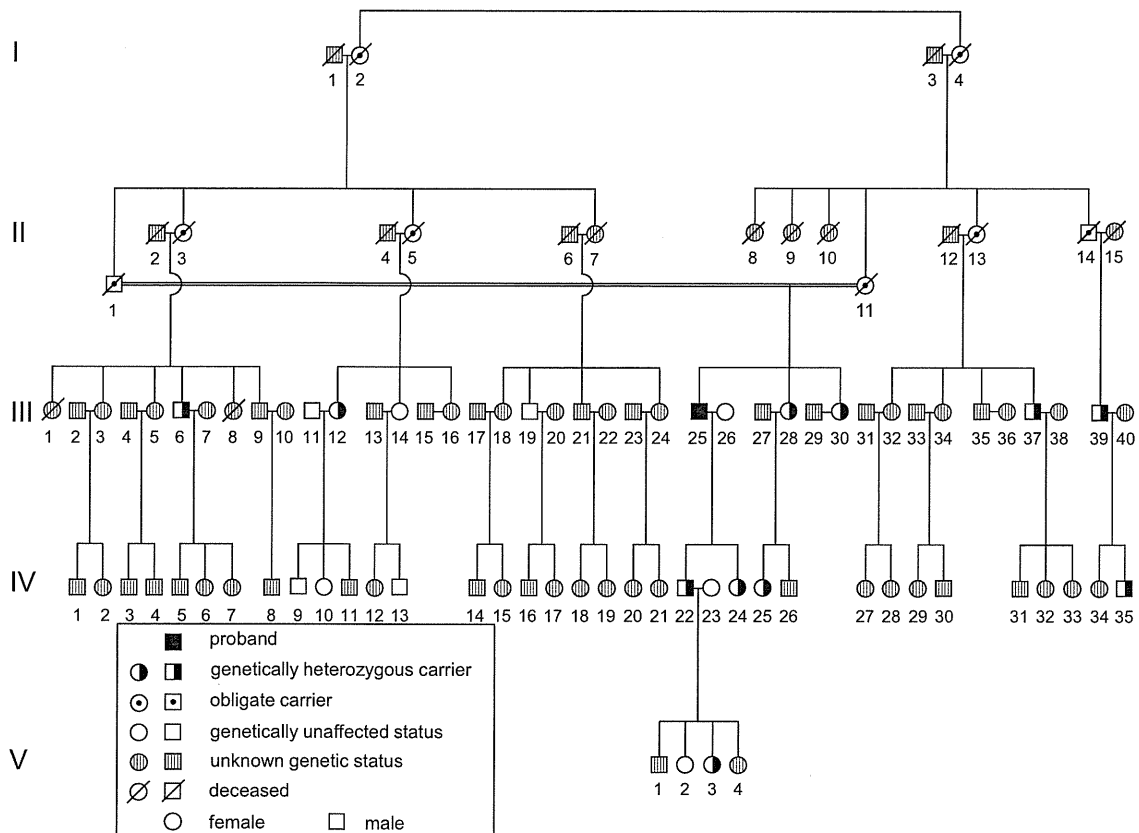
Figure 2. Pedigree of the autosomal recessive hypercholesterolemia patient. The proband was born to consanguineous parents (first cousins). The clinical data of the relatives, who were investigated further, are listed in online-only Data Supplement Table.

Results

Identification of ARH

A 68-year-old Japanese man presented at Kanazawa University Hospital for further examination of his hypercholesterolemia and severe tendon xanthomas (online-only Data Supplement Figure IA and IB). The proband was born to consanguineous parents (first cousins); neither parent had any signs of hypercholesterolemia or xanthomas. Large cutaneous and tendon xanthomas were identified on his fingers and foot, which had developed around 10 years of age. The thickness of his Achilles tendons reached 26 mm (online-only Data Supplement Figure IC). Initial serum TC and TG concentrations were high: 13.27 mmol/L and 3.39 mmol/L and were decreased to 5 mmol/L and 0.5 mmol/L after statin treatment for 8 years, respectively (online-only Data Supplement Figure ID). Several severe stenotic lesions including total occlusion of right common carotid artery were observed. Angiogram revealed total occlusion of bilateral external iliac arteries as well as left anterior descending artery (online-only Data Supplement Figure IE and IG). Bypass surgeries were conducted for both lesions (online-only Data Supplement Figure IF and IG). An abdominal aortic aneurysm, 33 mm in diameter, was observed. These extents of atherosclerosis are considered to be compatible with his high LDL-C level. Microscopic analysis revealed no specific findings in his liver (online-only Data Supplement Figure IH). Apo E phenotype of the ARH patient was E2/E3 in contrast to the result that those of control subjects were all E3/E3.

Although there was no mutation detected in LDLR and PCSK9 genes, homozygous mutation of an extra cytosine inserted into the region of the LDLRAP1 gene was found (c.606dup, previously described as ins C_{599}) in our proband (online-only Data Supplement Figure II), which is completely identical to that found in the first Japanese family identified with ARH.[19] An investigation, which extended back over 5 generations, failed to show any relationship between these 2 families, whose geographical origin were completely different. Using genetic analysis, we diagnosed 11 ARH heterozygous subjects and 6 normal subjects in the proband's family (Figure 2). Their lipid data and major clinical findings including the presence of coronary artery disease are listed in the online-only Data Supplement Table. As for LDLR activity, we found extremely accelerated LDLR activity (as much as 160% of normal control subjects) measured by the binding assay, using the measurement of 3,3′-dioctadecylindocarbocyanin (DiI)-labeled LDL uptake in blood peripheral lymphocytes (BML, Tokyo, Japan). In contrast, the value measured by our internalization assay using heparin showed that the activity was reduced to 14% of normal control subjects.

Lipoprotein Kinetic Study

At the time of the kinetic study (Table 1), the ARH patient showed higher serum TC levels (10.26 versus 4.87±0.58 mmol/L) and higher LDL-C levels (8.63 versus 2.95±0.49 mmol/L) than those of the control subjects.

The VLDL apoB, IDL apoB, and LDL apoB tracer/tracee ratio curves at baseline and after atorvastatin therapy, as well

Table 1.   Characteristics of ARH Patients and Control Subjects

Subjects	Sex	Age, y	BMI, kg/m^2	TC, mmol/L	TG, mmol/L	LDL-C, mmol/L	HDL-C, mmol/L	ApoB, g/L	ApoB/LDL-C	Lathosterol, μg/mL	LPL, ng/mL
Baseline	Male	68	26	10.26	1.26	8.63	1.06	1.90	0.56	6.3	324
After statin therapy		68	26	6.02	1.06	4.22	1.32	1.13	0.69	1.2	401
Control subjects (n=7)	All male	41±8	22±1	4.87±0.58	1.08±0.24	2.95±0.49	1.38±0.13	0.89±0.12	0.78±0.24	n.d.	n.d.

Values of control subjects are shown as mean±SD.

ARH indicates autosomal recessive hypercholesterolemia; BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; apoB, apolipoprotein B; LPL, lipoprotein lipase; n.d., not determined.

as those for the mean of the control subjects, are shown in Figure 3. Kinetic parameter of apoB within each lipoprotein fraction is shown in Table 2. Fractional catabolic rates (FCRs) of VLDL, IDL and LDL apoB were markedly slower in the ARH patient at baseline (3.153 1/day for VLDL, 1.414 1/day for IDL, 0.109 1/day for LDL) compared with those of the control subjects (8.408±2.697 1/day for VLDL, 8.326±3.467 1/day for IDL, 0.450±0.122 1/day for LDL). Production rates (PRs) of the ARH patient of the 3 fractions were within the mean value ±2 SD of those of control subjects. Therefore, the markedly increased concentrations of IDL and LDL apoB were primarily due to the decreased catabolism rate in the ARH patient.

Surprisingly, the FCR of LDL apoB significantly increased to within the normal range after statin therapy in the ARH patient (0.109–0.464 1/day), resulting in a 70% reduction of LDL apoB concentration. This result was completely differ-

ent from that seen with homozygous FH, where the FCR of LDL apoB was reported to be unchanged after statin therapy.[20] In addition to the response observed in FCR of LDL apoB, those of VLDL and IDL apoB also increased by the statin therapy in the ARH patient (3.153–7.881 1/day for VLDL, 1.414–2.525 1/day for IDL).

Remnant Fractions

Next, we investigated detailed metabolic channeling in the ARH patient (the results are summarized in Table 3). In the control subjects, the liver primarily secrets VLDL (87.0±11.0%), most of which (85.5±18.7%) was, in turn, converted to IDL by lipoprotein-mediated delipidation, thus leaving the VLDL remnant as a minor fraction (12.0±11.8% of total VLDL mass). Some crucial differences between the ARH patient and the control subjects were noted in VLDL metabolism. In the ARH patient: (1) only half of VLDL was converted to IDL (52.5%); (2) VLDL remnant mass comprised as much as 60.2%, resulting from an alteration in metabolic channeling in favor of the conversion to VLDL remnant (47.5% versus 1.8±2.1%, ARH versus control, respectively): (3) removal rate of VLDL remnant (k[0,12]) was increased (4.3 1/day) compared with that of the control subjects (1.3±0.9 1/day); and (4) direct removal of VLDL, including VLDL remnant, was much higher compared with that of the control subjects (47.5% versus 14.5±18.7%), a finding mirroring the decreased conversion to IDL as noted above. Furthermore, these tendencies were more pronounced after atorvastatin therapy. As shown in the middle panel of Table 3, most IDL was derived from VLDL and exclusively converted to LDL (97.8±3.1%) in the control subjects. In the ARH patient, however, about one-quarter of IDL was directly secreted from the liver and more IDL fractions were directed into remnant, again resulting in the increased remnant mass. These tendencies remained unchanged by atorvastatin therapy. Finally, the only notable difference in LDL metabolism was higher direct secretion of LDL with atorvastatin therapy, a finding consistent to higher tracer/tracee ratios during early time points (pink squares with dotted line in Figure 3C).

Changes in Lipoprotein Subfractions

As shown in online-only Data Supplement Figure III, relatively wide range of apoB-containing lipoproteins, including large VLDL, could be reduced by atorvastatin therapy in all fractions of lipids (cholesterol, triglyceride, free-cholesterol, and phospholipids) in the ARH patient.

Discussion

In this study, we performed an in vivo lipoprotein kinetic study, allowing us to assess detailed metabolic behavior of
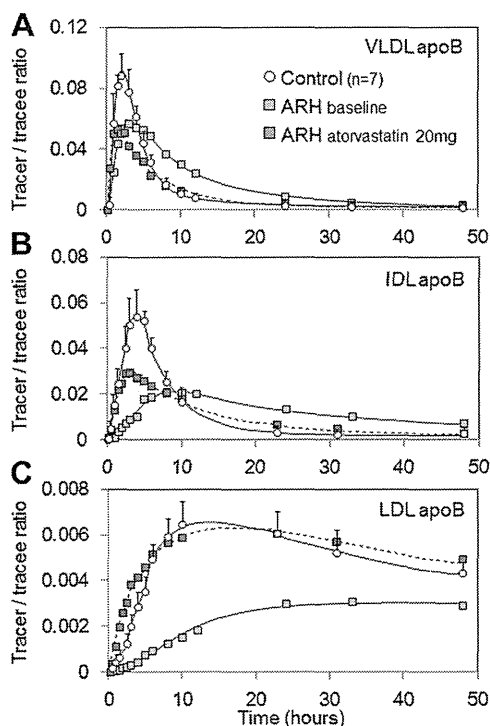

Figure 3. Tracer/trace ratios of apolipoprotein (apo)B-containing lipoproteins. Tracer/tracee ratios of very-low-density lipoprotein VLDL apoB (**A**), intermediate-density lipoprotein IDL apoB (**B**), and LDL apoB (**C**) in the autosomal recessive hypercholesterolemia patient at baseline (**blue squares**), on atorvastatin treatment (**pink squares with dotted line**), and in control subjects (**open circles**). Data were fitted by multicompartmental modeling using SAAMII. Bars represent standard error of the means.

Table 2. Kinetic Parameters of ApoB in the Study Subjects

Subjects	VLDL Conc, mmol/L	VLDL FCR, 1/Day	VLDL PR, mg/kg per Day	IDL Conc, mmol/L	IDL FCR, 1/Day	IDL PR, mg/kg per Day	LDL Conc, mmol/L	LDL FCR, 1/Day	LDL PR, mg/kg per Day
Baseline	0.340	3.153	9.180	0.657	1.414	9.560	7.730	0.109	6.980
After statin therapy	0.248	7.881	3.026	0.341	2.525	13.335	2.333	0.464	16.756
Control subjects (n=7)	0.104±0.033	8.408±2.697	13.172±4.664	0.091±0.052	8.320±3.467	10.562±5.194	2.037±0.315	0.450±0.122	13.947±3.636

Values of control subjects are shown as mean±SD.
Apo indicates apolipoprotein; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; Conc, concentration; FCR, fractional catabolic rate; PR, production rate.

apoB-containing lipoproteins in ARH. Our results demonstrated that in ARH there existed reduced LDL catabolism, which could be normalized by statin therapy and dramatically increased clearance of VLDL remnant as well as other remnant lipoprotein fractions in spite of the fact that our ARH patient has apoE2 isoform which could cause the disturbance in remnant clearance.[21] These unique metabolism of apoB-containing lipoprotein fractions, including VLDL and its remnant fractions were completely different from those reported in heterozygous/homozygous FH patients.[17,22]

One of the possible explanations for the paradoxical acceleration of remnant lipoprotein fractions in ARH is the existence of another pathway, which is independent from the FDNPVY internalization for VLDL and its remnants and does not require LDLRAP1 protein.[23] In addition, Altenburg et al[24] demonstrated that deficiency in the molecule which enhanced the affinity between ligands such as VLDL remnant and LDLR could accelerate the internalization of the remnants. This is consistent with the notion that remnants are passed from one cell surface molecule to the other before internalization.[24] If LDLRAP1 served as an anchor between VLDL remnant and LDLR, deficiency in this protein could result in the increased catabolism of VLDL remnant in ARH. Another possibility is that unknown pathways may exist that are inactivated in the presence of LDLRAP1. This hypothesis seems to be supported by the fact that the LDLR can transfer such remnants to an additional receptor for uptake by the liver when its internalization is impaired. These pathways are not always through LDLR, LDLR-related protein (LRP), and heparan sulfate proteoglycan.[25]

In contrast to homozygous FH patients, the ARH patient responded to statin therapy by an increasing rate of LDL apoB catabolism, resulting in about 70% reduction of LDL apoB pool size. Statin therapy also modulated LDL synthesis in favor of more direct secretion from the liver (11% at baseline to 16% with the treatment versus a mean of 7% for the control subjects). The rate of LDL catabolism is a function of LDLR activity or/and LDL particle affinity to the LDLR. Thus, our results indicate that atorvastatin upregulate LDLR activity in the absence of LDLRAP1. Another possibility for the increasing rate of LDL apoB catabolism seen in ARH is that directly secreted LDL may have a higher affinity for LDLR compared with LDL-processed delipidation/remodeling. Different ratio of apoB/LDL-C between the ARH patient and the control subjects suggest that different LDL processing occurred through delipidation/remodeling of LDL particles under the condition of the absence of this adaptor protein. We also provide additional information for the impact of atorvastatin on the distribution of lipoprotein subfractions in ARH. Relatively wide range of apoB-containing lipoproteins, including large VLDL, could be reduced by atorvastatin therapy. This may be explained by the statin-induced upregulation of possible pathway which could accelerate the clearance of remnant lipoprotein fractions in ARH.

As for the dramatic decrease in PR of VLDL apoB under atorvastatin therapy, one of the possible explanations is the upregulated activity of HMG-CoA reductase suggested by the relatively high level of lathosterol at baseline (Table 1). On

Table 3. Metabolic Channeling of ApoB in the Study Subjects

VLDL	Conversion to IDL, %	VLDL Direct Removal, %	Removal From Remnant, %	Remnant Mass, %
Baseline	52.5	47.5	47.5	60.2
After statin therapy	28.1	71.9	42.8	82.3
Control subjects (n=7)	85.5±18.7	14.5±18.7	1.8±2.1	12.0±11.8

IDL	Direct Production, %	Conversion to LDL, %	IDL Direct Removal, %	Removal From Remnant, %	Remnant Mass, %
Baseline	8.6	56.4	77.2	77.2	80.8
After statin therapy	12.2	85.5	17.0	17.0	29.6
Control subjects (n=7)	5.9±7.7	97.8±3.1	2.3±3.3	2.3±3.3	17.4±14.4

LDL	Direct Production, %	Via IDL, %
Baseline	11.0	90.0
After statin therapy	16.2	85.8
Control subjects (n=7)	7.3±6.1	92.3±6.1

Values of control subjects are shown as mean±SD.
Apo indicates apolipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; and LDL, low-density lipoprotein.

the other hand, the increase in the PR of LDL apoB during atorvastatin therapy could be partially explained by the elevation of LPL mass (Table 1), in accordance with the previous report.[26] Also, another study has shown that atorvastatin therapy is associated with an increase in LPL activity.[27] These data suggest that atorvastatin treatment may cause an increase in the conversion of VLDL to LDL.

Limitations
Our study has several limitations. First, only 1 ARH patient was included in this study because of the rarity of this disease, making it difficult to compare the results statistically. Also, the age of the control subjects were younger than the ARH patient, although all were male. Second, we did not measure apoE FCR in the ARH patient and thus could not draw any conclusion regarding the possibility of the clearance through VLDL receptor. However, the fact that the ARH patient has apoE2 isoform, which could cause the disturbance in remnant clearance, indicates the less influence of the apoE pathway on the catabolism of these lipoproteins. In this study, as much as 30% increase in HDL-C was achieved through atorvastatin therapy. Another kinetic study targeting apoA-I for the ARH patient may reveal the metabolic aspects about the increase in HDL-C.

Finally, it would be worthwhile to compare lipoprotein kinetics of ARH with that of FH directly. Although we cited previously published data on the apoB kinetics in FH patients to discuss the comparison between the kinetics of ARH and FH, further kinetic study comparing ARH and FH directly is needed to confirm this matter.

Conclusion
In summary, the first detailed lipoprotein kinetic study including remnant lipoprotein fractions in ARH before and after statin therapy revealed 2 important aspects of the lipoprotein metabolic basis of this disease. First, FCR of LDL apoB in ARH was decreased by about 76% that of normal control subjects at baseline; however, the catabolic parameter was elevated to normal range after statin therapy (atorvastatin 20 mg). Second, and possibly the major finding from this

investigation, is that the clearance of the VLDL remnant as well as other remnant fractions were dramatically increased compared with normal control subjects. We suggest that these results will provide new insights into the lipoprotein metabolism of ARH and the novel pharmacological target for LDLRAP1.

Disclosures
None.

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— 163 —

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CLINICAL PERSPECTIVE

Autosomal recessive hypercholesterolemia (ARH), which is due to mutations in an adaptor protein involved in low-density lipoprotein receptor internalization (LDLRAP1), is an extremely rare disorder, with only about 50 cases described in the literature. This defect appears to be a phenocopy of homozygous familial hypercholesterolemia; however, the clinical phenotype of ARH appears to be less severe and more responsive to statins—the mechanism for this observation still remains unknown. One of the possible mechanisms of great responsiveness of ARH to statins was elucidated by a metabolic study using LDLRAP1 knockout mice that showed a preserved ability for LDLR-dependent very-low-density lipoprotein (VLDL) clearance. However, few data exist regarding the metabolic basis of LDLRAP1 in clinical settings, especially the metabolism of remnant lipoprotein fractions. Therefore, we examined lipoprotein kinetics in the ARH patient by using a stable isotope methodology with kinetic modeling including several remnant lipoprotein fractions, before and after atorvastatin therapy. We demonstrate that ARH exhibits decreased LDL clearance associated with decreased fractional catabolic rates of LDL apoB and increased clearance for VLDL remnant; this observation indicates the lack of LDLRAP1-dependent modulation of VLDL metabolism, activating an alternate pathway that can remove VLDL remnant paradoxically. This preferred pathway could potentially contribute to the greater responsiveness of ARH to statins. Our results will provide new insights into the lipoprotein metabolism in ARH.

SUPPLEMENTAL MARERIAL

online-only Data Supplement Method 1. Determination of isotopic enrichment

Briefly, apoB isolated by isopropanol precipitation was hydrolyzed in 6N HCl (amino acid analysis grade, Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 110°C for 24 hours. The protein hydrolysates were lyophilized in a Speed-Vac evaporator (Savant Instrument Inc., Farmingdale, NY). Free amino acids were purified from plasma or protein hydrolysates by cation exchange chromatography (AG-50W-X8, Bio-Rad Laboratories, Richmond, CA), and then derivatized to the N-heptafluorobutyryl isobutyl esters, and analyzed by GC-MS on a 6890 gas chromatograph connected to a 5973 quadruple mass spectrometer (Hewlett Packard, Palo Alta, CA) in the chemical ionization mode, using methane as the reagent gas. Selective ion monitoring at 365 m/z (M+2 isotopomer) for unlabeled leucine and 366 m/z (M+3 isotopomer) for labeled leucine was used to determine the tracer/tracee ratio by regression analysis of standards of known tracer/tracee ratios (0-10%) as reported previously. Each sample was analyzed at least 2 times.

online-only Data Supplement Method 2. Kinetic modeling used in this study
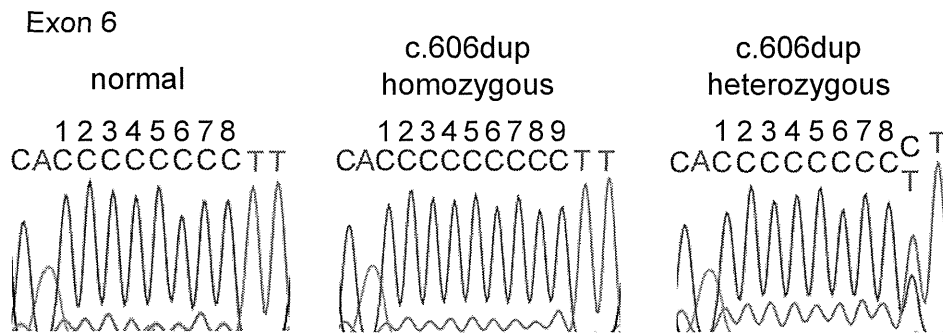
In this model, heterogeneity in VLDL is represented by large VLDL (VLDL1), small VLDL (VLDL2). For the VLDL1 fraction, supplementary heterogeneity was introduced in the model by 2 compartments: the first one, VLDL1 (compartment 11) was linked to VLDL2 (compartment 13) by delipidation cascade, and the second, VLDL remnants (compartment 12). This was performed to get a better fit of the data. ApoB-100 enters into plasma through VLDL secretion and direct production of IDL and LDL. ApoB-100 direct removal occurs from VLDL2 (k(0,13)), VLDL remnant (k(0,12)), IDL (k(0,21)), IDL remnant (k(0,22)), and LDL (k(0,31)). To make the model identifiable, the rate constant from VLDL1 to VLDL2 (k(13,11)), representing delipidation, was constrained to be equal to that from VLDL2 to IDL (k(21,13)). For comparison between 2 groups (ARH patient and controls) the VLDL1, VLDL2, and VLDL remnant data were presented as VLDL delipidation rate and VLDL FCR, which represents the sum of delipidation and direct removal rate. The VLDL conversion rate was calculated as VLDL2 delipidation flux divided by total VLDL mass. The VLDL direct removal was calculated as VLDL2 direct removal divided by total VLDL mass. The apoB100 PR in mg/kg per day represents the product of FCR and pool size of apoB100 in lipoprotein fractions assuming plasma volume equal to 4.5% of body weight.

online-only Data Supplement Figure 1. Large cutaneous and tendinous xanthomas in the proband's hand (A) and foot (B). Achilles tendons show significant thickness (right = 26 mm) with calcification (C).Clinical course is shown, open circles indicate TC, closed circles indicate TG, and open triangles indicate HDL-C (D). Bypass surgery was carried out on the bilateral external iliac artery because of the complaint of intermittent claudication (E and F). And bypass surgery for the coronary artery was conducted (G). Microscopic findings of the proband's liver stained with hematoxylin and eosin (H). There was no evidence of fatty liver or any other specific findings.
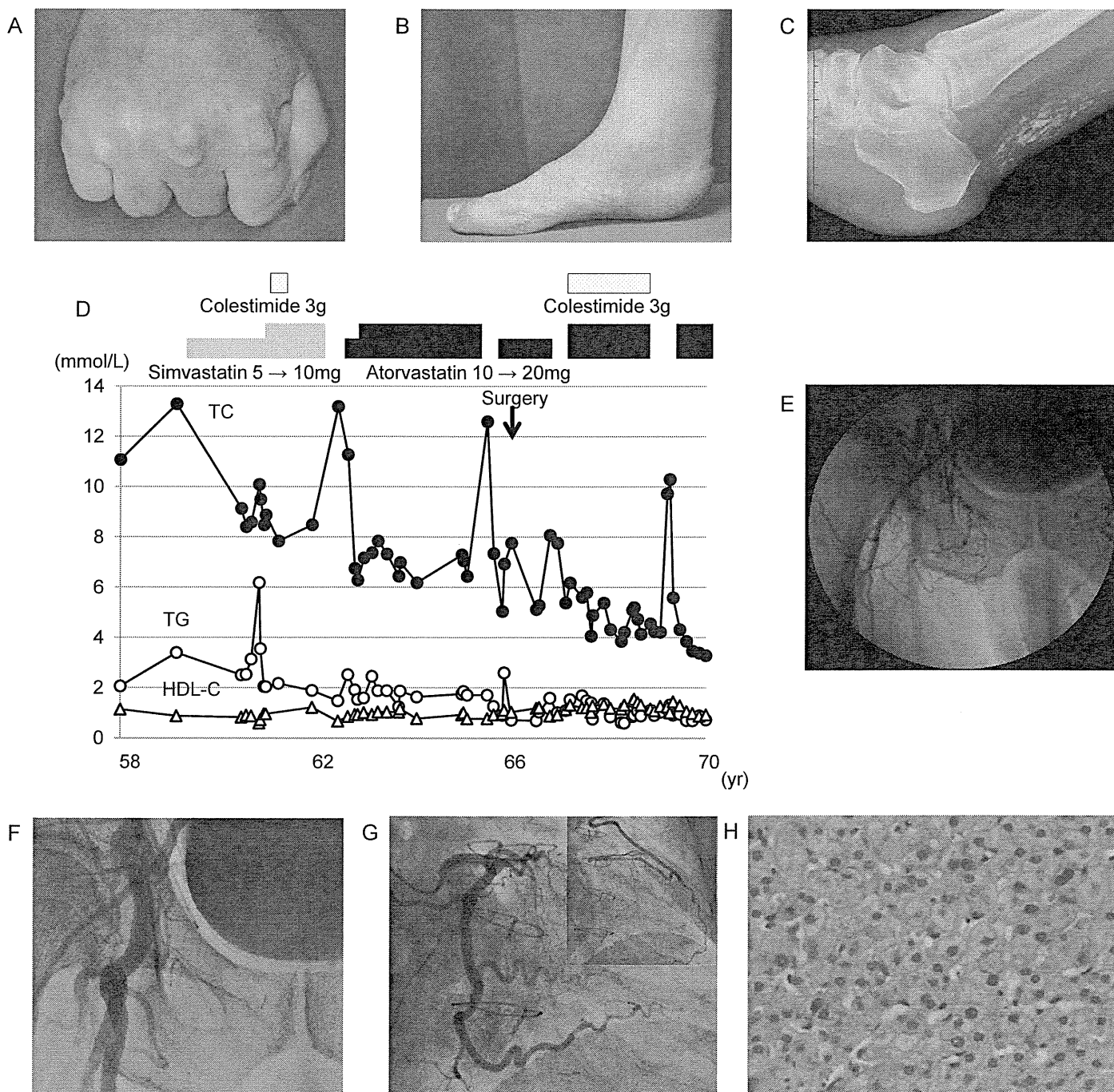
online-only Data Supplement Figure 2. DNA sequence data of the proband (central panel), his niece (right panel), and a control subject (left panel) for the LDLRAP1 gene exon 6.

Homozygosity for an extra cytosine insertion mutation in eight sequential cytosines between the nucleotide positions 599 and 606 (nucleotides are numbered from the first nucleotide that encodes the starting methyonine codon) was shown in the proband, with corresponding heterozygosity shown in his niece.

Exon 6

normal	c.606dup homozygous	c.606dup heterozygous
1 2 3 4 5 6 7 8	1 2 3 4 5 6 7 8 9	1 2 3 4 5 6 7 8
CACCCCCCCC TT	CACCCCCCCCC T T	CACCCCCCCCC

online-only Data Supplement Table. Clinical data of the proband's family

| Subjects[†]	Gender	Age (yr)	Condition	CAD	AT (mm)	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
III-6	male	70	heterozygous	-	6	6.13	0.94	4.73	0.96
III-11	male	75	normal	-	n.d.	4.68	1.32	2.76	1.29
III-12	female	72	heterozygous	-	6	7.32	1.88	5.04	1.40
III-14	female	77	normal	+	6	5.64	1.55	3.49	1.42
III-19	male	56	normal	-	8	5.90	1.95	3.57	1.42
III-25*	male	67	homozygous	+	26	13.27	3.39	10.84	0.88
III-26	female	65	normal	-	n.d.	6.34	1.24	3.90	1.86
III-28	female	60	heterozygous	-	6	6.28	2.16	3.85	1.42
III-30	female	53	heterozygous	-	5	6.54	1.97	4.06	1.58
III-37	male	55	heterozygous	-	8	7.37	1.05	4.89	1.99
III-39	male	59	heterozygous	-	4	4.89	1.67	5.48	1.22
IV-9	male	52	normal	-	n.d.	4.58	1.41	2.38	1.55
IV-10	female	48	normal	-	5	6.23	1.04	3.70	2.04
IV-13	male	53	normal	-	7	4.52	4.34	1.42	1.09
IV-22	male	42	heterozygous	-	n.d.	5.48	2.03	3.36	1.19
IV-24	female	39	heterozygous	-	5	4.60	1.41	2.51	1.45
IV-25	female	38	heterozygous	-	n.d.	4.60	1.39	2.69	1.23
IV-35	male	30	heterozygous	-	5	5.74	2.50	3.62	0.96
V-2	female	16	normal	-	n.d.	5.07	0.91	3.21	1.45
V-3	female	12	heterozygous	-	n.d.	4.60	0.78	2.46	1.79

CAD, coronary artery disease; AT, Achilles tendon; n.d., not done.
* Proband
† Generation-family member: see Supplemental Figure 1 for pedigree details of individual family members

online-only Data Supplement Figure 3. Changes in lipoprotein subfractions.

Changes in cholesterol (A), triglyceride (B), free-cholesterol (C), and phospholipids (D) in each lipoprotein subfraction was assessed by HPLC. Blue bars indicate the baseline value of the ARH patient. Pink bars indicate the value of the ARH patient after statin therapy.
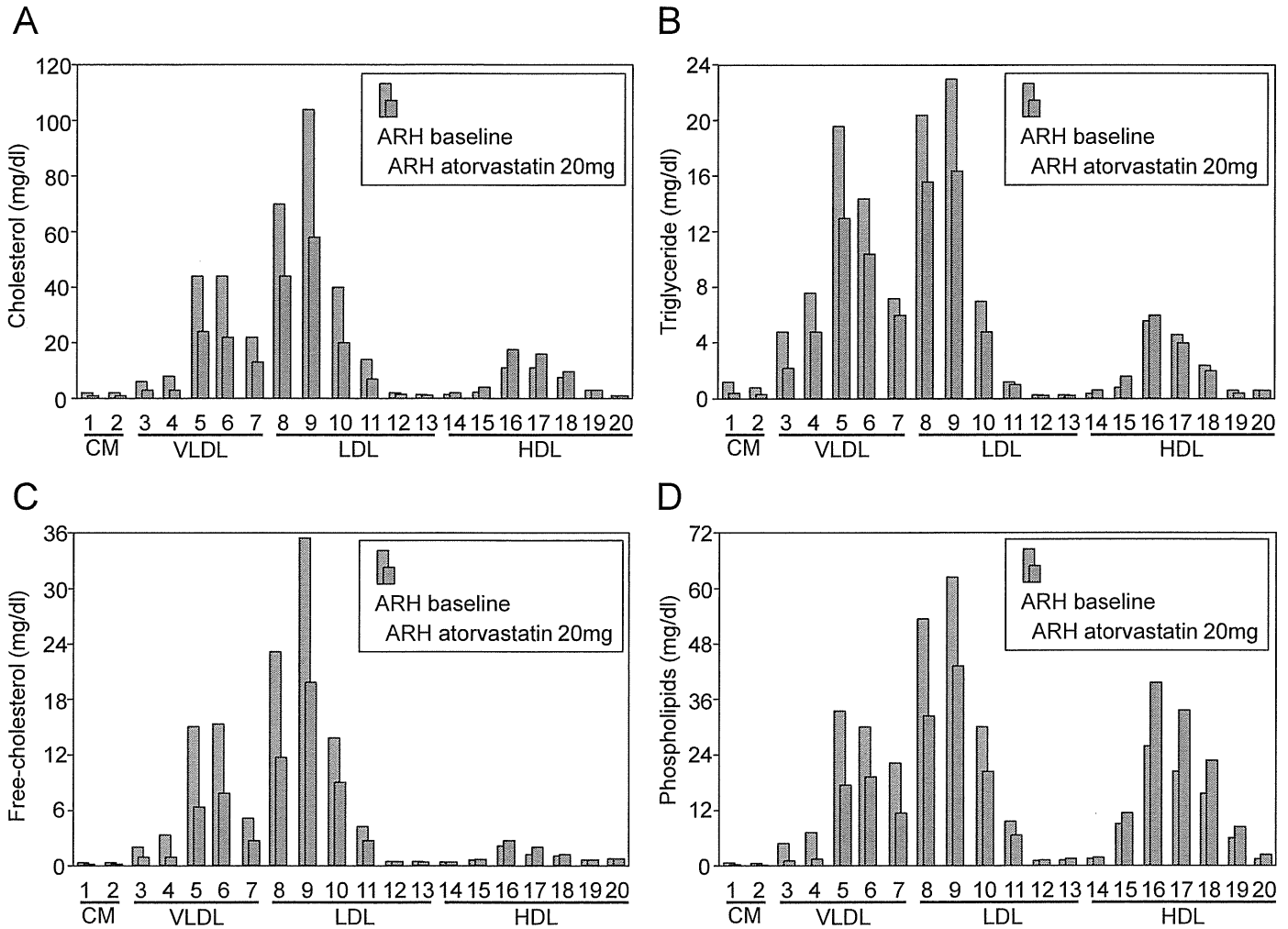
Original Article

Effect of body mass index-z score on adverse levels of cardiovascular disease risk factors

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Abstract   *Background*: Cardiovascular disease (CVD) risk factors are associated with body mass index z-score (BMISD) and/or insulin resistance (IR). However, the correlation between adverse levels of these risk factors and BMISD, and the effect of IR on these associations are not fully understood in children. The aim of this study was to evaluate the association between adverse levels of CVD risk factors and BMISD, and the effect of IR on these associations in schoolchildren. *Methods*: Conventional CVD risk factors, C-reactive protein (CRP), uric acid (UA) and adiponectin were determined in 757 boys and 494 girls aged between 7 and 12 years. IR was assessed by the homeostasis model approximation index. *Results*: BMISD were linearly associated with relative risks having adverse levels of all factors, except for glucose and low-density lipoprotein cholesterol (LDL-C) in boys, and except for glucose, LDL-C and adiponectin in girls ($P < 0.01$–0.001). These associations were weakened after adjustment for IR, but still significant in cases of UA and CRP in boys and UA, high-density lipoprotein cholesterol and CRP in girls ($P < 0.01$–0.001). *Conclusion*: The relative risk of having adverse levels of most CVD risk factors in school children increased across the entire range of BMISD. IR contributed to most of these relative risks, but BMISD itself also contributed to these relative risks. To prevent future development of CVD, it might be important for schoolchildren to maintain BMISD within normal range. However, in cases of hyper LDL-cholesterolemia, we should consider causes other than BMISD.

Key words   adipocytokine, cardiovascular disease risk factors, hypercholesterolemia, insulin resistance, obesity.

Many epidemiological studies have shown that overweight and obesity are increasing globally in both children and adults.[1] In Japan, the prevalence of obesity in schoolchildren has steadily increased in recent decades, possibly due to changes in dietary patterns and lifestyles among these children.[2] Must *et al.* reported that the risk of morbidity from coronary heart disease and atherosclerosis was increased among men and women who had been overweight as adolescents of 13–18 years old.[3] Given this finding, it seems rational to consider that the incidence of atherosclerotic cardiovascular disease (CVD) in Japan could increase dramatically in the near future. In contrast, a recent study reported that the overweight and obese show no increased risk for total mortality and cardiovascular mortality compared with those with a normal body mass index (BMI):[4] severely obese patients did not have increased total mortality, but they had the highest risk for cardiovascular mortality. These results suggest that the metabolic aberrations that coexisted with overweight and obesity may be more important than overweight and obesity themselves. In this regard, Barter *et al.* reported that overweight

people with normal plasma lipids might be at relatively low risk for developing diabetes and cardiovascular disease.[5]

In our previous studies, we showed that abnormal CVD risk factors, such as small dense low-density lipoproteins, dyslipidemia, hyperinsulinemia, high levels of inflammatory markers and low levels of adiponectin, were found in schoolchildren.[6–9] In addition, low-density lipoprotein particle size and serum concentrations of these CVD risk factors were closely associated with BMI.[6–9] However, these abnormal CVD risk factors may occur regardless of BMI.[7] As reported previously, genetic predispositions appear to contribute more to dyslipidemia in children than they do in adults.[7,10] Thus, it is important to clarify whether abnormal CVD risk factors are merely complications of overweight or obesity. It is generally accepted that many comorbidities with obesity, such as diabetes, dyslipidemia and hypertension, are attributed to insulin resistance.[11] Thus, in the present study, we investigated the correlations between adverse levels of CVD risk factors and BMI z-score (BMISD), and the effect of insulin resistance on these associations in Japanese schoolchildren.

Methods

Subjects

We studied 1251 Japanese children (757 boys and 494 girls) aged 7–12 years, who underwent screening and were enrolled in a care

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program for lifestyle-related diseases in Okinawa and Kumamoto, Japan. Sex-maturity stages in the children studied were equal or less than Tanner stage 3 (Tanner stage was evaluated by inspection of mammary development in girls, and by asking condition of pubic hair in boys and this evaluation was performed by a pediatrician). BMI was calculated as weight [kg]/height^2 [m^2]. BMISD adjusted for age and sex were obtained based on data on Japanese schoolchildren provided in 2000 by the Ministry of Education, Culture, Sports, Science and Technology (unpublished data). We employed BMISD to continuously evaluate BMI in the studied schoolchildren. None of the children was receiving therapy for weight reduction, or drugs that might affect lipid metabolism, and none had a smoking habit. Venous blood was drawn after an overnight fast. Informed consent was obtained from the parents of all of the children. This study was approved by the ethics committee of the Ryukyu University.

Laboratory measurements

The serum concentration of C-reactive protein (hCRP) was measured by a highly sensitive immunoturbidimetric assay using reagents and calibrators from Dade Behring Marbura GmbH (Marburg, Germany). The lower limit of detection for serum CRP concentration was 0.05 mg/L. Adiponectin was measured by enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA). Serum insulin was measured by two-step sandwich enzyme-linked immunosorbent assay (ELISA) (SRL Inc., Hachioji, Japan). Routine chemical methods were used to determine the serum concentrations of total cholesterol (TC), high-density-lipoprotein cholesterol (HDL-C), triglycerides (TG), uric acid and glucose. Low-density-lipoprotein cholesterol (LDL-C) was calculated as [TC – HDL-C – TG/5]. Apolipoprotein B (apoB) was measured by the turbidity immunoassay method.[12] Insulin resistance was calculated using the homeostasis model approximation index (HOMA-IR).[13]

Statistical evaluation

The significance of differences between boys and girls was determined by the Mann–Whitney U-test. Serum concentrations of insulin, TG and hCRP were markedly skewed. Thus, these parameters were normalized by log-transformation. Pearson and partial correlation coefficients were then computed to assess the associations between BMISD and various parameters. The logistic model was used to evaluate linear associations between adverse levels of variables and BMISD (continuous). The relative risks to have adverse variables (odds ratio) were adjusted for age by a multiple logistic regression analysis.

Results

Several indexes of overweight and/or abdominal obesity have been proposed for children, as in the case for adults. Among these, waist–height ratio is more strongly associated with CVD risk factors than is BMI;[14] however, a recent report found that BMISD and waist–height ratio did not differ in their ability to identify adverse risk factors.[15] Because waist circumference was not measured in our schoolchildren, we employed BMISD to evaluate the correlation between adverse levels of CVD risk factors and BMI.

As shown in Table 1, significant sex differences were found for several parameters; thus, we separated the data for boys and girls in the following analysis. Because age was significantly correlated with BMISD (boys: r = 0138, P < 0.001; and girls: r = 0.139, P < 0.01), age was adjusted by partial correlation. Table 2 shows age-adjusted correlations between BMISD and 10 parameters. All parameters except glucose were significantly associated with BMISD in both boys and girls (P < 0.01-0.001). BMISD showed a positive correlation with LDL-C and a negative correlation with HDL-C; therefore, we did not examine its correlation with TC. HOMA-IR and serum concentrations of insulin showed stronger correlations with BMISD than those of other factors in both boys and girls. HOMA-IR has recently been validated as a surrogate maker of insulin resistance, even in children.[16,17]

We then evaluated the correlation between adverse levels of CVD risk factors (except for glucose) and BMISD with a multiple logistic regression analysis. To date, there are no criteria to define adverse levels of these CVD risk factors in Japanese

Table 1 Clinical and chemical data

	Boys (n = 757)	P-value	Girls (n = 494)
Age (years)	10.0 ± 1.1	(NS)	10.0 ± 1.1
BMI SD	1.64 ± 1.12	(P < 0.01)	1.46 ± 1.12
TC (mg/dL)[‡]	182 ± 29	(P < 0.01)	176 ± 28
TG (mg/dL)[§]	79 ± 59	(NS)	80 ± 46
LDL-C (mg/dL)[‡]	107 ± 25	(NS)	104 ± 25
HDL-C (mg/dL)[‡]	59 ± 12	(P < 0.01)	56 ± 11
ApoB (mg/dL)	79 ± 18	(NS)	77 ± 18
Glucose (mg/dL)[†]	90 ± 6	(P < 0.01)	89 ± 7
Insulin (μU/mL)	12.21 ± 8.96	(P < 0.01)	14.10 ± 9.79
HOMA-IR	2.75 ± 2.24	(P < 0.01)	3.12 ± 2.36
Uric acid (mg/dL)	4.9 ± 1.0	(NS)	4.8 ± 1.0
Adiponectin (μg/mL)	8.7 ± 3.6	(NS)	8.6 ± 3.7
hCRP (mg/L)	1.65 ± 4.56	(NS)	1.24 ± 3.12

Values are expressed as mean ± standard deviation. [†]To convert to mmol/L, divided by 18. [‡]To convert to mmol/L, multiply by 0.0259. [§]To convert to mmol/L, multiply by 0.0113. ApoB, apolipoprotein B; BMI, body mass index; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; LDL-C, low-density-lipoprotein cholesterol; NS, not significant; TG, triglycerides; TC, total cholesterol.

Table 2   Age-adjusted correlations between body mass index z-score and cardiovascular disease risk factors

	Boys		Girls	
	r	P	r	P
Log TG	0.177	<0.001	0.218	<0.001
LDL-C	0.107	<0.01	0.150	<0.01
HDL-C	−0.277	<0.001	−0.399	<0.001
ApoB	0.178	<0.001	0.239	<0.001
Glucose	0.045	0.213	0.068	0.135
Log insulin	0.568	<0.001	0.647	<0.001
Log HOMA-IR	0.561	<0.001	0.634	<0.001
Uric acid	0.370	<0.001	0.437	<0.001
Adiponectin	−0.264	<0.001	−0.303	<0.001
Log hCRP	0.464	<0.001	0.333	<0.001

Bold indicates significant associations ($P < 0.05$). ApoB, apolipoprotein B; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides.

school children. Thus, when levels of CVD risk factors were greater than those of the 90th percentiles of our subjects, we tentatively considered the children to have adverse levels, except for HDL-C and adiponectin (boys: insulin > 20.8 μU/mL, HOMA-IR > 4.39, TG > 145 mg/dL, LDL-C > 138 mg/dL,

apoB > 101 mg/dL, uric acid > 6.3 mg/dL and hCRP > 3.41 mg/L; girls: insulin > 26.64 μU/mL, HOMA-IR > 5.62, TG > 148 mg/dL, LDL-C 133 mg/dL, apoB > 98 mg/dL, uric acid > 5.9 mg/dL and hCRP > 2.39 mg/L). HDL-C and adiponectin were considered to be adverse levels when their levels were less than those of the 10th percentiles (boys: HDL < 44 mg/dL and adiponectin < 4.2 μg/mL; girls: HDL-C < 43 mg/dL and adiponectin < 4.1 μg/mL). As shown in Table 3, we observed no linear association of BMISD with adverse levels of LDL-C in boys. The relative risk of having adverse levels of other CVD risk factors increased with increasing BMISD. Table 4 shows the case of girls. In contrast to the case of boys, BMISD did not show a linear correlation with adverse levels of adiponectin. As shown in Table 2, HOMA-IR showed stronger correlations with BMISD than those of other CVD risk factors in both boys and girls. Thus, to examine whether the correlations of adverse levels of CVD risk factors with BMISD were independent of insulin resistance, the findings were adjusted for HOMA-IR, in addition to age. After adjustment for HOMA-IR and age (Table 5), the relative risk of having adverse levels of uric acid and hCRP increased with increasing BMISD in boys. Significant associations of adverse levels of HDL-C, TG, apoB and adiponectin with BMISD were eliminated in boys after adjustment. In girls, the relative risk of having adverse levels of uric acid, HDL-C and

Table 3   Age-adjusted associations between body mass index z-score and adverse levels (above the 90th percentile or below the 10th percentile; HDL-C and adiponectin) in boys as assessed by a multiple logistic regression analysis

Dependent variable	β	Wald χ^2	P-value	Exp (β)	95%CI
LDL-C	0.133	1.64	0.201	1.14	0.93–1.40
HDL-C	0.351	11.56	<0.001	1.42	1.16–1.74
TG	0.230	4.87	0.027	1.26	1.03–1.54
ApoB	0.228	4.85	0.028	1.26	1.03–1.54
Insulin	1.009	68.55	<0.001	2.74	2.16–3.48
HOMA-IR	0.894	33.84	<0.001	2.44	1.95–3.07
Uric acid	0.680	40.99	<0.001	1.97	1.60–2.43
Adiponectin	0.387	14.49	<0.001	1.47	1.21–1.80
hCRP	0.745	45.05	<0.001	2.11	1.69–2.62

Bold type indicates a significant correlation ($P < 0.05$). ApoB, apolipoprotein B; CI, confidence interval; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides.

Table 4   Age-adjusted associations between body mass index z-score and adverse levels (above the 90th percentile or below the 10th percentile; HDL-C and adiponectin) in girls as assessed by a multiple logistic regression analysis

Dependent variable	β	Wald χ^2	P-value	Exp (β)	95%CI
LDL-C	0.216	2.65	0.103	1.24	0.96–1.61
HDL-C	0.831	32.98	<0.001	2.30	1.73–3.05
TG	0.451	11.21	<0.001	1.57	1.21–2.04
ApoB	0.392	8.62	<0.01	1.48	1.14–1.92
Insulin	0.846	33.28	<0.001	2.33	1.75–3.11
HOMA-IR	0.947	39.92	<0.001	2.58	1.92–3.46
Uric acid	0.931	42.75	<0.001	2.54	1.92–3.36
Adiponectin	0.203	2.53	0.112	1.23	0.95–1.57
hCRP	0.643	15.73	<0.001	1.90	1.39–2.62

Bold type indicates a significant correlation ($P < 0.05$). ApoB, apolipoprotein B; CI, confidence interval; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides.

Table 5 Age- and homeostasis model approximation index-adjusted significant associations between body mass index z-score and adverse levels (above the 90th percentile or below the 10th percentile; HDL-C and adiponectin) in schoolchildren as assessed by a multiple logistic regression analysis

Dependent variable	β	Wald χ^2	P-value	Exp (β)	95%CI
Boys					
hCRP	0.666	27.46	<0.001	1.95	1.52–2.50
Uric Acid	0.559	20.85	<0.001	1.75	1.38–2.22
Girls					
Uric acid	0.827	25.28	<0.001	2.29	1.66–3.16
HDL-C	0.591	12.49	<0.001	1.81	1.30–2.51
hCRP	0.530	9.48	<0.01	1.70	1.21–2.38

CI, confidence interval; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol.

hCRP showed an increase with increasing BMISD. Significant associations of adverse levels of TG and apoB with BMISD were eliminated in girls after adjustment.

Discussion

Based on the findings of the present study, adverse levels of CVD risk factors can be divided into three groups (Table 6): (i) independent of BMISD (boys: glucose and LDL-C; girls: glucose, LDL-C and adiponectin); (ii) dependent on BMISD and independent of insulin resistance (boys: uric acid and hCRP; girls: uric acid, HDL-C and hCRP); and (iii) dependent on BMISD and insulin resistance (boys: insulin, HOMA-IR, HDL-C, TG, apoB and adiponectin; girls: insulin, HOMA-IR, TG and apoB).

It is generally accepted that many comorbidities with obesity, such as diabetes, dyslipidemia and hypertension, are attributed to insulin resistance.[11] In the present study, BMISD was strongly correlated with insulin resistance. The relative risk of having an adverse level of insulin resistance was linearly increased across the normal range. The risk of an adverse level of insulin resistance was significantly higher for children at BMISD 1.0 compared with those at BMISD 0.0, with an odds ratio of adverse level of insulin resistance ranging from 2.44 to 2.58 (Tables 3 and 4). The present findings suggest that in schoolchildren, a slight shift of BMISD from normal ranges affects insulin resistance.

Correlations of BMISD and adverse levels of CVD risk factors are generally reported only in studies regarding hypertension,[18,19] in which the risk of hypertension has been found to be significantly higher in obese children than in non-obese children, with an odds ratio of hypertension ranging from 2.4 to 2.5; however, it has been noted that the prevalence of hypertension in children increases across the entire range of BMI values and

cannot be defined by a simple threshold effect. The effects of BMISD on adverse levels of LDL-C, HDL-C, TG and apoB are not yet clear. Unexpectedly, LDL-C was not associated with BMISD in both boys and girls. However, adverse levels of TG and apoB were associated with BMISD in both boys and girls. These significant associations were lost after adjustment for insulin resistance. Different findings of LDL-C and apoB were consistent with our previous report that LDL size was inversely associated with BMI in school children.[6] In the case of HDL-C, a strong association between BMISD and adverse level of HDL-C was found in both boys and girls; however, after adjustment for insulin resistance, a significant association was only retained in girls. As reported previously, hypercholesterolemia (hyper LDL-C) in school children commonly occurs regardless of BMISD.[6,7] Familial hypercholesterolemia and familial combined hyperlipidemia should not be overlooked in school children with overweight and obesity. Effect of genetic factors on hyper LDL-C may be greater than that of environmental factors. In addition to hyper LDL-C in school children, low HDL-C in schoolgirls should not be diagnosed as complications of overweight and obesity before clarifying the genetic background.

Serum concentrations of adiponectin were inversely correlated with BMISD in both boys and girls. However, the association between the relative risk of having an adverse level of adiponectin and BMISD was only significant in boys. This association was completely dependent on insulin resistance. In other words, relative risk of an adverse level of adiponectin is not increased in obese boys without insulin resistance, thereby indicating a close correlation between adverse adiponectin level and insulin resistance in schoolboys. In girls, factors other than BMISD and insulin resistance seemed to regulate adverse levels

Table 6 Correlation between BMISD and adverse levels of cardiovascular disease risk factors

	Boys	Girls
Independent of BMISD	Glucose, LDL-C	Glucose, LDL-C, Adiponectin
Dependent on BMISD		
Independent on IR	Uric acid, hCRP	Uric acid, HDL-C, hCRP
Dependent of IR	Insulin, HOMA-IR, HDL-C, TG, apoB, adiponectin	Insulin, HOMA-IR, TG, apoB

apoB, apolipoprotein B; BMISD, body mass index z-score; hCRP, serum concentration of C-reactive protein; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model approximation index; IR, insulin resistance; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides.

of adiponectin. Recently, Magge *et al.* also reported similar findings that adiponectin levels are independent of insulin resistance in adolescents.[20]

With respect to hCRP, the association between the relative risk of adverse levels of hCRP and BMISD was unaffected by the adjustment for insulin resistance in both boys and girls. The underlying mechanism behind the correlation between hCRP and BMISD has not been clarified in the present study; however, our data suggest that subclinical inflammation as expressed by hCRP did occur even in school children, and that the degree of inflammation was associated with BMISD. According to a recent report, serum concentrations of uric acid are associated with all-cause and cardiovascular disease mortality.[21] Association between an adverse level of uric acid and BMISD was unexpectedly high and was unaffected by insulin resistance in both boys and girls. In addition, the association was unaffected by hCRP (data not shown). Although further studies are needed to clarify the physiological role of uric acid in children, the strong association between the relative risk of having adverse levels of uric acid and BMISD should be highlighted as a complication of overweight and obesity.

Conclusion

In the present study, hyper LDL-cholesterolemia in school children cannot be explained by BMISD. However, the relative risk of having adverse levels of other CVD risk factors in school children increases across the entire range of BMISD. Relative risks of adverse levels of UA and CRP in boys, and those of UA, HDL-C and CRP in girls are independent of insulin resistance. Not only obese children but also overweight children seem to be high-risk for the future development of CVD. To prevent future development of CVD, it is quite important for school children to maintain BMISD within normal range. However, we should also consider causes other than BMISD, especially in cases of hyper LDL-cholesterolemia in school children.

Acknowledgment

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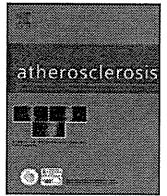
Non-high-density lipoprotein cholesterol is a practical predictor of long-term cardiac death after coronary artery bypass grafting

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ABSTRACT

Background: Recent studies have demonstrated that non-high-density lipoprotein cholesterol (non-HDL-C) can predict the risk of cardiovascular events among general population without coronary heart disease (CHD). However, few studies have investigated the predictive value of non-HDL-C for long-term prognosis in patients with CHD. The purpose of this study was to investigate whether non-HDL-C can predict long-term cardiovascular events in patients with CHD who underwent coronary artery bypass grafting (CABG).

Methods: We enrolled 1074 consecutive patients who underwent CABG at Juntendo University Hospital between 1984 and 1994, and obtained mortality data through 2000. We divided the patients into 2 groups by the median non-HDL-C level at baseline (180 mg/dL) and used Kaplan–Meier method with log-rank test for survival analyses. Cox proportional-hazard regression model was used to calculate the relative risk (RR) of cardiac death.

Results: The mean follow-up period was 10.6 ± 3.5 years. The survival rate of cardiac death was significantly lower in the high non-HDL-C group than that in the low non-HDL-C group (log-rank test; $p = 0.006$). Furthermore, in proportional regression analysis adjusted for conventional coronary risk factors, metabolic syndrome, statin treatment, and use of artery bypass graft, the increased levels of non-HDL-C were significant and independent predictor of cardiac death beyond other lipid parameters (RR1.22; by 10 mg/dL non-HDL-C increasing, 95% confidence interval 1.03–1.44; $p < 0.05$).

Conclusions: The increased levels of non-HDL-C were significantly associated with an increased risk of cardiac death. Baseline non-HDL-C levels may be a practical predictor of long-term cardiac death in patients with CHD after CABG.

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1. Introduction

Low-density lipoprotein cholesterol (LDL-C) has been well established as a primary target for lipid-lowering therapy in both primary and secondary prevention of coronary heart disease (CHD). Regardless of LDL-C levels, atherogenic dyslipidemia characterized by increased triglyceride (TG)-rich lipoproteins, increased small dense LDL and/or decreased high-density lipoprotein cholesterol (HDL-C), which are mostly caused by insulin resistance in metabolic syndrome and type 2 diabetes mellitus (DM), has been also extensively explored their attribution to the development of CHD [1,2].

A possible surrogate marker of this atherogenic dyslipidemia in addition to LDL-C has been considered to be non-high-density

lipoprotein cholesterol (non-HDL-C) [3], calculated by subtracting HDL-C levels from total cholesterol (TC) levels. Non-HDL-C represents cholesterol in all of the apolipoprotein B (apoB)-containing lipoproteins, composed of LDL as main part, TG-rich lipoproteins, and lipoprotein (a) [Lp (a)] [4]. Therefore, non-HDL-C levels are strongly correlated with total apoB levels and directly reflect the total number of circulating atherogenic lipoprotein particles [5].

Based on this correlation, several population-based studies have demonstrated the usefulness of non-HDL-C levels to predict the incidence of cardiovascular events such as fatal or non-fatal myocardial infarction and cardiac death [6–10]. Consequently, the National Cholesterol Educational Program (NCEP) Adult Treatment Panel (ATP) III guidelines identify non-HDL-C as a secondary target for lipid-lowering therapy after achieving target LDL-C levels in patients with increased TG levels (≥ 200 mg/dL) [11,12]. Recently in Japan, some epidemiological cohort studies have demonstrated that non-HDL-C levels can predict the risk of cardiovascular events

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among the general population without CHD [13–15]. However, few studies have investigated the predictive value of non-HDL-C for long-term prognosis in patients with CHD in Japan even in other countries. The purpose of this study was to investigate whether non-HDL-C levels can predict long-term cardiovascular prognosis in patients with CHD who underwent coronary artery bypass grafting (CABG).

2. Methods

2.1. Subjects

A total of 1074 consecutive patients who underwent CABG at Juntendo University Hospital (Tokyo, Japan) between January 1984 and December 1994 were enrolled in this retrospective cohort study. Patients who had achieved complete revascularization, i.e., those who had no un-bypassed major vessels with stenosis >50% [16,17] were included, and those who received hemo-dialysis were excluded. This study was performed in according to the principles of the Declaration of Helsinki and the ethics policies of the institution.

2.2. Collection of baseline data

Demographic data, including age, gender, body mass index (BMI), coronary risk factors, medication, CABG procedure-related factors, and comorbidities were retrospectively collected using our institutional database. Fasting blood samples were obtained before coronary artery angiography on admission. Plasma TC, HDL-C and TG levels were determined using enzymatic methods. LDL-C levels were calculated by using the Friedewald formula [18]. Non-HDL-C levels were calculated by subtracting HDL-C levels from TC levels. Hemoglobin A1c (HbA1c) was expressed by Japan Diabetes Society (JDS) value.

Hypertension was defined as: systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg, or treatment with antihypertensive agents. DM was defined as: fasting plasma glucose level ≥126 mg/dL [19] or treatment with oral hypoglycemic drugs or insulin injections. Metabolic syndrome was defined using the following modified American Heart Association/National Heart Lung and Blood Institute (AHA/NHLBI) statement [20]. Waist circumference substituted with BMI ≥25 kg/m^2 based on the established Japanese criteria for obesity [21] as abdominal obesity in the AHA/NHLBI definition. BMI has been reported to correspond well to the Asian criterion for waist circumference (≥90 cm in men, ≥80 cm in women) [19].

2.3. Outcomes

Mortality data were collected by serial contact with the patients or from their families through telephone interviews or letters of response to questionnaires sent out every 5 years. Details relating to cause of death were further obtained from the medical records of hospitals or by direct contact with general physicians. Mortality data were ascertained through 2000.

Non-HDL-C levels were not skewed and had an almost normal distribution. The median non-HDL-C level was 180 mg/dL at baseline. To investigate the predictive value of non-HDL-C for long-term mortality, patients were divided into 2 groups, the high and the low non-HDL-C groups, based on the median non-HDL-C level at baseline. To confirm the predictive value of LDL-C for long-term mortality, we also divided the patients into 2 groups, the high and the low LDL-C groups, based on the mean LDL-C level, which was 146 mg/dL at baseline. The main outcome was cardiac death,

including death associated with CHD, cardiogenic shock or cardiac sudden death. The secondary outcome was all-cause death.

2.4. Statistical analysis

In the comparison of characteristics of the patients, categorical data were tabulated as frequencies and percentages and continuous variables were expressed as mean ± standard deviation (SD). The former data were analyzed using the Chi-square tests and the latter were analyzed using Student's t-test.

Survival analyses for 2 groups were constructed using Kaplan–Meier method and compared by the log-rank test. The predictive values of plasma lipid parameters for long-term mortality were determined using Cox proportional-hazard regression analysis. The model was constructed by forward stepwise method and adjusted for various confounding factors. Relative risk (RR) and confidence intervals (CIs) were calculated and p-value < 0.05 was considered significant. All statistical analyses were performed using JMP8.0 MDSU statistical software (SAS Institute, Cary, NC).

3. Results

The comparison of baseline characteristics between the low and the high non-HDL-C groups are shown in Table 1. In the high non-HDL-C group, BMI and the prevalence of metabolic syndrome were significantly higher and use of left internal thoracic artery (LITA) and statin treatment were significantly lower than those in the low non-HDL-C group. In contrast, the prevalence of male, smoker, and hypertension and values of blood pressure, ejection fraction, fasting blood glucose, and HbA1c were not different between 2 groups. Age and the prevalence of DM were even significantly lower in the high non-HDL-C group. In comparison of baseline lipid parameters between 2 groups, the plasma levels of TC, LDL-C, and TG and the LDL-C/HDL-C ratio were significantly higher and the HDL-C levels were significantly lower in the high non-HDL-C group than those in the low non-HDL-C group.

Cumulative survival curves for cardiac death and all-cause death in 2 groups divided by the median non-HDL-C and the mean LDL-C levels at baseline are shown in Figs. 1 and 2. A total of 1074 patients who underwent CABG were followed up for a mean of 10.6 ± 3.5 years. During this observational period, we confirmed that cardiac deaths were 90 (8.4%) and all-cause deaths were 297 (27.7%). The cumulative survival rate for cardiac death in the high non-HDL-C group was significantly lower than that in the low non-HDL-C group (log-rank test; p = 0.006, Fig. 1A). However the cumulative survival rate for cardiac death was not significantly different between 2 groups divided by the mean LDL-C levels (Fig. 1B). Furthermore, the cumulative survival rate for all-cause death was not significantly different between 2 groups divided by mean LDL-C level but also those divided by median non-HDL-C level (Fig. 2A and B).

Fig. 3 shows the predictive value for cardiac death across quintiles of non-HDL-C levels using Cox proportional-hazard regression model. RR adjusted for conventional coronary risk factors including sex, age, current smoker, hypertension and DM as confounders, tended to increase with dose dependent manner across quintiles of non HDL-C levels (p trend = 0.07). In the highest quintile of non-HDL-C levels, RR was significantly higher and almost double of that in the lowest quintile of non-HDL-C levels (p < 0.01).

Table 2 shows the predictive values of plasma lipid parameters for cardiac death using Cox proportional-hazard regression analysis. In Model 1, RR was adjusted for conventional coronary risk factors as confounders and calculated by increasing plasma lipid levels by 10 mg/dL, except for LDL-C/HDL-C ratio. The increased levels of non-HDL-C, TC, TG and LDL-C/HDL-C ratio were significantly correlated with an increased risk of cardiac death [RR (95%CI);

Table 1
Comparison of the baseline characteristics of the patients between low and high non-HDL-C groups.

	Low non-HDL-C	High non-HDL-C	p-value
Number	534	540	
Age (Years)	60 ± 8	59 ± 9	<0.001
Male (%)	82.4	85.4	0.185
BMI (kg/m^2)	23.3 ± 2.6	23.8 ± 2.5	<0.01
SBP (mmHg)	130 ± 17	129 ± 17	0.694
DBP (mmHg)	75 ± 13	76 ± 12	0.081
Smoker (%)	70.2	75.0	0.079
Hypertension (%)	71.0	69.1	0.497
Diabetes (%)	39.5	33.7	<0.05
MetS (%)	39.3	52.4	<0.0001
FH of CHD (%)	30.2	28.2	0.470
EF (%)	63 ± 13	63 ± 14	0.499
Use of LITA (%)	57.5	46.5	<0.001
Statin treatment (%)	10.5	7.0	<0.05
FBG (mg/dL)	107 ± 30	111 ± 36	0.079
HbA1c (%)	5.8 ± 0.9	5.9 ± 0.8	0.058
Non-HDL-C (mg/dL)	149 ± 22	209 ± 20	Designated
TC (mg/dL)	193 ± 25	250 ± 22	<0.0001
LDL-C (mg/dL)	120 ± 23	172 ± 22	<0.0001
HDL-C (mg/dL)	44 ± 14	42 ± 12	<0.01
TG (mg/dL)	150 ± 69	193 ± 99	<0.0001
LDL-C/HDL-C	3.0 ± 1.2	4.4 ± 1.5	<0.0001

Continuous data were expressed as mean ± SD. LDL-C was estimated using the Friedewald formula. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MetS, metabolic syndrome; FH, family history; CHD, coronary heart disease; EF, ejection fraction; LITA, left internal thoracic artery; FBG, fasting blood glucose; HbA1c, Hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

1.09 (1.029–1.161), 1.09 (1.021–1.153), 1.02 (1.002–1.042), 1.14 (1.007–1.282), respectively]. In Model 2, in which all lipid variables were added simultaneously to confounding factors in Model 1, only increased levels of non-HDL-C significantly correlated with increased risks of cardiac death [RR (95%CI); 1.31 (1.124–1.525), $p < 0.001$]. Furthermore in Model 3, we analyzed the predictive values of these lipid parameters by adjusting for metabolic syndrome, statin treatment and use of LITA as confounders in addition
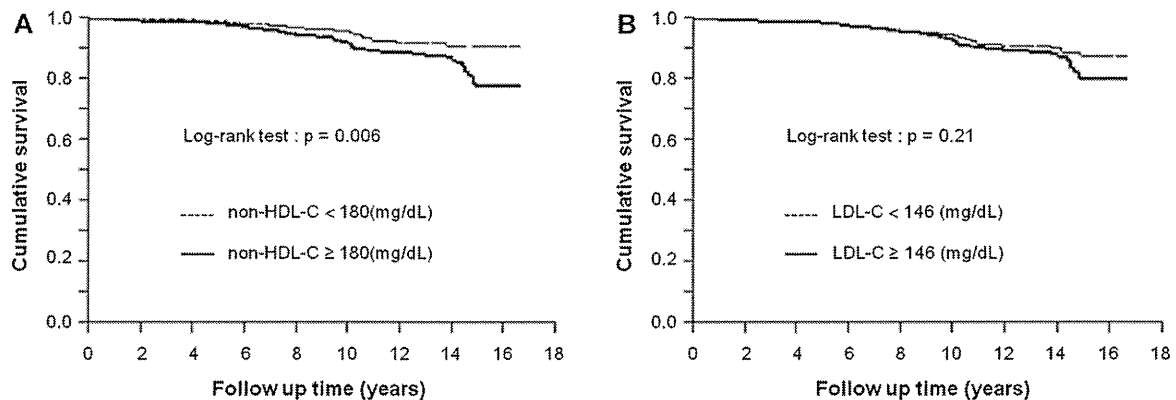

Fig. 1. Cumulative survival curves for cardiac death in patients after CABG. (A) Cumulative survival curves for cardiac death in 2 groups divided by median non-HDL-C level (180 mg/dL). (B) Cumulative survival curves for cardiac death in 2 groups divided by mean LDL-C level (146 mg/dL).
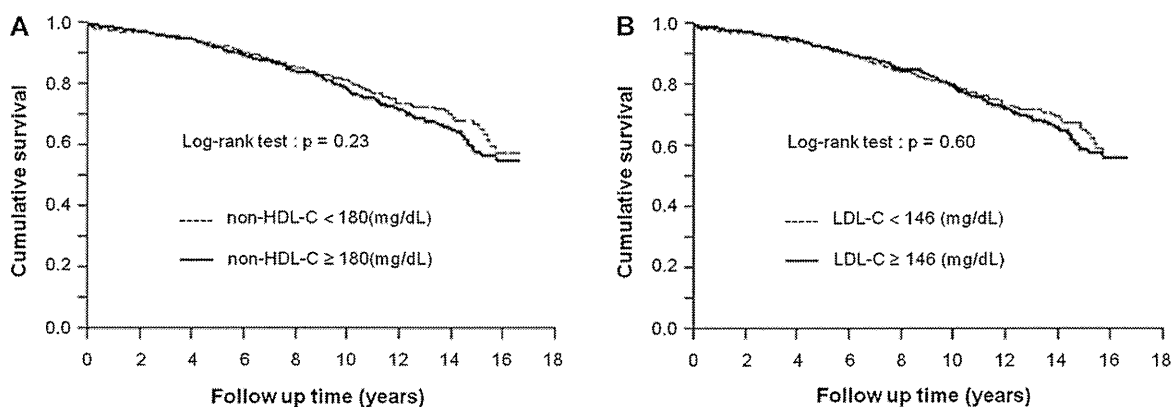

Fig. 2. Cumulative survival curves for all-cause death in patients after CABG. (A) Cumulative survival curves for all-cause death in 2 groups divided by median non-HDL-C level (180 mg/dL). (B) Cumulative survival curves for all-cause death in 2 groups divided by mean LDL-C level (146 mg/dL).

Table 2
Predictive values of plasma lipid parameters for cardiac death in regression analysis.

	Model 1		Model 2		Model 3	
	RR (95% CI)		RR (95% CI)		RR (95% CI)	
Non-HDL-C	1.09	(1.029–1.161)**	1.31	(1.124–1.525)***	1.22	(1.029–1.442)*
TC	1.09	(1.021–1.153)**	1.09	(0.996–1.182)	1.05	(0.964–1.147)
LDL-C	1.06	(0.999–1.120)	1.02	(0.925–1.126)	1.02	(0.923–1.118)
HDL-C	0.93	(0.778–1.106)	0.99	(0.966–1.024)	0.99	(0.813–1.199)
TG	1.02	(1.002–1.042)*	1.02	(0.995–1.049)	1.01	(0.984–1.042)
LDL-C/HDL-C	1.14	(1.007–1.282)*	1.17	(0.866–1.503)	1.17	(0.854–1.489)

Model 1: adjusted for conventional coronary risk factors at baseline as continuous variables using cox proportional hazard models. Model 2: adjusted for all lipid levels in addition to Model 1 at baseline. Model 3: adjusted for metabolic syndrome, using an artery bypass graft, and use of statin in addition to Model 2 at baseline. Conventional coronary risk factors are composed of sex, age, current smoker, hypertension, and diabetes mellitus. RR was calculated by increasing plasma lipid levels by 10 mg/dL, except for LDL-C/HDL-C ratio. RR; relative risk; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

to Model 2. Because these three factors were demonstrated significant difference between the low and high non-HDL-C groups and are also considered to be associated with cardiovascular events ordinarily. Nevertheless, increased levels of non-HDL-C remained a significant and independent predictor of cardiac death [RR (95%CI); 1.22 (1.029–1.442), $p < 0.05$].

4. Discussion

Our study demonstrated that non-HDL-C is a practical and distinct predictor of long-term cardiac death in patients with CHD who underwent CABG. However, LDL-C levels could not predict cardiac death in our study population. Moreover, increased levels of non-HDL-C were significantly associated with the risk of cardiac death in a dose-dependent manner across quintiles of non-HDL-C levels.

Recent studies have demonstrated that the predictive value of non-HDL-C for cardiovascular risk is similar to or better than that of LDL-C [6,7,13–15,22]. Although, the subjects in most studies were no history of CHD individuals as the target of primary prevention for the incidence of CHD, few studies have investigated the predictive value of non-HDL-C for cardiovascular outcomes in CHD patients [23]. To the best of our knowledge, this is the first study to demonstrate an association between long-term cardiovascular

Fig. 3. Comparison of predictive values for cardiac death across quintiles of non-HDL-C levels. RR for cardiac death across quintiles of non-HDL-C levels using Cox proportional-hazard model. The RR was adjusted for conventional coronary risk factors, such as sex, age, current smoker, hypertension and diabetes mellitus.

death and non-HDL-C levels in CHD patients for secondary prevention in Japan. Taken together, our results suggest that non-HDL-C may be a practical and distinct predictor of cardiovascular outcomes and a target for lipid-lowering therapy for both primary and secondary prevention.

Non-HDL-C levels may increase in metabolic syndrome, which result in not only increased TG-rich lipoproteins but also decreased HDL-C mostly through insulin resistance [24,25]. Insulin resistance is a key feature of metabolic syndrome and often progresses to DM. It becomes emerging worldwide problem that the number of metabolic syndrome and DM are increasing rapidly even in Japan. Therefore, compared with LDL-C, non-HDL-C expects to reflect a broad range of dyslipidemia including LDL-C levels and is currently considered to be the more important coronary risk factor. However, it should be noticed that the specificity of non-HDL-C can be weakened for the diagnosis and treatment. Because non-HDL-C incorporates TG-rich lipoproteins, LDL and Lp (a), we need to carefully evaluate which of the atherogenic components increases non-HDL-C levels.

As shown in Table 1, comparison of baseline characteristics between 2 groups divided by median non-HDL-C level demonstrated that the high non-HDL-C group had higher BMI, higher prevalence of metabolic syndrome, higher TC, LDL-C, TG levels, and lower HDL-C levels than the low non-HDL-C group. These results indicated that the high non-HDL-C group had apparently higher coronary risk, so that it seems reasonable that this group demonstrated lower survival rate for cardiac death (Fig. 1A). However, between 2 groups divided by mean level of LDL-C, there were no significant differences in their conventional risk factors and metabolic syndrome (data not shown). Moreover, there was no significant difference in the cumulative survival rate for cardiac death between these 2 groups (Fig. 1B). These discrepant results may be caused in part by the different implication of cholesterol in non-HDL and LDL. This difference could be induced by the exchange of cholesterol in LDL particles to TG in VLDL particles, which can be dynamically transferred and enhanced with increasing a number of TG rich VLDL particles. Thus, in patients with hypertriglyceridemia, LDL-C levels may be decreased by this enhanced exchange and may underestimate the atherogenic risk of the patients. In contrast, non-HDL-C levels are not affected by this exchange and can consistently estimate the atherogenic risk even in hypertriglyceridemia.

Besides high risk of the high non-HDL-C group, the other conventional risk factors, such as age, sex, smoking, hypertension, and DM, were not associated with the risk of the high non-HDL-C groups, as shown in the results of comparison between 2 groups. Focused on the situation of this study enrolling the patients from 1984 to 1994, the rate of statin treatment at baseline were relatively
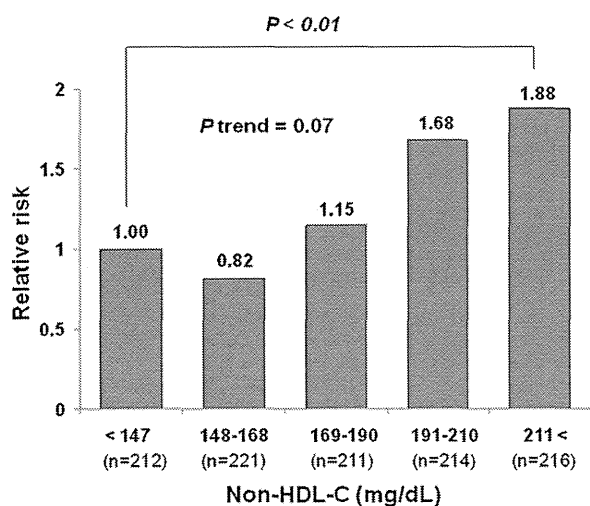
low in 2 groups. In Japan, the reason was considered that statin was released for treatment at clinical practice in 1989 and afterward the prescription rate of statin for the patients with CHD was gradually increased. In the high non-HDL-C group, moreover, the rate of statin treatment was significantly lower than that in the low non-HDL-C group. As a possible cause, it is considered that the patients with high LDL-C levels without statin treatment could be incorporated into the high non-HDL-C group due to the classification by non-HDL-C levels. Finally, in considering these differences of risk factors, we conducted Cox proportional-hazard regression analysis by adjusting for metabolic syndrome, statin treatment, and use of LITA in addition to the conventional risk factors as confounders in Table 2. The results of this analysis demonstrated that increased levels of non-HDL-C were significant and independent predictor of cardiac death.

There has been no clinical trial to prove the beneficial effects of non-HDL-C lowering therapy for preventing CHD. A recent meta-analysis, including both primary and secondary prevention studies, reported that non-HDL-C is an important target for CHD prevention and most lipid-lowering drugs have an almost 1:1 relationship between percent non-HDL-C lowering and CHD risk reduction [26]. The structured lipid-lowering treatments with statin may contribute to decrease not only in LDL-C but also in non-HDL-C by reducing all atherogenic apoB containing lipoprotein particles. Although NCEP ATP III guidelines recommend non-HDL-C levels as a secondary target for lipid-lowering therapy after achieving target levels of LDL-C, the NCEP Evaluation Project Utilizing Novel E-technology II (NEPTUNE II) [27] and the Atorvastatin Cholesterol Efficacy and Safety Study (ACCESS) [28] reported that the frequency of achievement of non-HDL-C goals was much lower than that for LDL-C even with statin treatment. Thus, a more aggressive lipid-lowering therapy is needed to achieve non-HDL-C goals and clinical trials are needed to determine whether non-HDL-C lowering therapy further reduce CHD risk followed by the achievement of LDL-C goals. Future Japan Atherosclerosis Society guidelines for prevention of atherosclerotic cardiovascular diseases will set management target levels of non-HDL-C applying NCEP III guidelines and European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) guidelines [29].

Because this was a retrospective study, it had some limitations. First, data on the progression of each coronary risk factor including onset of metabolic syndrome, DM, hypertension and dyslipidemia were lacking. Second, in comparison with recent pharmacological interventions, the infrequent use of several essential drugs for the prevention of cardiac events, such as angiotensin-converting enzyme inhibitors, angiotensin-receptor II antagonists, β-blockers, and statins was different from the current situation because our findings were based on subjects from 1984 to 1994. Therefore, the incidence rate of cardiac death and all-cause death may not be comparable with those in recent years. This should be investigated further to clarify whether non-HDL-C remains a practical and distinct predictor of CHD events conducting a recent clinical data.

In conclusion, the increased levels of non-HDL-C were significantly associated with an increased risk for cardiac death in this study. Baseline non-HDL-C levels are possible independent predictor of long-term cardiac death in patients with CHD after CABG. Therefore in clinical practice, non-HDL-C may be a target of lipid-lowering therapy for both primary and secondary prevention of CHD. It should be needed to examine whether non-HDL-C lowering therapy decreases further cardiac events in patients with or without CHD.

Conflict of interest

Nothing to declare.

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