References

1) Zilversmit DB: Atherogenesis: a postprandial phenomenon. Circulation, 1979; 60:473-485
2) Groot PH, van Stiphout WA, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofman A, Cresswell SR, Havekes L: Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. Arterioscler Thromb, 1991; 11:653-662
3) Patsch JR, Miesenböck G, Hopferwieser T, Mühlberger V, Knapp E, Dunn JK, Gotto AM, Jr, Patsch W: Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb, 1992; 12:1336-1345
4) Karpe F, Hamsten A: Postprandial lipoprotein metabolism and atherosclerosis. Curr Opin Lipidol, 1995; 6:123-129
5) Davignon J, Cohn JS: Triglycerides: a risk factor for coronary heart disease. Atherosclerosis, 1996; 124(Suppl):S57-S64
6) Yu KC, Cooper AD: Postprandial lipoproteins and atherosclerosis. Front Biosci, 2001; 6:D332-D354
7) Kugiyama K, Doi H, Takazoe K, Kawano H, Soejima H, Mizuno Y, Tsunoda R, Sakamoto T, Nakano T, Nakajima K, Ogawa H, Sugiyama S, Yoshimura M, Yasue H: Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. Circulation; 1999; 99:2858-2860
8) Nakamura T, Takano H, Umetani K, Kawabata K, Obata JE, Kitta Y, Kodama Y, Mende A, Ichigi Y, Fujioka D, Saito Y, Kugiyama K: Remnant lipoproteinemia is a risk factor for endothelial vasomotor dysfunction and coronary artery disease in metabolic syndrome. Atherosclerosis, 2005; 181:321-327
9) Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ: Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. Circulation, 2004; 109:1918-1925
10) Redgrave TG: Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. J Clin Invest, 1970; 49:465-471
11) Imaizumi K, Fainaru M, Havel RJ: Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. J Lipid Res, 1978; 19:712-722
12) Cooper AD: Hepatic uptake of chylomicron remnants. J Lipid Res, 1997; 38:2173-2192
13) Mahley RW, Ji ZS: Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. J Lipid Res, 1999; 40:1-16
14) Sakai N, Uchida Y, Ohashi K, Hibuse T, Saika Y, Tomari Y, Kihara S, Hiraoka H, Nakamura T, Ito S, Yamashita S, Matsuzawa Y: Measurement of fasting serum apo B-48 levels in normolipidemic and hyperlipidemic subjects by ELISA. J Lipid Res, 2003; 44:1256-1262
15) Arai H, Yamamoto A, Matsuzawa Y, Saito Y, Yamada N, Oikawa S, Mabuchi H, Teramoto T, Sasaki J, Nakaya N, Itakura H, Ishikawa Y, Ouchi Y, Horibe H, Shirahashi N, Kita T: Prevalence of metabolic syndrome in the general Japanese population in 2000. J Atheroscler Thromb, 2006; 13:202-208
16) Hirano T, Ito Y, Yoshino G: Measurement of small dense low-density lipoprotein particles. J Atheroscler Thromb, 2005; 12:67-72
17) Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, Adachi M, Tanaka A, Tada N, Nakamura H, Campos E, Havel RJ: Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. Clin Chim Acta, 1993; 223:53-71
18) Miyauchi K, Kayahara N, Ishigami M, Kuwata H, Mori H, Sugiuchi H, Irie T, Tanaka A, Yamashita S, Yamamura T: Development of a homogeneous assay to measure remnant lipoprotein cholesterol. Clin Chem, 2007; 53:2128-2135
19) Sato I, Taniguchi T, Ishikawa Y, Kusuki M, Hayashi F, Mukai M, Kawano S, Kondo S, Yamashita S, Kumagai S: The lipoprotein fraction between VLDL and LDL detected by biphasic agarose gel electrophoresis reflects serum remnant lipoprotein and Lp(a) concentrations. J Atheroscler Thromb, 2006; 13:55-61
20) Kido T, Kurata H, Matsumoto A, Tobiyama R, Musha T, Hayashi K, Tamai S, Utsunomiya K, Tajima N, Fidge N, Itakura H, Kondo K: Lipoprotein analysis using agarose gel electrophoresis and differential staining of lipids. J Atheroscler Thromb, 2001; 8:7-13
21) Sato I, Hyakuta M, Hayashi F, Mukai M, Kondo S, Maeda E, Kumagai S: Usefulness of examination of the cholesterol versus triglyceride ratio for lipoprotein fractions in a patient with marked hyper-triglyceridemia. Rinsho Byori, 2002; 50:987-991
22) Karpe F: Postprandial lipoprotein metabolism and atherosclerosis. J Intern Med, 1999; 246:341-355
23) Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN: Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. J Lipid Res, 2003; 44:2065-2272
24) Mahley RW: Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science, 1988; 240:622-630
25) Fujioka Y, Cooper AD, Fong LG: Multiple processes are involved in the uptake of chylomicron remnants by mouse peritoneal macrophages. J Lipid Res, 1998; 39:2339-2349
26) Yamashita S, Sakai N, Hirano K, Ishigami M, Maruyama T, Nakajima N, Matsuzawa Y: Roles of plasma lipid transfer proteins in reverse cholesterol transport. Front Biosci, 2001; 6:D366-D387
27) Taskinen MR, Smith U: Lipid disorders in NIDDM: implications for treatment. J Intern Med, 1998; 244:361-370
28) Nakada Y, Kurosawa H, Tohyama J, Inoue Y, Ikewaki K: Increased remnant lipoprotein in patients with coronary artery disease--evaluation utilizing a newly developed remnant assay, remnant lipoproteins cholesterol homogenous assay (RemL-C). J Atheroscler Thromb, 2007; 14:56-64
29) Shachter NS: Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. Curr Opin Lipidol, 2001; 12:297-304
30) Westerterp M, de Haan W, Berbée JF, Havekes LM, Rensen PC: Endogenous apoC-I increases hyperlipidemia in apoE-knockout mice by stimulating VLDL production and inhibiting LPL. J Lipid Res, 2006; 47:1203-1211

31) Fojo SS, Brewer HB: Hypertriglyceridaemia due to genetic defects in lipoprotein lipase and apolipoprotein C-II. J Intern Med, 1992; 231:669-677
32) Ogita K, Ai M, Tanaka A, Ito Y, Hirano T, Yoshino G, Shimokado K: Serum concentration of small dense low-density lipoprotein-cholesterol during oral glucose tolerance test and oral fat tolerance test. Clin Chem Acta, 2008; 387:36-41

DOI: 10.1111/j.1365-2362.2009.02163.x

ORIGINAL ARTICLE

Ezetimibe improves postprandial hyperlipidaemia in patients with type IIb hyperlipidaemia

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ABSTRACT

Background Postprandial hyperlipidaemia is known to be a high-risk factor for atherosclerotic disease because of rapid and lasting accumulations of triglyceride-rich lipoproteins and remnants. The Niemann-Pick C1-Like 1 (NPC1L1) protein acts as an intestinal cholesterol transporter and ezetimibe, which inhibits NPC1L1, has been used in patients with hypercholesterolaemia. We investigated effects of ezetimibe on fasting lipid and lipoprotein profiles and postprandial hyperlipidaemia in patients with type IIb hyperlipidaemia.

Materials and methods Ezetimibe 10 mg per day was administered in ten patients with type IIb hyperlipidaemia for 2 months, and lipid and lipoprotein profiles were examined during fasting and after an oral fat loading (OFL) test.

Results In the fasting state, ezetimibe significantly decreased not only total cholesterol, low density lipoprotein (LDL)-cholesterol and apolipoproteinB-100 (apoB-100) levels but triglycerides (TG), apoB-48 and remnant lipoprotein cholesterol (RemL-C) levels. High performance liquid chromatography analysis showed that ezetimibe decreased cholesterol and TG levels in the very low density lipoprotein (VLDL) and LDL size ranges as well as apoB-100 levels, suggesting a decrease in numbers of VLDL and LDL particles. After OFL, ezetimibe decreased the area under the curve for TG, apoB-48 and RemL-C. Ezetimibe decreased postprandial elevations of cholesterol and TG levels in the chylomicrons (CM) size range, suggesting that the postprandial production of CM particles was suppressed by ezetimibe.

Conclusions These findings suggest that ezetimibe improves fasting lipoprotein profiles and postprandial hyperlipidaemia by suppressing intestinal CM production in patients with type IIb hyperlipidaemia and such treatment may prove to be effective in reducing atherosclerosis.

Keywords Apolipoprotein B-48, atherosclerosis, ezetimibe, postprandial hyperlipidaemia, remnants, triglycerides-rich lipoproteins.

Eur J Clin Invest 2009; 39 (8): 689–698

Introduction

Plasma triglycerides (TG) are mainly found in triglyceride-rich lipoproteins (TRL) consisting of chylomicrons (CM) and very low density lipoproteins (VLDL). TRL constitute a population of particles of heterogeneous size, origin and apolipoprotein (apo) and lipid content. CM assemble dietary cholesterol, TG and apoB-48 in enterocytes and VLDL assemble endogenous hepatic TG, cholesterol and apoB-100 in hepatocytes. These lipoprotein particles undergo partial hydrolysis predominantly by lipoprotein lipase (LPL) into smaller and more dense particles know as remnants, which are believed to be more atherogenic than the larger TRL. CM are produced in enterocytes, primarily through the use of exogenous lipid sources and apoB-48 recruitment and are secreted into thoracic lymph, from which they flow into the systemic circulation. LPL hydrolyses CM-TG to free fatty acids (FFA), and residual particles become CM remnants (CM-R) which are taken up by the liver via remnant receptors. VLDL assemble endogenous hepatic TG, cholesterol and apoB-100 in hepatocytes, which are secreted directly into the blood stream. There, LPL hydrolyses VLDL-TG to FFA, and residual particles become VLDL remnants. The liver takes up VLDL remnants and further hydrolysed particles, and the low density lipoproteins (LDL) are taken up via LDL receptors while these particles are supplying energy and lipids to peripheral tissues. In the postprandial state, blood levels of

CM and CM-R quickly rise to reflect the increased exogenous lipid supply. This subsequently activates endogenous lipid synthesis in the liver by increasing the hepatic lipid inflow, leading to augmented hepatic VLDL production. Postprandial hypertriglyceridaemia is caused by overproduction and/or impaired clearance of TRL and TRL remnants, leading to rapid accumulation and sustained blood levels after dietary intake. Both fasting and postprandial hypertriglyceridaemia are known to be risk factors for coronary heart disease [1,2].

Recently Niemann-Pick C1 Like 1 (NPC1L1) protein has been reported to play a central role in cholesterol absorption in enterocytes [3,4]. Genetic inactivation of NPC1L1 protein decreases cholesterol levels and atherosclerotic lesions in hyperlipidaemic apoE knockout mice fed a western diet [5,6]. Ezetimibe, a novel lipid-lowering compound, selectively inhibits intestinal cholesterol absorption by binding to NPC1L1 protein, reducing total cholesterol (TC) and TG levels and also reducing the development of atherosclerosis in apoE knockout mice [7,8]. Clinically, it has already been shown that administration of ezetimibe diminished fasting levels of total and LDL-cholesterol in patients with primary hypercholesterolemia in Japan and the United States [9,10]. Due to the nature of its medicinal properties, the investigation into the pharmacological effects of ezetimibe has focused primarily on the metabolism of sterols, including cholesterol, rather than on TG or TRL. However, ezetimibe has been reported to decrease fasting TG levels significantly in patients with combined hyperlipidaemia [10], and its underlying mechanism of action has not yet been elucidated. As fasting and postprandial TG levels are closely related, it is essential to understand the effects of ezetimibe in combined hyperlipidaemic patients with reference to postprandial TRL and remnant metabolism. In this study, we administered ezetimibe 10 mg day^{-1} orally to 10 patients with type IIb hyperlipidaemia who have both hypercholesterolaemia and hypertriglyceridaemia, and used oral fat loading (OFL) tests to evaluate changes in fasting and postprandial lipid and lipoprotein profiles.

Materials and methods

Subjects

Ten Japanese patients (two female, eight male) were enrolled in this study. All patients had been diagnosed with type IIb hyperlipidaemia according to the Japanese criteria (fasting TC level ≥220 mg dL^{-1} and fasting TG level ≥150 mg dL^{-1}). Ezetimibe (Bayer Yakuhin Ltd. (Tokyo, Japan) and Schering-Plough K.K. (Tokyo, Japan)) 10 mg was administered once daily to all patients for 2 months. None of the patients took any other drugs that might affect lipid or lipoprotein metabolism. Every medication other than ezetimibe was continued unchanged throughout the study period. Total calorie intake and composition of the diet were kept constant for each patient. All subjects gave written informed consent before participating in this study, and the ethics committee of the Osaka University Hospital approved the study design.

Measurement of serum samples

Fasting blood samples were drawn from each of the 10 enrolled patients before the start and after the conclusion of ezetimibe administration. Serum was separated by low-speed centrifugation (1200 g, 15 min, at 4°C) and stored at 4°C until measurement within a week. All samples were treated in accordance with the Helsinki Declaration. Concentrations of TC, TG and FFA were measured using the enzymatic method. Concentrations of LDL-cholesterol (LDL-C) and high density lipoprotein cholesterol were measured using the direct method. Concentrations of apoAI, AII, B, CII, CIII, and E were measured using the immunoturbidity method. Concentrations of high sensitivity C-reactive protein were measured using the immunonephelometric assay (Sekisui Medical Co., Ltd., Tokyo, Japan). Haemoglobin A1c levels were measured using high performance liquid chromatography (HPLC) method. Fasting plasma glucose levels were measured using a hexokinase UV method. Concentrations of fasting plasma insulin were measured using a chemiluminescent enzyme immunoassay (CLEIA) method (SRL Inc., Tokyo, Japan). HOMA-IR (homeostasis model assessment of insulin resistance) index was calculated as [fasting plasma insulin (μIU mL^{-1}) × fasting plasma glucose (mg dL^{-1})]/405. Concentrations of apoB-48 were measured using a sandwich CLEIA (Fuji Rebio Inc., Tokyo, Japan) [11]. Remnant lipoprotein cholesterol (RemL-C) levels were measured using a RemL-C homogenous assay, RemL-C (Kyowa Medex, Tokyo, Japan), which enabled separation of CM-R and VLDL remnants from other lipoproteins with higher specificity than the remnant like particle-cholesterol method [12,13]. Before ezetimibe administration, RemL-C and apoB-48 levels were higher in enrolled patients than in normolipidaemic subjects, in conjunction with higher levels of TC, TG, apoB and LDL-C (patients vs. normolipidemic subjects shown in the previous studies: RemL-C 18·7 ± 10·5 vs. 3·5 ± 1·2 mg dL^{-1} in [13]; apoB-48 6·8 ± 4·3 vs. 5·2 ± 3·8 μg mL^{-1} in [11]).

Oral fat loading test

The OFL test was performed before and after the administration of ezetimibe. After an overnight fast for 12 h, oral fat tolerance test (OFTT) cream which was prepared from milk and adjusted to contain 35% fat without sugar (JOMO Foods, Gunma, Japan) was loaded to each patient sufficient to provid a fat load of 30 g fat m^{-2} body surface area. Blood samples were drawn before and 1, 2, 3, 4, 6 and 8 h after OFL and concentrations of TC, TG, apo B-48, FFA, RemL-C and apoB-100 were measured. To compare the net postprandial change in

these parameters, areas under the curve (AUC) for TC, TG, apo B-48, FFA, RemL-C and apoB-100 were calculated using the trapezoidal method and incremental AUC (ΔAUC) values by ignoring area beneath the fasting level.

Lipoprotein profiles assessed by HPLC

The effect of ezetimibe on lipoprotein profile during fasting and 4 h after OFL was evaluated using the HPLC method. Samples of 200 microlitres of serum (fasting state and 4 h after OFL before and after administration of ezetimibe) were analysed at Skylight Biotech Inc. (Akita, Japan) and dissolved with the loading buffer (0·05 mol L^{-1} Tris-buffered acetate, pH 8·0). These samples were loaded into two tandem connected TSK-gel Lipopropak XL columns and concentrations of TC and TG in the flow-through of each sample were measured continuously and simultaneously [14]. The flow-through of dissolved serum (n = 10) which was drawn 4 h after OFL was collected serially every 1 min into collection tubes (tube No. 1-20) both before and after administration of ezetimibe. The apoB-48 levels of tube No. 1-11 which were supposed to contain lipoproteins in the size range of CM (tube No.1-2), VLDL (tube No. 3-7) and LDL (tube No. 8-10) were measured using the method as mentioned above. The beginning and ending time of the collection of the flow-through was shown in the chromatographic pattern using grey bars in Fig. 3a. We calculated cholesterol and TG concentrations of lipoprotein fractions in the size categories of CM, VLDL, LDL and HDL, based on findings from a prior investigation that confirmed the correspondence of lipoprotein fractions in CM, VLDL, LDL, and HDL-sizes and the elution time, by comparing the HPLC pattern of each lipoprotein separated using ultracentrifugation [15]. Those categories were as follows: CM-size, estimated particle size > 80 nm, elution time 15–17 min; VLDL size 30–80 nm, 17–22 min; LDL size, 16–30 nm, 22–25·5 min; HDL-size, 8–16 nm, 25·5–28·5 min.

Statistical analyses

The results were expressed as mean ± SD. The Student's paired t-test was used for pairwise comparisons between values before and after administration of ezetimibe. A value of $P < 0·05$ was considered to be statistically significant.

Results

Effect of ezetimibe on fasting serum levels of lipid biomarkers in patients with type IIb hyperlipidaemia

Table 1 shows fasting serum levels of lipid biomarkers before and after administration of ezetimibe for 2 months. Ezetimibe effectively reduced serum levels of TC, TG, apoB and LDL-C in the fasting state as we expected. LDL-C reducing response varied between 9·8% (reducing from 151 to 136 mg dL^{-1}) and 33·2% (from 152 to 101 mg dL^{-1}). However, the mean rate of reduction in TG was larger than previously reported for ezetimibe treatment in patients with primary hypercholesterolaemia (mean reduction rates: TC -16·5%, TG -24·5%, apoB -15·7, LDL-C -20·3%). It is especially striking that fasting levels of apoB-48, and RemL-C were also significantly decreased after the administration of ezetimibe (mean reduction rates: RemL-C -22%, apoB-48 -31%) in type IIb hyperlipidaemic patients. These results suggest that ezetimibe may affect not only VLDL and LDL particles containing apoB-100, but also CM and CM-R particles containing apoB-48. There was no difference in body weight and waist circumference through the treatment. Ezetimibe treatment did not alter serum levels of HDL cholesterol, apoAI, apoAII, apoCIII, apoE, FFA and diabetic parameters, fasting plasma glucose, plasma insulin or haemoglobin A1c

Table 1 Fasting levels of lipid biomarkers before and after administration of ezetimibe

		Ezetimibe(−)	Ezetimibe(+)	*P*-value
TC	(mg dL^{-1})	231 ± 43	194 ± 26	0·001
TG	(mg dL^{-1})	218 ± 83	178 ± 85	0·031
LDL-C	(mg dL^{-1})	145 ± 42	120 ± 25	0·005
HDL-C	(mg dL^{-1})	53 ± 14	52 ± 13	0·394
FFA	(μEq L^{-1})	508 ± 187	483 ± 184	0·270
RemL-C	(mg dL^{-1})	18·7 ± 10·5	12·0 ± 6·3	0·006
apoAI	(mg dL^{-1})	144 ± 29	142 ± 31	0·130
apoAII	(mg dL^{-1})	32·2 ± 8·0	30·8 ± 7·6	0·071
apoB-100	(mg dL^{-1})	116 ± 22	101 ± 13	0·004
apoB-48	(μg mL^{-1})	6·8 ± 4·3	4·7 ± 2·3	0·019
apoCII	(mg dL^{-1})	5·3 ± 2·8	4·3 ± 2·1	0·043
apoCIII	(mg dL^{-1})	11·7 ± 4·3	10·5 ± 3·8	0·082
apoE	(mg dL^{-1})	6·2 ± 1·3	5·6 ± 1·4	0·054
Glucose	(mg dL^{-1})	107 ± 21	104 ± 19	0·165
Insulin	(μIU mL^{-1})	12·1 ± 5·5	14·5 ± 5·5	0·231
HOMA-IR		3·2 ± 1·6	3·7 ± 3·6	0·165
HbA1c	(%)	5·6 ± 0·4	5·5 ± 0·4	0·165
hs-CRP	mg dL^{-1}	0·11 ± 0·08	0·16 ± 0·15	0·17

TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; FFA, free fatty acid; RemL-C; remnant lipoprotein cholesterol; apo, apolipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, haemoglobin A1c; hs-CRP; high sensitivity C reactive protein.
HOMA-IR index was calculated as [fasting plasma insulin (μ IU mL^{-1}) × fasting plasma glucose (mg dL^{-1})]/405.
Data were shown as mean ± SD and statistical significance was calculated using paired t-test.

levels as well as HOMA-IR index (Table 1). In this study, there was no significant change in the levels of hs-CRP which is an independent marker for the development of atherosclerotic cardiovascular diseases by ezetimibe treatment.

Effect of ezetimibe on fasting lipoprotein profiles in patients with type IIb hyperlipidaemia

To evaluate the effect of ezetimibe on fasting lipoprotein profiles, serum samples were analysed by HPLC, and cholesterol and TG levels were measured. Representative chromatographic patterns of cholesterol and TG before and after ezetimibe treatment are shown in Fig. 1a. For each patient cholesterol and TG levels in the indicated pooled fractions corresponding to CM-, VLDL-, LDL- or HDL-sized particles were summed and averages were calculated. The levels of cholesterol and TG decreased in the VLDL and LDL fractions after ezetimibe treatment, and the LDL peak in cholesterol tended to shift slightly to the left (lower elution time, greater apparent size), which may represent large LDL particles (before vs. after administration of ezetimibe: VLDL-C 46 ± 13 vs. 32 ± 12 mg dL^{-1}, $P = 0{\cdot}0016$; LDL-C 150 ± 33 vs. 120 ± 27, $P = 0{\cdot}0018$; VLDL-TG 176 ± 67 vs. 116 ± 54, $P = 0{\cdot}0027$; LDL-TG 49 ± 12 vs. 41 ± 7, $P = 0{\cdot}034$). However, this shift was not observed in all specimens. Findings for cholesterol and TG content in CM- and HDL-size particles after ezetimibe treatment were similar to the treatment baseline.

OFL test before and after ezetimibe administration

As shown in Fig. 2, postprandial changes in lipid profiles were determined by OFL testing with OFTT cream before and after administration of ezetimibe in 10 patients with type IIb hyperlipidaemia. Initial values for serum TC and apoB-100 after

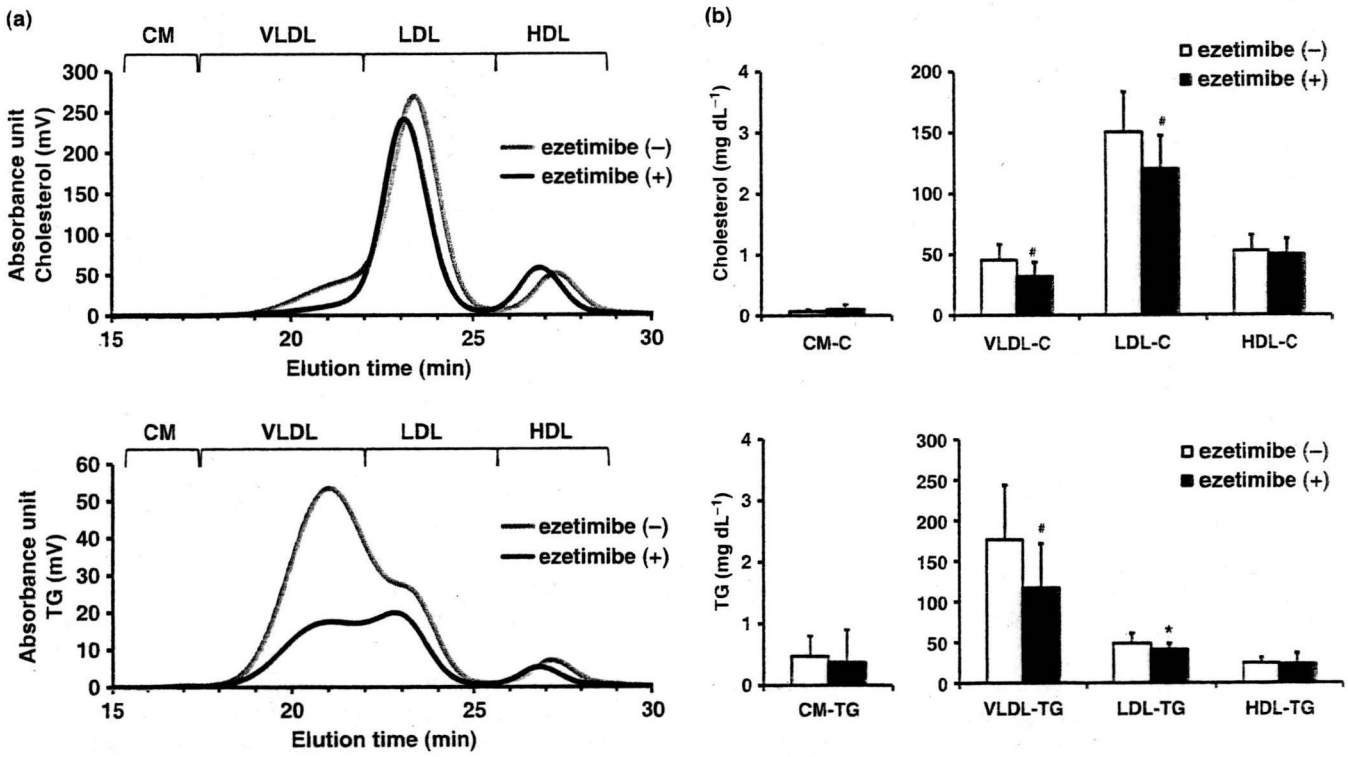

Figure 1 Lipoprotein profiles in the fasting state before and after administration of ezetimibe. Ezetimibe 10 mg was administered in patients with type IIb hyperlipidaemia ($n = 10$, two females and eight males) for 2 months. Two hundred microlitres of serum were separated from blood drawn in the fasting state before and after administration of ezetimibe. Lipoprotein profiles were analysed by high performance liquid chromatography. The concentrations of cholesterol and triglyceride (TG) in the flow-through of each sample were measured continuously and simultaneously. (a) Representative chromatograms of cholesterol and TG of fasting serum before (grey line) and after (black line) administration of ezetimibe were shown with approximate elution times of chylomicrons (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). (b) For each patient cholesterol and TG levels in the indicated pooled fractions corresponding to CM-, VLDL-, LDL- or HDL-size particles were summed and averages were calculated before (open squares) and after (closed squares) administration of ezetimibe. $*P < 0{\cdot}05$, $\#P < 0{\cdot}005$.

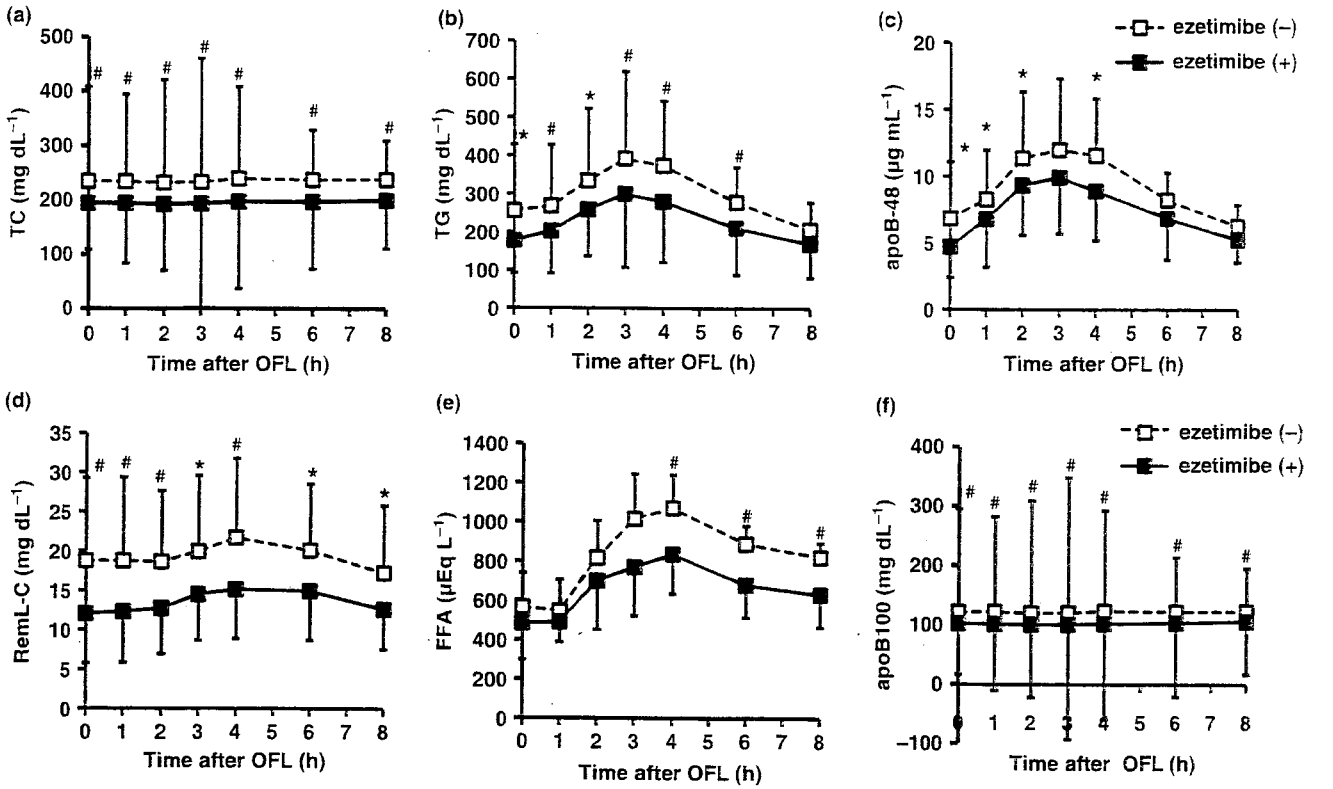

Figure 2 Oral fat loading (OFL) test before and after administration of ezetimibe. Patients with type IIb hyperlipidaemia (n = 10, two females and eight males) were given OFTT cream (containing 35% fat without sugar, 30 g fat m^{-2} body surface area) after overnight fasting before (open squares) and after (closed squares) administration of ezetimibe. Blood samples were drawn during fasting and 1, 2, 3, 4, 6 and 8 h after OFL, and serum and plasma were separated immediately. Concentrations of (a) total cholesterol (TC), (b) triglyceride (TG), (c) apolipoprotein B-48(apoB-48), (d) remnant lipoprotein cholesterol (RemL-C), (e) free fatty acids (FFA) and (f) apoB-100 were measured as described in Materials and methods. *P < 0·05, #P < 0·01.

ezetimibe treatment were significantly lower than before the treatment. Serum TC and apoB-100 levels remained constant throughout the 8-h OFL test. TG, apoB-48 and RemL-C levels rose for the first 3 or 4 h, and returned to fasting levels 8 h after OFL. Ezetimibe significantly diminished fasting and peak levels for these parameters and for AUC, which reflects the postprandial integrated response (AUC-TC 1892 ± 350 vs. 1570 ± 204 mg dL^{-1} 8 h, P = 0·0001; AUC-apoB-100 2167 ± 649 vs. 1519 ± 488 mg dL^{-1} 8 h, P = 0·023; AUC-TG 2448 ± 1130 vs. 1863 ± 1012 mg dL^{-1} 8 h, P = 0·003; AUC-apoB-48 75 ± 23 vs. 61 ± 22 μg dL^{-1} 8 h, P = 0·044; AUC-RemL-C 156 ± 72 vs. 110 ± 46 mg dL^{-1} 8 h, P = 0·008). However, incremental AUCs (ΔAUCs), which are thought to describe postprandial integrated response more accurately, after ezetimibe administration were comparable to the corresponding values before ezetimibe administration for TG, apoB-48 and RemL-C (ΔAUC-TC 11 ± 98 vs. 15 ± 61 mg dL^{-1} 8 h, P = 0·448; ΔAUC-apoB-100 483 ± 334 vs. 236 ± 318 mg dL^{-1} 8 h, P = 0·168; ΔAUC-TG 405 ± 442 vs. 443 ± 553 mg dL^{-1} 8 h, P = 0·442; ΔAUC-apoB-48 21 ± 33 vs. 23 ± 17 μg dL^{-1} 8 h, P = 0·394; ΔAUC-RemL-C 6·5 ± 22 vs. 14 ± 14 mg dL^{-1} 8 h, P = 0·432). Ezetimibe intervention reduced peak level, AUC and ΔAUC for FFA after OFL (AUC-FFA 6856 ± 1362 vs. 5433 ± 1231 mg dL^{-1} 8 h, P = 0·004; ΔAUC-FFA, 2329 ± 1159 vs. 1564 ± 1249 mg dL^{-1} 8 h, P = 0·017), indicating a possible decrease in FFA production and/or increase in FFA clearance. There were no changes in serum levels for other apolipoproteins (apoAI, AII, CII, CIII, and E) throughout the OFL test, either before or after ezetimibe treatment (data not shown).

HPLC analysis of postprandial lipoprotein profiles

To further elaborate on postprandial lipid changes, HPLC analysis was conducted 4 h after the OFL test to compare cholesterol and TG concentrations of lipoprotein fractions in the CM, VLDL, LDL and HDL-size ranges before and after administration of ezetimibe. Chromatographic patterns of

serum 4 h after OFL revealed that three peaks were observed in the size range of CM, VLDL and LDL by the detection of cholesterol and TG levels. The VLDL peak by the detection of TG after OFL was shifted to the left (lower elution time, greater apparent size) compared with that in the fasting state, suggesting that any other lipoprotein particles which were contained in the size range of VLDL and larger than VLDL observed in the fasting state were produced after OFL. HPLC analysis of serum which was obtained 4 h after OFL before and after ezetimibe treatment showed that three peaks by the detection of TG in the size range of CM, VLDL and LDL tended to decrease after ezetimibe treatment (Fig. 3a). By the calculation of average cholesterol and TG levels in the size range of CM, VLDL, LDL and HDL, HPLC analysis 4 h after OFL revealed that the reduction in serum TC and TG after ezetimibe treatment was mainly due to cholesterol and TG changes in the size range of CM and VLDL, not due to those in the size range of LDL (CM-C 0·63 ± 0·26 vs. 0·31 ± 0·09 mg dL^{-1}, P = 0·0029; VLDL-C 50 ± 14 vs. 37 ± 11 mg dL^{-1}, P = 0·0022; LDL-C 138 ± 41 vs. 116 ± 2 mg dL^{-1}, P = 0·059; CM-TG 10·2 ± 5·4 vs. 4·7 ± 2·2 mg dL^{-1}, P = 0·014; VLDL-TG 251 ± 93 vs. 180 ± 88 mg dL^{-1}, P = 0·0009, LDL-TG 50 ± 13 vs. 43 ± 8 mg dL^{-1}, P = 0·056) (Fig. 3b). Furthermore, to evaluate whether CM-R were contained in the size range of VLDL and LDL 4 h after OFL and their contents were changed before and after ezetimibe treatment, we measured apoB-48 levels of serially collected flow-through of dissolved serum (n = 10) which was drawn 4 h after OFL, as shown in Materials and methods (Fig. 3a). Both before and after ezetimibe treatment, apoB-48 was detected in the fractioned flow-through which was suggested to contain lipoproteins in the size range of not only CM but also VLDL and LDL (Fig. 1a). Before ezetimibe treatment, we can see two peaks of apoB-48 levels at the position of tube No. 5 and No. 8, which was coincided with peaks by the detection of TG in the size range of VLDL and LDL 4 after OFL. These findings suggested that CM-R particles existed in various size ranges, from the size of CM to HDL, and the peak of the size of CM-R particles existed both in the size range of VLDL and LDL. After ezetimibe treatment, apoB-48 levels were decreased in all size ranges and the peak of apoB-48 levels in the size range of VLDL had disappeared. However, the decreases in apoB-48 levels by ezetimibe treatment were significant in tube No.1, No. 7 and No. 8, but not significant in other tubes. (No.1:before vs after treatment, 0·012 ± 0·008 vs. 0·003 ± 0·001 μg dL^{-1}, P = 0·020, No. 7; 0·031 ± 0·020 vs. 0·013 ± 0·003 μg dL^{-1}, P = 0·043, No.8; 0·044 ± 0·018 vs. 0·018 ± 0·006 μg dL^{-1}, P = 0·021). These results suggested that the decreases in particle numbers of CM and CM-R by the ezetimibe treatment occurred significantly in the size range of CM and small VLDL, but relatively in the size range of large VLDL particles. To address whether suppression of lipoprotein production resulted in any reduction of TG and cholesterol in the size range of CM and VLDL particles after ezetimibe administration, we calculated differences in cholesterol and TG levels in the size range of CM and VLDL particles between fasting and 4-h OFL, and compared these differences before and after ezetimibe treatment (Fig. 3c). Ezetimibe attenuated the increase in cholesterol level at the CM-size (0·56 ± 0·25 vs. 0·21 ± 0·11 mg dL^{-1}, P = 0·0008), which might reflect the inhibition of cholesterol absorption in the intestine in accordance with the mechanism of action of ezetimibe. In a particularly striking finding, the increase in CM-size TG was also attenuated after the administration of ezetimibe (9·7 ± 5·4 vs. 4·4 ± 2·3 mg dL^{-1}, P = 0·017) (Fig. 3c) along with the significant decrease in apoB-48 level in the size range of CM 4 h after OFL (Fig. 3a), which raised the possibility that the decreased intestinal cholesterol absorption associated with ezetimibe administration might also influence the intestinal production of CM. There were no significant differences before and after ezetimibe treatment in increased VLDL particle size levels for cholesterol or TG between fasting and 4-h OFL, even though ezetimibe decreased the fasting and postprandial (4 h after initiating OFL test) TG levels for the VLDL size range (Figs 1 and 3). These findings suggest that VLDL metabolism, at least during the 4-h OFL test, was unaffected by ezetimibe treatment.

Discussion

In this study, we elucidated the fasting and postprandial lipid and lipoprotein profiles of patients with type IIb hyperlipidaemia before and after ezetimibe administration. We clearly showed that ezetimibe treatment decreased the fasting apoB-48 and RemL-C levels as well as TC, TG, and apoB-100 levels. When we subtracted apoB-48 levels from the apoB levels, the resulting values also showed decreases in apoB-100 after ezetimibe administration. HPLC analysis showed reduced levels of cholesterol and TG in VLDL and LDL fractions at fasting after ezetimibe administration, suggesting that the levels of apoB-100-containing lipoproteins such as VLDL, VLDL remnants and LDL particles were reduced in conjunction with decreased serum apoB-100 levels. Telford *et al.* demonstrated, in a study of miniature pigs, that ezetimibe decreased the intrahepatic cholesterol pool through inhibition of intestinal cholesterol absorption, leading to the suppression of hepatic VLDL production and enhanced LDL clearance by upregulation of LDL receptor expression in hepatocytes [16]. As a consequence, serum levels of apoB-100-containing lipoproteins were reportedly reduced after ezetimibe administration in that experiment. Unlike rodents, humans express high levels of NPC1L1 protein in the liver as well as in the intestine. A study using liver-specific NPC1L1 transgenic mice has indicated that the function of liver NPC1L1 was to take up cholesterol from

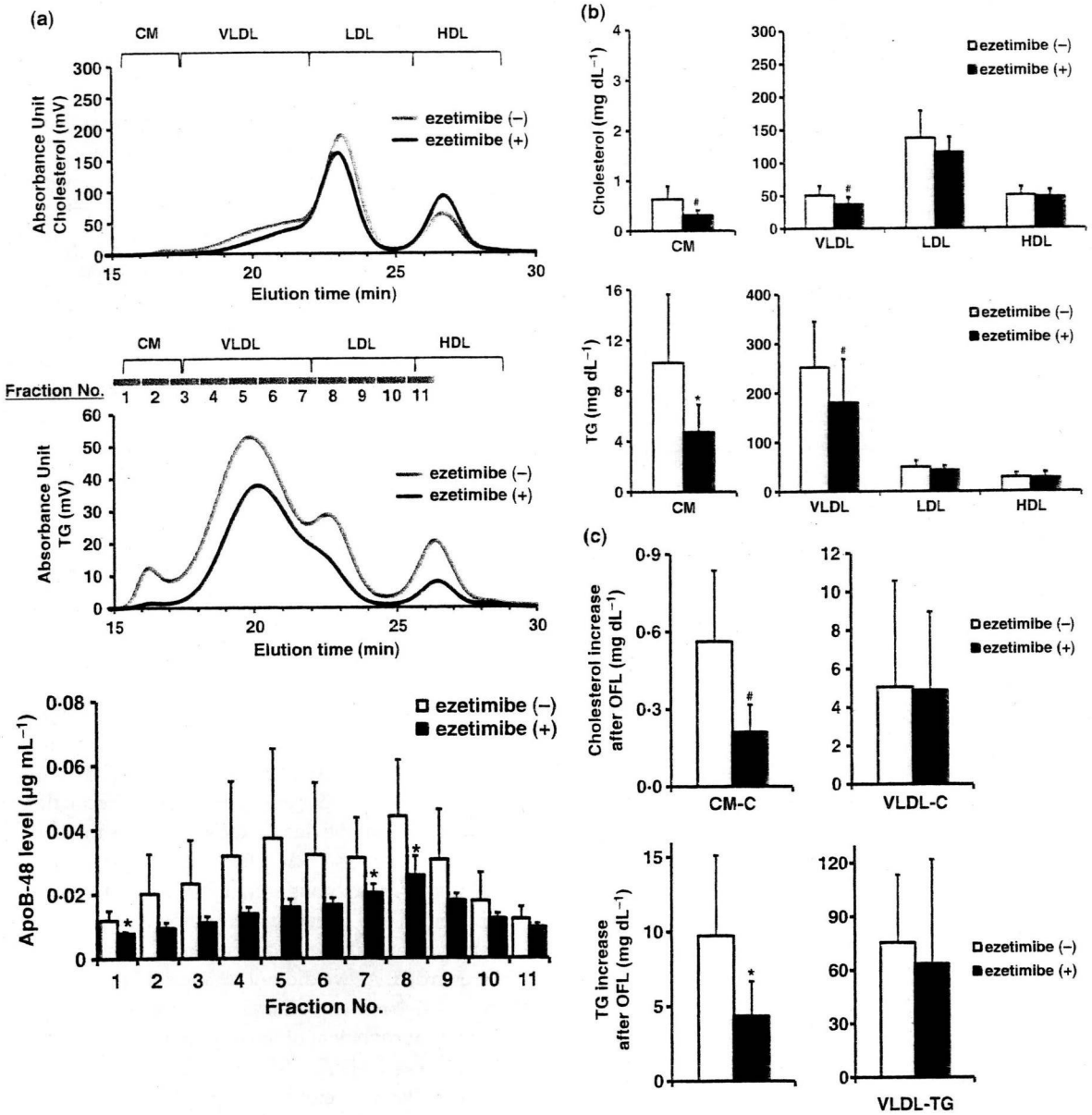

Figure 3 Lipoprotein profiles in postprandial state and incremental fasting/postprandial serum cholesterol and triglyceride (TG) levels before and after administration of ezetimibe. Two hundred microlitres of serum were separated from blood samples drawn 4 h after oral fat loading (OFL) before (open squares) and after (closed squares) administration of ezetimibe for 2 months in patients with type IIb hyperlipidaemia (n = 10, two females and eight males). (a) Representative chromatograms of cholesterol and TG of serum 4 h after the OFL before (grey line) and after (black line) administration of ezetimibe are shown with approximate elution times of chylomicrons (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). The flow-through of dissolved serum was collected serially every 1 min into collection tubes (tube No. 1-20), apoB-48 levels of tube No. 1-11 which were supposed to contain lipoprotein in the size range of CM, VLDL and LDL were measured using a chemiluminescent enzyme immunoassay method. Grey bars indicate the beginning and ending time of the collection of the flow-through. (b) For each patient (n = 10), cholesterol and TG concentrations of lipoprotein fractions in the size range of CM, VLDL, LDL and HDL were calculated before (open squares) and after (closed squares) administration of ezetimibe. (c) Incremental serum cholesterol and TG levels in the indicated pooled fractions corresponding to CM- or VLDL size particles between fasting and postprandial (4 h after OFL) states were calculated before (open squares) and after (closed squares) ezetimibe treatment. $^*P < 0{\cdot}05$, $\#P < 0{\cdot}005$.

bile acids and return it to the liver, and that hepatic NPC1L1 was also targeted by ezetimibe [17]. Inhibition of hepatic NPC1L1 by ezetimibe would result in attenuation of the hepatic cholesterol pool caused by a relative increase of cholesterol secretion into bile acids. It would be appropriate to contextualize these reports, at least partially, by explaining the mechanism that we detected, whereby apoB-100-containing lipoprotein levels were diminished at fasting after ezetimibe treatment. ApoB-48 incorporated into CM and CM-R was also reduced at fasting, whereas cholesterol and TG contents in the CM fraction were unaltered on HPLC analysis. In our previous study, we reported that apoB-48 protein was also detected by western blotting, in the flow-through analytes for elution time between 19 and 22 min which overlapped the VLDL fraction. In this study, we were able to detect apoB-48 in these subfractions by a CLEIA method. Those findings proved that the lipoprotein fraction in the VLDL size range contained CM-R as well [18] in the HPLC system that we used. This can explain our findings of apoB-48 reduction with no alteration of cholesterol or TG content in the CM-size fraction, and taken together with the findings from this study, demonstrates that ezetimibe treatment decreased the level of fasting CM-R. Similar to the ezetimibe-induced modification of the metabolism of apoB-100-containing lipoproteins, the inhibition of cholesterol inflow into the liver might cause upregulation of remnant receptors, which would improve clearance of CM-R and reduce serum CM-R levels. As the fasting levels of CM-size particles remained unchanged after ezetimibe treatment, there are some remaining issues to be addressed regarding whether ezetimibe may facilitate LPL activity, although we did not measure LPL activity in this study.

These discoveries are relevant to the alteration of fasting TRL serum levels, and imply that ezetimibe might modify postprandial TRL metabolism as well, as sustained accumulation of TRL particles in the blood after a meal induces high fasting levels for TRL. Our results from the OFL test conducted in patients with type IIb hyperlipidaemia supported this hypothesis. It is well documented that TC, apoB-100 and LDL-cholesterol levels are unaffected by the OFL test under normal conditions. Consistent with the previous observation, serum TC and apoB-100 levels showed constant values throughout the 8-h OFL test both before and after ezetimibe treatment, although initial values for serum TC after administration were significantly lower than before administration, reflecting decreased fasting TC and apoB-100 levels. Ezetimibe intervention significantly diminished fasting and peak levels for TG, RemL-C and apoB-48, and those respective AUC values in the OFL test, whereas the corresponding ΔAUCs were comparable to those values before ezetimibe treatment. These findings suggest that very few additional effects other than reduction of initial levels were observed in this experiment. However, further detailed examination of lipoprotein profiles by HPLC, performed 4 h after the OFL test, revealed striking evidence that ezetimibe did incrementally attenuate both cholesterol and TG levels with regard to the size of CM but not the size of VLDL particles. Especially, we measured the apoB-48 levels of serially collected flow-through of dissolved serum which was drawn 4 h after OFL and evaluated changes of CM-R particles by ezetimibe treatment in the size range of CM, VLDL and LDL (Fig. 3a). As a result, CM-R particles existed in various size ranges, from the size of CM to HDL and their peak existed both in the size range of VLDL and LDL. After ezetimibe treatment, apoB-48 levels were decreased in all size ranges and the peak of apoB-48 levels in the size range of VLDL disappeared. However, the decreases in apoB-48 levels by ezetimibe treatment were significant only in the size range of CM and small VLDL, but not in the size range of large VLDL (Fig. 3a). These results suggested that the decreases in particle numbers of CM and CM-R by the ezetimibe treatment occurred significantly in the size range of CM and small VLDL, but relatively in the size range of large VLDL particles. It can be speculated that, because both production of CM and catabolism of CM and CM-R may be accelerated with ezetimibe treatment, the reduction of CM was apparent based upon the reduction of apoB-48 levels in CM-size range; however, the reduction of CM-R in the size range of VLDL was not apparent. This suggests the possibility that intestinal CM production was reduced significantly and CM-R which were in the size range of VLDL and LDL were relatively decreased, but hepatic VLDL production was unaffected by ezetimibe during the 4-h OFL as there was no increase in apoB-100 levels by the OFL both with and without ezetimibe treatment. These changes in lipoprotein profiles were substantial, and the effect of ezetimibe on postprandial TLR metabolism could be underestimated if those changes were disregarded. As we did not measure LPL activity or compounds like retinyl palmitate, we could not deny the possibility for the improvement of the impaired catabolism of CM and CM-R.

The only parameter showing reduced ΔAUC after ezetimibe administration was FFA. Recently Labonte ED *et al.* reported that ezetimibe-treated mice absorbed only 86·9% of the fat from a high-fat, high-sucrose diet compared with 94·9% of fat absorption in untreated mice [19]. Our loading fat, OFTT cream, contains 35% fat and has a main fatty acid composition of C16:0, C18:1 and C14:0. According to the Labonte experiments, absorption of palmitate, oleate and myristate was decreased from 89·0, 95·9 and 93·5% in the controls to 79·2, 91·2, and 87·7% respectively in ezetimibe-treated mice. In addition, there was a 50% reduction in expression of FATP4 protein in intestinal preparations from ezetimibe-treated mice in comparison with the control mice and a 35% reduction in CD36 protein expression. Both of these proteins are considered to play

important roles in FFA transport. These observations might apply under our experimental conditions as well, although mice received chronic exposure to a high-fat and high-sucrose diet and FFA measurements were fasting values. Once FFA are absorbed by the enterocytes, it is used for the resynthesis of TG, along with monoacylglycerols that are believed to be absorbed by passive diffusion from the gut lumen. TG is incorporated into CM and released into the thoracic lymph, a process that involves many molecules related to the assembly and secretion of CM. Next, CM passes into the bloodstream and is exposed to LPL, resulting in the discharge of FFA from CM to serum. We could speculate that decreased FFA absorption after ezetimibe treatment in acute fat loading led to the reduction in ΔAUC for FFA. FFA are also taken up in the adipose tissue for energy storage and in striated muscles for combustion. This might be less likely to play a role in the ezetimibe-induced reduction of postprandial ΔAUC for FFA, as there were no changes in patient body weight or waist circumference during the 2 months of the study and as fasting FFA levels also remained unchanged. The reduction in postprandial intestinal CM production that was associated with ezetimibe treatment could be a consequence of chronic cholesterol shortage and reduced FFA absorption in the intestinal epithelium.

Our findings in this study suggested some treatment options for patients with combined hyperlipidaemia. There has been an ongoing argument regarding whether patients with type IIb hyperlipidaemia should be treated with statins for hypercholesterolaemia or fibrates for hypertriglyceridaemia. (Dual therapy is not an attractive option, as the combined use of statins and fibrates is associated with a higher frequency of the severe life-threatening side effect of rhabdomyolysis.). In this study, the administration of ezetimibe improved endogenous and exogenous TRL profiles by suppressing postprandial intestinal production of CM and possibly by reducing the fasting hepatic cholesterol pool. Ezetimibe administration can thus be a favourable option for the treatment of patients with elevated VLDL, LDL and remnant lipoproteins. Several studies have shown that ezetimibe improved lipid metabolism in obese patients with dyslipidaemia and in animal models for metabolic syndrome [20–22] and one of those studies also showed a concomitant improvement in insulin response. Moreover, ezetimibe has been reported to inhibit elevation of hs-CRP [23] and to improve endothelium-dependent acetylcholine-induced vasodilatation in patients with metabolic syndrome [24]. There thus appear to be numerous pleiotrophic effects of ezetimibe on ameliorating cardiovascular risk factors. More evidence from mega-trials can be expected to clarify the anti-atherogenic effects of ezetimibe in cardiovascular disease accompanied by accumulation of remnant lipoproteins.

Acknowledgments

This work was supported by the following grants: a grant-in-aid for Scientific Research (No. 18659267) to S. Yamashita from the Ministry of Education, Science, Sports and Culture in Japan; a grant from Mitsui Life Social Welfare Foundation to S. Yamashita; a Takeda Medical Research Foundation Grant to S. Yamashita. Authors gratefully acknowledge Hiromi Nishinaka and Kaori Hizu for their excellent technical assistance.

Disclosures

From Bayer Yakuhin Ltd. (Tokyo, Japan) and Schering-Plough K.K. (Tokyo, Japan) in Japan, S. Yamashita received a grant for research support for general purposes and an honoraria and travel expenses for his lectures in conferences supported by both companies. S. Yamashita is an advisory board of (Otsuka Pharmaceutical Co. Tokyo, Japan) and Skylight Biotech Inc. (Tokyo, Japan).

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Received 1 December 2008; accepted 24 April 2009

References

1 Iso H, Naito Y, Sato S, Kitamura A, Okamura T, Sankai T *et al.* Serum triglycerides and risk of coronary heart disease among Japanese men and women. *Am J Epidemiol* 2001;**153**:490–9.
2 Eberly LE, Stamler J, Neaton JD, Multiple Risk Factor Intervention Trial Research Group. Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease. *Arch Intern Med* 2003;**163**:1077–83.
3 Altmann SW, Davis HR Jr, Zhu LJ, Yao X, Hoos LM, Tetzloff G *et al.* Niemann-pick C1 like 1 protein is critical for intestinal cholesterol absorption. *Science* 2004;**303**:1201–4.
4 Huff MW, Pollex RL, Hegele RA. NPC1L1: evolution from pharmacological target to physiological sterol transporter. *Arterioscler Thromb Vasc Biol* 2006;**26**:2433–8.

5 Davis HR Jr, Hoss LM, Tetzloff G, Maguire M, Zhu LJ, Graziano MP *et al.* Deficiency of Niemann-Pick C1 like 1 prevents atherosclerosis in ApoE−/− mice. *Arterioscler Thromb Vasc Biol* 2007; **27**:841–9.

6 Davies JP, Scott C, Oishi K, Liapis A, Ioannou YA. Inactivation of NPC1L1 causes multiple lipid transport defects and protects against diet-induced hypercholesterolemia. *J Biol Chem* 2005;**280**:12710–20.

7 Davis HR Jr, Compton DS, Hoos L, Tetzloff G. Ezetimibe, a potent cholesterol absorption inhibitor, inhibits the development of atherosclerosis in ApoE knockout mice. *Arterioscler Thromb Vasc Biol* 2001;**21**:2032–8.

8 Garcia-Calvo M, Lisnock J, Bull HG, Hawes BE, Burnett DA, Braun MP *et al.* The target of ezetimibe is Niemann-Pick C1-like 1 (NPC1L1). *Proc Natl Acad Sci USA* 2005;**102**:8132–7.

9 Knopp RH, Dujovne CA, Le Beaut A, Lipka LJ, Suresh R, Veltri EP. Evaluation of the efficacy, safety, and tolerability of ezetimibe in primary hypercholesterolaemia: a pooled analysis from two controlled phase II clinical studies. *Int J Clin Pract* 2003;**57**:363–8.

10 Saito Y, Yamada N, Nakatani K, Teramoto T, Oikawa S, Yamashita S *et al.* Phase III clinical study of ezetimibe – double-blind comparative study with colestilan. *J Clin Ther Med* 2007;**23**:493–522.

11 Sakai N, Uchida Y, Ohashi K, Hibuse T, Saika Y, Tomari Y *et al.* Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA. *J Lipid Res* 2003;**44**:1256–62.

12 Miyauchi K, Kayahara N, Ishigami M, Kuwata H, Mori H, Sugiuchi H *et al.* Development of a homogeneous assay to measure remnant lipoprotein cholesterol. *Clin Chem* 2007;**53**:2128–35.

13 Nakada Y, Kurosawa Y, Tohyama J, Inoue Y, Ikewaki K. Increased remnant lipoprotein in patients with coronary artery disease – evaluation utilizing a newly developed remnant assay, remnant lipoprotein cholesterol homogenous assay (RemL-C). *J Atheroscler Thromb* 2007;**14**:56–64.

14 Usui S, Hara Y, Hosaki S, Okazaki M. A new on-line dual enzymatic method for simultaneous quantification of cholesterol and triglycerides in lipoproteins by HPLC. *J Lipid Res* 2002;**43**:805–14.

15 Okazaki M, Usui S, Fukui A, Kubota I, Tomoike H. Component analysis of HPLC profiles of unique lipoprotein subclass cholesterols for detection of coronary artery disease. *Clin Chem* 2006;**52**:2049–53.

16 Telford DE, Sutherland BG, Edwards JY, Andrews JD, Barrett PH, Huff MW. The molecular mechanisms underlying the reduction of LDL apoB-100 by ezetimibe plus simvastatin. *J Lipid Res* 2007;**117**:1968–78.

17 Temel RE, Tang W, Ma Y, Rudel LL, Willingham MC, Ioannou YA *et al.* Hepatic Niemann-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. *J Clin Invest* 2007;**117**:1968–78.

18 Masuda D, Hirano KI, Oku H, Sandoval JC, Kawase R, Yuasa-Kawase M *et al.* Chylomicron remnants are increased in the postprandial state in CD36 deficiency. *J Lipid Res* 2009;**50**:999–1011.

19 Labonté ED, Camarota LM, Rojas JC, Jandacek RJ, Gilham DE, Davies JP *et al.* Reduced absorption of saturated fatty acids and resistance to diet-induced obesity and diabetes by ezetimibe-treated and Npc1l1−/− mice. *Am J Physiol Gastrointest Liver Physiol* 2008;**295**:G776–83.

20 González-Ortiz M, Martinez-Abundis E, Kam-Ramos AM, Hernández-Salazar E, Ramos-Zavala MG. Effect of ezetimibe on insulin sensitivity and lipid profile in obese and dyslipidaemic patients. *Cardiovasc Drugs Ther* 2006;**20**:143–6.

21 van Heek M, Austin TM, Farley C, Cook JA, Tetzloff GG, Davis HR. Ezetimibe, a potent cholesterol absorption inhibitor, normalizes combined dyslipidemia in obese hyperinsulinemic hamsters. *Diabetes* 2001;**50**:1330–5.

22 Deushi M, Nomura M, Kawakami A, Haraguchi M, Ito M, Okazaki M *et al.* Ezetimibe improves liver steatosis and insulin resistance in obese rat model of metabolic syndrome. *FEBS Lett* 2007;**581**:5664–70.

23 Sager PT, Capece R, Lipka L, Strony J, Yang B, Suresh R *et al.* Effects of ezetimibe coadministered with simvastatin on C-reactive protein in a large cohort of hypercholesterolemic patients. *Atherosclerosis* 2005;**179**:361–7.

24 Bulut D, Hanefeld C, Bulut-Streich N, Graf C, Mügge A, Spiecker M. Endothelial function in the forearm circulation of patients with the metabolic syndrome – effect of different lipid-lowering regimens. *Cardiology* 2005;**104**:176–80.

OBSERVATIONS

Changes in Serum Adiponectin Concentrations Correlate With Changes in BMI, Waist Circumference, and Estimated Visceral Fat Area in Middle-Aged General Population

Adiponectin was identified as an adipocytokine in the human adipose tissue cDNA library. It has antiatherosclerotic and antidiabetic properties in experimental studies, and its blood levels are low in obesity, diabetes, cardiovascular diseases, and metabolic syndrome. Several studies have reported that weight reduction in massively obese subjects is associated with a rise in serum adiponectin (APN) concentration (1–3). However, the relationship between changes in APN and BMI, waist circumference (WC), and visceral fat accumulation (VFA) in general population has not been reported. The present study investigated 1-year change in APN (ΔAPN) in relation to changes in BMI (ΔBMI), WC (ΔWC), and estimated visceral fat area (ΔeVFA) in middle-aged general population.

The study subjects were 2,024 middle-aged Japanese (1,619 men [45.7 ± 10.4 years] and 405 women [45.6 ± 9.3 years], mean ± SD) who were employees of Amagasaki city office and had undergone annual health checkup in both 2004 and 2005 and were not taking any medications for diabetes, hypertension, or dyslipidemia. The study was approved by the human ethics committee of Osaka University, and a signed informed consent was obtained from each participant. Height, weight, and WC were measured in standing position. BMI was calculated as weight in kilograms divided by the square of height in meters. WC at umbilical level was measured with a nonstretchable tape in late expiration while standing (in cm). VFA was estimated noninvasively by bioelectrical impedance analysis (BIA) (4). Briefly, the voltage recorded at the flank to the flow of current between the umbilicus and the back correlates significantly with VFA and is not influenced by subcutaneous fat. We reported previously that VFA estimated by BIA correlates significantly with that determined by computed tomography (4). APN was measured using latex particle–enhanced turbidimetric assay (5). ΔAPN correlated negatively with ΔBMI, both in men ($r = -0.256$, $P < 0.0001$) and women ($r = -0.223$, $P < 0.0001$) and with ΔWC in men only ($r = -0.191$, $P < 0.0001$), but no correlation was found in women ($P = 0.097$), and ΔAPN correlated negatively with ΔeVFA in both men ($r = -0.189$, $P < 0.0001$) and women ($r = -0.121$, $P = 0.015$).

APN is likely influenced by genetic and environmental factors. It has been reported that APN is associated with single nucleotide polymorphisms in adiponectin gene. The present study demonstrated that changes in body fat, i.e., reduction in BMI, WC, and eVFA, correlated with a rise in APN in middle-aged general population, which to our knowledge is the first such report. We used BIA to evaluate VFA in the present study, and further research on both visceral and subcutaneous fat areas measured by computed tomography scan is needed to clarify the effects of visceral and subcutaneous adiposity on APN.

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DOI: 10.2337/dc09-1130. Clinical trial reg. no. UMIN000002084, www.umin.ac.jp.

Acknowledgments— No potential conflicts of interest relevant to this article were reported.

References

1. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595–1599
2. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 2001;86:3815–3819
3. Ng TW, Watts GF, Barrett PH, Rye KA, Chan DC. Effect of weight loss on LDL and HDL kinetics in the metabolic syndrome: associations with changes in plasma retinol-binding protein-4 and adiponectin levels. Diabetes Care 2007;30:2945–2950
4. Ryo M, Maeda K, Onda T, Katashima M, Okumiya A, Nishida M, Yamaguchi T, Funahashi T, Matsuzawa Y, Nakamura T, Shimomura I. A new simple method for the measurement of visceral fat accumulation by bioelectrical impedance. Diabetes Care 2005;28:451–453
5. Nishimura A, Sawai T. Determination of adiponectin in serum using a latex particle-enhanced turbidimetric immunoassay with an automated analyzer. Clin Chim Acta 2006;371:163–168

J Epidemiol 2010;20(1):62-69
doi:10.2188/jea.JE20081041

Original Article

Association Between Stroke and Metabolic Syndrome in a Japanese Population: Jichi Medical School (JMS) Cohort Study

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Received December 26, 2008; accepted July 13, 2009; released online December 5, 2009

ABSTRACT

Background: Metabolic syndrome increases the morbidity and mortality of cardiovascular diseases. However, few studies have examined the association between the incidence of stroke and metabolic syndrome, as defined by Japanese criteria. The aim of this study was to identify the association between stroke and metabolic syndrome, as defined by criteria used in Japan.

Methods: A total of 2205 subjects (920 men and 1285 women) were examined between 1992 and 1995 as part of the Jichi Medical School Cohort Study. Metabolic syndrome was defined using the Japanese criteria. Medical records, computed tomography, and magnetic resonance imaging were used to diagnose stroke. The Cox proportional-hazards model was used to analyze the association between metabolic syndrome and incident stroke.

Results: The prevalence of metabolic syndrome at baseline was 9.0% in men and 1.7% in women. There were 96 incident strokes during an 11.2-year follow-up period, 14 of which occurred in subjects with metabolic syndrome. Among subjects with metabolic syndrome, the age-adjusted hazard ratio (95% confidence interval) for stroke was 1.93 (0.94–3.96) in men and 6.85 (2.68–17.47) in women. After adjusting for age, smoking status, and alcohol drinking status, the hazard ratio was 1.89 (0.88–4.08) in men and 7.24 (2.82–18.58) in women. Age-adjusted hazard ratios associated with having 2 or more components of metabolic syndrome, with and without central obesity, were 2.93 (1.21–7.08) and 3.20 (1.23–8.31) in men and 1.75 (0.69–4.44) and 8.64 (2.82–28.03) in women, respectively.

Conclusions: The presence of metabolic syndrome, as defined by Japanese criteria, increases the risk of stroke; this effect was highly significant among women.

Key words: metabolic syndrome X; stroke; cohort studies; incidence; cardiovascular diseases

INTRODUCTION

Metabolic syndrome is defined as a cluster of risk factors—central obesity, hypertension, hyperlipidemia, and impaired glucose tolerance—that increases cardiovascular disease morbidity and mortality.[1,2] The third revision of the US Adult Treatment Panel guidelines for cholesterol testing and management was published by the National Cholesterol Education Program in 2001.[3] In 2005, the Examination Committee of Criteria for Metabolic Syndrome in Japan proposed a new set of criteria for the diagnosis of metabolic syndrome.[4] In the same year, the International Diabetes Federation presented a new criterion that became an essential component—race- and ethnic-specific measurement of waist circumference.[5] This parameter was modified to establish a Japanese set point for waist circumference in 2007.[6] In addition, the American Heart Association/National Heart, Lung, and Blood Institute modified the National Cholesterol Education Program criteria.[7]

In Japan, Health Checkups and Healthcare Advice with a Particular Focus on the Metabolic Syndrome were first implemented in April 2008.[8] The aims of this program are to prevent middle-aged men and women from developing chronic diseases, thereby reducing medical costs for individuals and the health care system. There is an urgent need for evidence from studies of the Japanese general population regarding the effects of metabolic syndrome, as defined by the Japanese criteria.

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Several studies have used various criteria to investigate the prevalence of metabolic syndrome in the Japanese general population; however, few have examined the association between metabolic syndrome, as defined by Japanese criteria, and stroke incidence.[9-11] In a previous report, our study group observed no significant association between all-cause mortality and metabolic syndrome, defined by Japanese criteria.[12]

The purpose of the present study was to examine the association between incident stroke and metabolic syndrome, as defined by Japanese criteria, among the Japanese general population.

METHODS

The Jichi Medical School (JMS) Cohort Study is a prospective population-based study that aims to clarify the risk factors of cardiovascular disease in a Japanese rural population. Details on the JMS Cohort Study design and some descriptive data were published previously.[13,14] Baseline data were collected between 1992 and 1995 in 12 rural communities. A total of 12 490 subjects (4911 men and 7579 women) participated in the 12 districts and the waist circumferences of 2286 subjects in 3 of these districts (Takasu, Wara, and Sakuma) were measured. From these 2286 subjects, we excluded 40 subjects who had a previous history of stroke or coronary heart disease, 40 from whom a blood sample could not be obtained, and 1 who was lost to follow-up. Ultimately, 2205 subjects (920 men and 1285 women) were available for observation in the present study. The participation rate for people invited to the mass screening examination was 56%.[15]

Mass screening examinations for cardiovascular disease have been conducted in Japan since 1983, in accordance with the Health and Medical Service Law for the Aged, and the same system was used to collect the present data. In each community, the local government office mailed a personal invitation to all subjects who were enrolled in this study. Trained interviewers used a standardized questionnaire to obtain information about their medical history and lifestyle. Smoking status was classified as current smoker, ex-smoker, or never-smoker; while alcohol drinking was classified as current drinker, ex-drinker, or never-drinker.

Body mass index was calculated as weight (kg) divided by the square of body height (m). Waist circumference was measured at the highest point of the iliac crest. Systolic and diastolic blood pressures were measured with a fully automated sphygmomanometer, the BP203RV-II (Nippon Colin, Komaki, Japan). All blood samples were collected after fasting for at least 8 hours. Serum total cholesterol and triglyceride levels were measured by an enzymatic method (Wako, Osaka, Japan; interassay coefficient of variation (CV): 1.5% for total cholesterol, and 1.7% for triglyceride). High-density lipoprotein cholesterol was measured by phosphotungstate precipitation (using an instrument by Wako, Osaka, Japan; interassay CV: 1.9%). Fasting plasma glucose was measured enzymatically (Kanto Chemistry, Tokyo, Japan; interassay CV: 1.9%).

The subjects who were enrolled in this study were followed-up and their cardiovascular events were investigated and recorded. If they were hospitalized for any reason, their medical records, including duplicate computed tomography scans and magnetic resonance imaging, were checked for evidence of stroke. Each municipal government annually obtained information about subjects who had relocated out of the area. Death certificates were collected from public health centers until the end of 2005, with official permission from the Agency of General Affairs and the Ministry of Health, Labour and Welfare.

The criteria for stroke were sudden onset of a focal and nonconvulsive neurological deficit that persisted for longer than 24 hours; stroke subtype was determined according to the criteria of the National Institute of Neurological Disorders and Stroke.[16] In this study, cerebral infarction and cerebral hemorrhage were defined as stroke, but cases of subarachnoid hemorrhage were not. All probable cases of stroke in this study were evaluated independently by a diagnosis committee composed of a radiologist and a neurologist, with the aid of computed tomography and magnetic resonance imaging.[13]

Written informed consent for participation in the study was obtained from each responder at the mass screening health checkup. We explained that data would be gathered by using the questionnaire and blood samples, that participants' health status would be checked, and that their hospital medical records would be examined if a stroke was suspected. All responders agreed to join the study. The Institutional Review Board of Jichi Medical School for Ethical Issues approved this study.

Metabolic syndrome

The original diagnostic definition of metabolic syndrome in Japan was promulgated by the Examination Committee of Criteria for Metabolic Syndrome in April 2005.[4] For the purposes of this study, metabolic syndrome was defined as a waist circumference of at least 85 cm in men or 90 cm in women, plus at least 2 of the following: (1) triglycerides ≥1.7 mmol/L (150 mg/dL) or high-density lipoprotein cholesterol <1.0 mmol/L (40 mg/dL), (2) systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg, and (3) fasting plasma glucose ≥6.1 mmol/L (110 mg/dL). Subjects who were being treated for diabetes and hypertension were identified by questionnaire at baseline and were included in the study; however, treatment for hyperlipidemia was not taken into account because the questionnaire was not equipped to differentiate those treated for elevated total cholesterol, triglyceride, and lower high-density lipoprotein cholesterol.

Table 1. Clinical characteristics of subjects with and without metabolic syndrome

	Without metabolic syndrome	With metabolic syndrome	*P*-value[a]
Males			
n (%)	837 (91.0)	83 (9.0)	
Age (year)	56.3 ± 12.4	57.9 ± 12.1	N.S.
BMI (kg/m^2)	22.4 ± 2.6	26.4 ± 2.1	<0.001
Waist circumference (cm)	77.9 ± 7.8	90.2 ± 4.6	<0.001
Systolic blood pressure (mm Hg)	127.4 ± 21.2	143.1 ± 17.9	<0.001
Diastolic blood pressure (mm Hg)	76.8 ± 12.2	86.1 ± 11.2	<0.001
Fasting plasma glucose (mmol/L)	5.3 ± 0.9	6.0 ± 1.5	<0.001
Total cholesterol (mmol/L)	4.8 ± 0.9	5.0 ± 0.8	0.01
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.0 ± 0.2	<0.001
Triglyceride (mmol/L)	1.3 ± 1.0	2.2 ± 1.2	<0.001
Current smoking, *n* (%)	404 (49.4)	36 (43.9)	N.S.
Current alcohol drinking, *n* (%)	636 (77.8)	61 (74.4)	N.S.
Diabetes mellitus, *n* (%)	66 (7.9)	32 (38.6)	<0.001
Hypertension, *n* (%)	352 (42.1)	76 (91.6)	<0.001
Females			
n (%)	1263 (98.3)	22 (1.7)	
Age (year)	55.9 ± 12.1	62.0 ± 10.7	0.01
BMI (kg/m^2)	22.9 ± 3.0	28.9 ± 4.5	<0.001
Waist circumference (cm)	73.6 ± 8.7	93.4 ± 3.7	<0.001
Systolic blood pressure (mm Hg)	130.8 ± 22.5	151.0 ± 19.5	<0.001
Diastolic blood pressure (mm Hg)	76.9 ± 13.1	86.4 ± 9.5	<0.001
Fasting plasma glucose (mmol/L)	5.1 ± 0.9	6.3 ± 2.0	<0.01
Total cholesterol (mmol/L)	5.1 ± 0.9	5.5 ± 0.8	0.02
HDL cholesterol (mmol/L)	1.3 ± 0.3	1.1 ± 0.2	<0.001
Triglyceride (mmol/L)	1.1 ± 0.6	2.0 ± 0.9	<0.001
Current smoking, *n* (%)	56 (4.5)	1 (4.5)	N.S.
Current alcohol drinking, *n* (%)	411 (33.1)	7 (31.8)	N.S.
Diabetes mellitus, *n* (%)	80 (6.3)	8 (36.4)	<0.001
Hypertension, *n* (%)	613 (48.5)	21 (95.5)	<0.001

BMI: body mass index.
HDL cholesterol: high-density lipoprotein cholesterol.
N.S.: not significant.
[a]*P*-values were calculated using the *t*-test for variables and the chi-square test for rates.
Data are expressed as the mean ± standard deviation (SD) for variables and as a percentage for rates.

Statistical analysis

All statistical analyses were performed on a personal computer with the Statistical Package for Social Science® (SPSS) for Windows (SPSS Japan Inc., version 11.5, Tokyo, Japan). The results are expressed as the mean ± standard deviation (SD). *P* values were calculated using the *t*-test for variables. Smoking status, alcohol-drinking status, and histories of hypertension and diabetes mellitus were tested using the chi-square test.

The Cox proportional-hazards model was used to calculate the hazard ratios (HRs) for stroke incidence after adjustment for age, smoking status, and alcohol-drinking status, with or without metabolic syndrome, using the Japanese criteria. The crude stroke incidence was calculated per 1000 person-years. A *P* value <0.05 was considered significant.

RESULTS

The total number of person-years of observation was 24 653, the mean follow-up period (± SD) was 11.2 ± 2.4 years, and the mean age at baseline ± SD was 56.2 ± 12.2 (56.5 ± 12.4 in men and 56.0 ± 12.1 in women). There were 96 incident strokes during the observation period: 54 (5.9%) in men and 42 (3.3%) in women.

Table 1 shows the characteristics of subjects with and without metabolic syndrome (stratified by sex). At baseline, the prevalence of metabolic syndrome, as per the Japanese definition, was 9.0% in men and 1.7% in women. There were no significant differences in smoking or alcohol drinking status between the subjects with and without metabolic syndrome in either sex. The women with metabolic syndrome were older than the women without metabolic syndrome; however, among men, there was no such age difference. With the exception of high-density lipoprotein cholesterol, the values for other parameters were significantly higher in subjects with, as compared to without, metabolic syndrome.

Table 2 shows the crude stroke incidence rate and HRs for metabolic syndrome, calculated by the Cox proportional-hazards model, with the absence of metabolic syndrome as reference. There were 96 incident strokes (in 54 men and

Table 2. Adjusted hazard ratios in men and women with and without metabolic syndrome

	Males		Females	
	Without metabolic syndrome	With metabolic syndrome	Without metabolic syndrome	With metabolic syndrome
All subjects, *n*	920		1285	
Subjects with metabolic syndrome, *n* (%)	837 (91.0)	83 (9.0)	1263 (98.3)	22 (1.7)
Stroke incidence, *n*	45	9	37	5
Crude incidence rate[a]	4.9	10.3	2.6	22.0
HR - model 1[b] (95% CI)	reference	1.93 (0.94–3.96)	reference	6.85 (2.68–17.47)
HR - model 2[c] (95% CI)	reference	1.89 (0.88–4.08)	reference	7.24 (2.82–18.58)

Metabolic syndrome was defined using Japanese criteria.
HR: hazard ratio.
CI: confidence interval.
[a]per 1000 person-years.
[b]Hazard ratio adjusted for age.
[c]Hazard ratio adjusted for age, smoking status, and alcohol drinking status.

Table 3. Hazard ratios for stroke, by number of supplementary components of metabolic syndrome, presence of central obesity, and sex

No. of supplementary components	*n*	No. of strokes	Crude incidence rate[a]	Model 1[b]		Model 2[c]	
				HR	(95% CI)	HR	(95% CI)
Males							
Central obesity (−)	685						
0	259	8	2.8	1.00	reference	1.00	reference
1	298	20	6.2	1.81	(0.79–4.14)	1.73	(0.75–3.97)
≥2	128	13	9.8	2.93	(1.21–7.08)	2.53	(1.02–6.24)
Central obesity (+)	235						
0	36	0	0	—	—	—	—
1	116	4	3.0	1.24	(0.37–4.12)	0.91	(0.24–3.42)
≥2	83	9	10.3	3.20	(1.23–8.31)	2.83	(1.05–7.59)
Females							
Central obesity (−)	1213						
0	510	8	1.4	1.00	reference	1.00	reference
1	503	19	3.3	1.31	(0.57–3.01)	1.31	(0.59–3.10)
≥2	200	10	4.4	1.75	(0.69–4.44)	1.83	(0.72–4.68)
Central obesity (+)	72						
0	14	0	0	—	—	—	—
1	36	0	0	—	—	—	—
≥2	22	5	22.0	8.64	(2.82–26.51)	9.09	(2.95–28.03)

HR: hazard ratio.
CI: confidence interval.
Central obesity: waist circumference ≥85 cm in males or ≥90 cm in females.
[a]per 1000 person-years.
[b]Hazard ratio adjusted for age.
[c]Hazard ratio adjusted for age, smoking status, and alcohol drinking status.

42 women) during the follow-up period. The age-adjusted HRs (95% confidence intervals [CI]) were 1.93 (0.94–3.96) in men and 6.85 (2.68–17.47) in women. After further adjustment for current smoking and alcohol drinking statuses, the HRs were 1.89 (0.88–4.08) for men and 7.24 (2.82–18.58) for women.

Next, we classified all subjects into 6 groups by the presence of 0, 1, and 2 or more supplementary components of metabolic syndrome, in men and women with and without central obesity (Tables 3 and 4). Table 3 shows the crude stroke incidence rates and HRs calculated by using the Cox proportional-hazards model. Subjects are classified by the number of supplementary components of metabolic syndrome, the presence of central obesity, and by sex. After adjustment for age and further adjustment for current smoking and alcoholic statuses, the HRs increased in both men and women with 2 or more supplementary components of metabolic syndrome, regardless of central obesity; however, in women, HRs markedly increased in those with central obesity and 2 or more supplementary components of metabolic syndrome, as compared to women without central obesity. There were no strokes among men with central

Table 4. Hazard ratios of stroke incidence with metabolic components with or without central obesity (using various cut-off value of waist circumference) by sex

No. of supplementary components	WC ≥80 cm					WC ≥85 cm					WC ≥90 cm				
	No. of strokes/total	Model 1[a]		Model 2[b]		No. of strokes/total	Model 1[a]		Model 2[b]		No. of strokes/total	Model 1[a]		Model 2[b]	
		HR	(95% CI)	HR	(95% CI)		HR	(95% CI)	HR	(95% CI)		HR	(95% CI)	HR	(95% CI)
Males															
Central obesity (−)															
0	5/208	1.00	Reference	1.00	Reference	8/259	1.00	Reference	1.00	Reference	8/280	1.00	Reference	1.00	Reference
1	17/215	2.58	(0.94–7.04)	2.52	(0.92–6.91)	20/298	1.81	(0.79–4.14)	1.73	(0.75–3.97)	23/374	1.83	(0.81–4.10)	1.75	(0.78–3.95)
≥2	9/80	3.76	(1.26–11.27)	3.24	(1.05–9.98)	13/128	2.93	(1.21–7.08)	2.53	(1.02–6.24)	17/169	2.99	(1.28–6.95)	2.50	(1.05–5.95)
Central obesity (+)															
0	3/87	1.88	(0.45–7.88)	2.08	(0.49–8.73)	0/36	—	—	—	—	0/15	—	—	—	—
1	7/199	1.65	(0.52–5.20)	1.41	(0.43–4.63)	4/116	1.24	(0.37–4.12)	0.91	(0.24–3.24)	1/40	0.98	(0.12–7.85)	—	—
≥2	13/131	4.17	(1.48–11.70)	3.83	(1.34–10.92)	9/83	3.20	(1.23–8.31)	2.83	(1.05–7.59)	5/42	4.10	(1.34–12.53)	4.05	(1.32–12.44)
Females															
Central obesity (−)															
0	8/437	1.00	Reference	1.00	Reference	8/481	1.00	Reference	1.00	Reference	8/510	1.00	Reference	1.00	Reference
1	16/388	1.32	(0.56–3.09)	1.27	(0.54–3.00)	17/455	1.31	(0.56–3.05)	1.26	(0.54–2.94)	19/503	1.35	(0.59–3.10)	1.31	(0.57–3.01)
≥2	8/127	1.83	(0.69–4.89)	1.96	(0.73–5.27)	9/171	1.76	(0.68–4.58)	1.84	(0.71–4.79)	10/200	1.75	(0.69–4.44)	1.83	(0.72–4.64)
Central obesity (+)															
0	0/87	—	—	—	—	0/43	—	—	—	—	0/14	—	—	—	—
1	3/151	0.52	(0.14–1.96)	0.53	(0.14–2.12)	2/82	0.62	(0.13–2.95)	0.70	(0.15–3.34)	0/36	—	—	—	—
≥2	7/95	2.30	(0.88–6.38)	2.42	(0.87–6.72)	6/51	3.83	(1.33–11.07)	4.38	(1.51–12.75)	5/22	8.64	(2.82–26.51)	9.09	(2.95–28.03)

HR: hazard ratio.
CI: confidence interval.
WC: Waist circumference.
[a]Hazard ratios adjusted for age.
[b]Hazard ratio adjusted for age, smoking status, and alcohol drinking status.

obesity but no supplementary components of metabolic syndrome. There were also no strokes among women with central obesity and fewer than 2 supplementary components of metabolic syndrome.

Table 4 shows HRs calculated using the Cox proportional-hazards model for stroke incidence with 0, 1, and 2 or more supplementary components of metabolic syndrome, in men and women with and without central obesity, using subjects with no supplementary components of metabolic syndrome as a reference. The HRs for stroke were calculated for both sexes using different cutoffs for waist circumference (80, 85, and 90 cm) and the number of supplementary components of metabolic syndrome, as defined by the Japanese criteria. The HRs for stroke among subjects with 2 or more supplementary components of metabolic syndrome were higher in subjects with, as compared to without, central obesity, when the cutoffs for waist circumferences were 90 cm in men and 85 cm in women.

DISCUSSION

We investigated the associations between stroke and metabolic syndrome, as defined by Japanese criteria, in the general Japanese population. Our findings suggest that metabolic syndrome was associated with an increased incidence of stroke; this effect was statistically significant in women.

Several studies have examined the association between stroke incidence and metabolic syndrome in Japan.[9–11,17,18] In the Hisayama Study, metabolic syndrome, as defined by the modified National Cholesterol Education Program Adult Treatment Panel III criteria, was associated with increased morbidity for cardiovascular diseases, including stroke.[18] In the NIPPON DATA 80, metabolic syndrome, as defined by the modified National Cholesterol Education Program Adult Treatment Panel III using body mass index instead of waist circumference, was associated with higher incidences of ischemic stroke and ischemic heart disease.[17] However, few such studies have used the Japanese definition of metabolic syndrome.[9–11] Saito et al reported that the overall prevalence of metabolic syndrome, as defined by the Japanese criteria, was 6.4%, that the sex- and age-adjusted HR for stroke was 0.82, and that metabolic syndrome was not associated with stroke.[9] They suggested that the Japanese criteria for metabolic syndrome should include 1 requisite component—waist circumference. The use of such a definition weakens the effects of atherosclerotic risk factors (eg, glucose intolerance, hypertension, and dyslipidemia). Takahashi et al reported that the prevalence of metabolic syndrome, defined by the Japanese criteria, was 11.0% in men and 1.1% in women, and that the HR adjusted for age and smoking status was 23.1 in women. They suggested that metabolic syndrome, as defined by the Japanese criteria, was associated with stroke in women but not in men.[10] Our findings were consistent with theirs; however, their study had a smaller sample size and wider 95% CIs for the HRs.

In the Suita Study, the frequencies of metabolic syndrome in men and women, based on the Japanese criteria, were 17.7% and 5.0%, and the age-adjusted HRs were 1.21 and 2.09; after further adjustment for current smoking and alcohol drinking status, the HRs were 1.27 and 2.05.[11] In addition, they noted that metabolic syndrome, defined by Japanese criteria, was associated with cardiovascular disease, myocardial infarction, and all-stroke incidence only in women. The investigators suggested that the number of metabolic components might be more strongly associated with cardiovascular disease incidence than the requisite waist circumference criterion. They observed elevated HRs in both sexes, as was the case in the present study; however, they observed a statistically significant association between metabolic syndrome and stroke only in women.

Table 3 shows that, in men, the HRs for stroke incidence in men with 2 or more supplementary components of metabolic syndrome were similar in men with and without central obesity; however, among women, the HRs in women with 2 or more supplementary components of metabolic syndrome and central obesity had a higher risk of stroke than did those without central obesity. The prevalence of central obesity, which is included in the Japanese diagnostic criteria for metabolic syndrome, was 25.5% in men and 5.6% in women. As compared with other populations, the proportion of women with metabolic syndrome was not low.[12] However, the 95% CIs of the HRs are likely to be wider for women because of the low prevalence of metabolic syndrome.

Some have reported that waist circumference is positively associated with the risk of cardiovascular events.[19,20] In our study, there were no strokes either among men with central obesity and no supplementary components of metabolic syndrome or among women with central obesity and 0 or 1 supplementary component of metabolic syndrome (Table 3). Different waist circumference cutoffs (80, 85, and 90 cm) were used to divide the subjects into 6 groups (Table 4). When the waist circumference cutoff was 90 cm for men and 85 cm for women, the HRs for stroke were higher among subjects with central obesity and 2 or more supplementary components of metabolic syndrome than those for subjects without central obesity. Consequently, we believe that the appropriate cutoffs for waist circumference in the Japanese criteria for metabolic syndrome are 90 cm in men and 85 cm in women; moreover, our findings indicate that, in addition to increased waist circumference, the combination of central obesity and 2 or more supplementary components of metabolic syndrome is associated with a higher risk for stroke.

Waist circumference is a requisite in the diagnostic definitions of both the Japanese and International Diabetes Federations; however, the World Health Organization and National Cholesterol Education Program Adult Treatment Panel III criteria include waist circumference as only one of

several components. The Japan Diabetes Complication Study (JDCS) observed that there was an association between metabolic syndrome and stroke when the World Health Organization or National Cholesterol Education Program Adult Treatment Panel III diagnostic criteria were used[21]; however, in the same patient group (ie, those observed in the JDCS), there was no significant association with stroke when the diagnostic criteria advanced by the International Diabetes Federation were used.[22] Their results show that different diagnostic definitions of metabolic syndrome can lead to substantially different assessments of the risks for cardiovascular events in the same population.

In the present study, using waist circumference cutoffs of 90 cm in men and 80 cm in women, which the International Diabetes Federation recommend for Japanese, we re-examined the association between stroke and metabolic syndrome (Table 4). The HRs for stroke increased in both sexes; however, the results were significant only when men with no supplementary components of metabolic syndrome were used as the reference.

The strengths of this study are: (1) it was a longitudinal population-based study, (2) there was almost complete follow-up of subjects who developed cardiovascular disease (including stroke), (3) the follow-up period was long, and (4) fasting blood samples were collected.

The most notable limitation of this study is its small sample size. Because of this, the 95% CIs for the HRs are relatively wide; however, the study is valuable because few longitudinal studies have investigated metabolic syndrome in the Japanese general population. We believe that a longer period of follow-up will solve the problem of small sample size.

Other limitations include: (1) waist circumference was measured at the highest level of the iliac crest; (2) the subjects resided in only 3 rural districts; and (3) drug therapy for dyslipidemia was not identified on the questionnaire. Measurement of waist circumference was not common at health examinations between 1992 and 1995, when the baseline data for the general population were obtained. Even at present, various methods are used to measure waist circumference. According to the Japanese criteria, it should be measured at the level of the umbilicus while the subject is standing and breathing normally. We measured it using the method that is utilized to obtain the waist-to-hip ratio, which is endorsed by the World Health Organization.[23] However, the use of this method may have underestimated waist circumference.

In conclusion, metabolic syndrome, as defined by the original Japanese criteria, was positively associated with stroke. Furthermore, in women, there was a statistically significant difference in stroke incidence between women with and without metabolic syndrome. We hope that there will be larger and more comprehensive prospective studies of cardiovascular morbidity and mortality in the Japanese general population.

ACKNOWLEDGEMENT

This study was supported by a grant from the Foundation for the Development of the Community, Tochigi, Japan.

REFERENCES

1. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15:539–53.
2. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988;37:1595–607.
3. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001;285:2486–97.
4. Definition and the diagnostic standard for metabolic syndrome—Committee to Evaluate Diagnostic Standards for Metabolic Syndrome. Nippon Naika Gakkai Zasshi. 2005;94:794–809 (in Japanese).
5. Alberti KG, Zimmet P, Shaw J; IDF Epidemiology Task Force Consensus Group. The metabolic syndrome—a new worldwide definition. Lancet. 2005;366:1059–62.
6. The IDF consensus worldwide definition of the metabolic syndrome. available from: http://www.idf.org/webdata/docs/MetS_def_update2006.pdf [cited 2008 Nov 24].
7. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112:2735–52.
8. Yamamoto H. Health Checkups and Healthcare Advice with a Particular Focus on the Metablic Syndrome in the Health Care System Reform. J Natl Inst Public Health. 2008;57:3–8 (in Japanese).
9. Saito I, Konishi M, Watanabe K, Kondo H, Fujimotos K, Okada K. The metabolic syndrome and risk of stroke in a rural community in Japan. Nippon Koshu Eisei Zasshi. 2007;54:677–83 (in Japanese).
10. Takahashi K, Bokura H, Kobayashi S, Iijima K, Nagai A, Yamaguchi S. Metabolic syndrome increases the risk of ischemic stroke in women. Intern Med. 2007;46:643–8.
11. Kokubo Y, Okamura T, Yoshimasa Y, Miyamoto Y, Kawanishi K, Kotani Y, et al. Impact of metabolic syndrome components on the incidence of cardiovascular disease in a general urban Japanese population: the suita study. Hypertens Res. 2008;31:2027–35.
12. Niwa Y, Ishikawa S, Gotoh T, Kayaba K, Nakamura Y, Kajii E. Metabolic syndrome mortality in a population-based cohort study: Jichi Medical School (JMS) Cohort Study. J Epidemiol. 2007;17:203–9.
13. Ishikawa S, Kayaba K, Gotoh T, Nago N, Nakamura Y, Tsutsumi A, et al. Incidence of Total Stroke, Stroke Subtypes, and Myocardial Infarction in the Japanese Population: The JMS Cohort Study. J Epidemiol. 2008;18:144–50.