metabolism and atherogenic dyslipidemia[1], including elevated triglyceride (TG) in the fasting state.

Besides the early hours of the day before breakfast, we are constantly in a non-fasting state. Accumulating evidence concerning nonfasting TG levels as a predictor of cardiovascular diseases[2] and stroke[3] suggest atherosclerosis as a postprandial phenomenon in which intestine-derived TG-rich lipoproteins, such as chylomicron (CM) and CM remnants, would play an important role[4-6], which Zilversmit stated three decades ago[7].

CD36, or fatty acid translocase, is an 88 kD scavenger receptor class B that is expressed in many cells, such as monocytes, macrophages, microvascular endothelial cells, adipocytes, skeletal and cardiac myocytes and enterocytes. It binds multiple ligands, including long-chain fatty acids (FAs) and oxidized low density lipoprotein[8]. Patients with CD36 deficiency present with increased remnant lipoproteins and decreased high density lipoprotein (HDL)-cholesterol, as well as impaired glucose metabolism based upon insulin resistance. All these findings suggest that CD36 deficiency may be considered a monogenic form of MetS[9]. CD36 knockout (CD36KO) mice present with an excessive postprandial plasma TG and FA response after acute oral fat loading compared to wild-type (WT) mice[10]. Previous studies in our laboratory using CD36KO mice reported a postprandial increase in plasma CM and CM remnants with enhanced TG synthesis in the small intestines, suggesting that the main cause of postprandial hypertriglyceridemia (PHTG) in CD36KO mice was the increased *de novo* synthesis of small CM in enterocytes[11]. These findings established CD36KO mice as a model to evaluate PHTG in a MetS environment.

Ezetimibe, a cholesterol absorption inhibitor that acts by blocking the sterol-induced internalization of the key cholesterol transporter, Niemann-Pick C1 Like 1 (NPC1L1), in enterocytes[12] has been demonstrated to lower total and LDL-cholesterol levels significantly in patients with primary and mixed hypercholesterolemia as a coadjuvant therapy to either statins[13] or fibrates[14]. In these studies, ezetimibe was also found to decrease other important atherogenic factors significantly, such as fasting TG and total apolipoprotein B (ApoB) levels in plasma. Moreover, ezetimibe has been demonstrated to reduce PHTG in combination with low-dose statins in patients with obesity and metabolic syndrome comparable to high-dose statins alone[15].

Recently, our group reported that ezetimibe alone significantly reduced PHTG in Japanese subjects with type IIb hyperlipidemia[16], suggesting that ezetimibe might also play a role in regulating the production of TG-rich lipoproteins in addition to act as a cholesterol absorption inhibitor. Since investigations concerning ezetimibe and its mechanism of action on lipid metabolism have primarily focused on sterol metabolism, we prioritized the need to establish molecular mechanisms that participate in the TG-lowering effect of ezetimibe in the postprandial state. For that purpose, we performed oral fat loading in ezetimibe-treated and non-treated wild-type (WT) mice fed a western diet and CD36KO mice fed a chow diet as an animal model of PHTG. We demonstrated that ezetimibe reduces PHTG by decreasing the absorption of both cholesterol and long-chain FAs through enterocytes, which affected intestinal FA transport, TG production, and CM formation in both mice strains.

Materials and Methods

Animals

Male C57BL6/J WT and CD36KO mice created on a C57BL6/J background (kindly provided by Mason. W. Freeman, M.D., Ph.D., Professor of Harvard Medical School)[17], 8–10 weeks of age were used for this experiment. Each mouse strain was separated into two groups in the following manner: CD36KO mice were fed a chow diet (MF; Oriental BioLaboratories, Chiba, Japan) either with or without supplementation of 10 mg/kg ezetimibe (Schering-Plough, USA), and WT mice were fed a western diet either with or without supplementation of 10 mg/kg ezetimibe. The animals were housed in a temperature-controlled environment at 12-hour dark-light cycles with free access to food and water. After 3 weeks of treatment, mice in each group were divided into 2 subgroups. One subgroup was euthanized after fasting for 12 h and the other was fasted for 12 h followed by acute ingestion of 17 μL/g body weight of olive oil (Nacalai Tesque, Kyoto, Japan) by intragastric gavage, and then euthanized 3 h after initiating oral fat loading. Plasma, intestinal lymph and tissues were collected from both subgroups at the time of euthanization. Additionally, WT mice fed a standard chow diet were used as controls for the TG determination study. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka University Graduate School of Medicine (IEXAS).

Lipid Determination and Lipoprotein Analysis of Plasma and Intestinal Lymph

Cholesterol and TG concentrations in plasma and intestinal lymph for each mouse were measured

using an enzymatic method (Wako Pure Chemical Industries, Tokyo, Japan) according to the manufacturer's protocol. Plasma and lymph lipoprotein profiles were analyzed by an online dual enzymatic method using high performance liquid chromatography (HPLC) at Skylight Biotech Inc. (Akita, Japan)[18]. Two hundred microliters of plasma or lymph were dissolved in loading buffer and loaded onto TSK gel Lipopropak XL columns; TG concentrations in the flow-through were measured continuously and simultaneously. The correspondence of the size of lipoprotein fractions (CM, very low density lipoprotein (VLDL), LDL, and HDL-sized fractions) and the elution time were; CM (particle diameter >80 nm, elution time: 15-17 min), VLDL (particle diameter: 30-80 nm, elution time: 17-22 min), LDL (particle diameter: 16-30 nm, elution time: 22-28 min), and HDL (particle diameter: 8-16 nm, elution time: 28-37 min).

Collection of Intestinal Lymph in Postprandial State

Five mice from each group, previously fasted for 12 h, were gavaged with olive oil (17 μL/g body weight). Three hours later, the animals were anesthetized and the intestinal lymphatic trunk was cannulated with a 27-gauge needle connected to a polyethylene tube (PE-50), which was pretreated with EDTA-containing water. The procedure was performed in accordance with the modified method described by Bollman *et al.*[19]. The collected intestinal lymph was used for HPLC and protein detection by western blot.

Determination of Labeled Triolein Absorption

Mice from each group were fasted for 12 h and gavaged with 3 μCi of [9,10-^{3}H(N)] triolein (PerkinElmer, MA, USA) mixed into 17 μL/g body weight of olive oil. Three hours after fat loading, the mice were euthanized and blood samples were collected from the inferior vena cava. The activity of radiolabeled tritium in 250 μL plasma was determined by scintillation counting using a WALLAC Winspectral™ 1414 Liquid Scintillation Counter.

Protein Detection by Western Blot

One microliter of sample (plasma or lymph) was subjected to 4-12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE; TEFCO, Tokyo, Japan), later transferred onto an Immobilon-P transfer membrane (Millipore Co., USA), and blocked by Blocking One (Nacalai Tesque, Kyoto, Japan). The blotted membrane was then incubated with anti-mouse ApoB-48/B-100 antibody (BIODESIGN International, ME, USA) and anti-rabbit IgG as a secondary antibody (NA934V; GE Healthcare Backinghamshire, UK). Bands corresponding to ApoB-48 were detected with the ECL Advance Detection Kit (GE Healthcare, UK).

RNA Extraction, cDNA Synthesis and Quantitative Real-Time PCR

Mice were fasted for 12 h, gavaged with olive oil as previously stated, and their small intestines were removed, flushed with ice-cold phosphate-buffered saline and divided into three sections of equal length; the proximal two-thirds of mucosa were gently scraped and stored in RNAlater RNA stabilization reagent (QIAGEN GmbH, Germany) at −20℃.

Total RNA from tissue samples were extracted and purified using the RNeasy Plus Mini Kit (cat. 74134; QIAGEN GmbH, Germany). Two micrograms of total RNA were primed with 50 pmol anchored-oligo $(dT)_{18}$ and transcribed with the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Germany), according to the manufacturer's protocol. Quantitative RT-PCR was performed; DNA polymerase and SYBR Green I (Finnzymes Oy, Espoo, Finland) were set in a reaction volume of 20 μL containing gene-specific primers (5 μM) and cDNA (corresponding to ~50 ng total RNA). The reaction was performed using the DNA engine Opticon 2 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA). The $2^{-\Delta\Delta CT}$ method of relative gene expression was employed and standard deviation with a ct value of <0.3 was accepted. Results are expressed as arbitrary units in comparison with the expression of GAPDH.

Primers Used for This Study

The sequence data of the genes were found with GenBank and the sequences of primers were designed with Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). GAPDH was used as a housekeeping gene. The sequence and information for primers used in this study are as follows: CD36 (GenBank accession number NM_001159558): 5'-gagcaactggtggatggttt-3' and 5'-gcagaatcaagggagagcac-3', FATP-4 (NM_011989): 5'-atcaacaccaaccttaggcg-3' and 5'-aacccttgtctgggtgactg-3', FABP1 (NM_017399): 5'-catccagaaagggaaggaca-3' and 5'-ttttccccagtcatggtctc-3', FABP2 (NM_007980): 5'-ttgctgtccgagaggtttct-3' and 5'-gctttgacaaggctggagac-3', FAS (NM_007988): 5'-gctgcggaaacttcaggaaat-3' and 5'-agagacgtgtcactcctggactt-3', SCD1 (NM_009127): 5'-ccttcccccttcgactactctg-3' and 5'-gccatgcagtcgatgaagaa-3', DGAT-1 (NM_010046): 5'-gtgcacaagtggtgcatcag-3' and 5'-cagtgggatctgagccatc-3', DGAT-2 (NM_026384): 5'-agtggcaatgctatcatcatcgt-3' and 5'-aaggaataagtgggaaccagatca-3', MGAT-2 (NM_

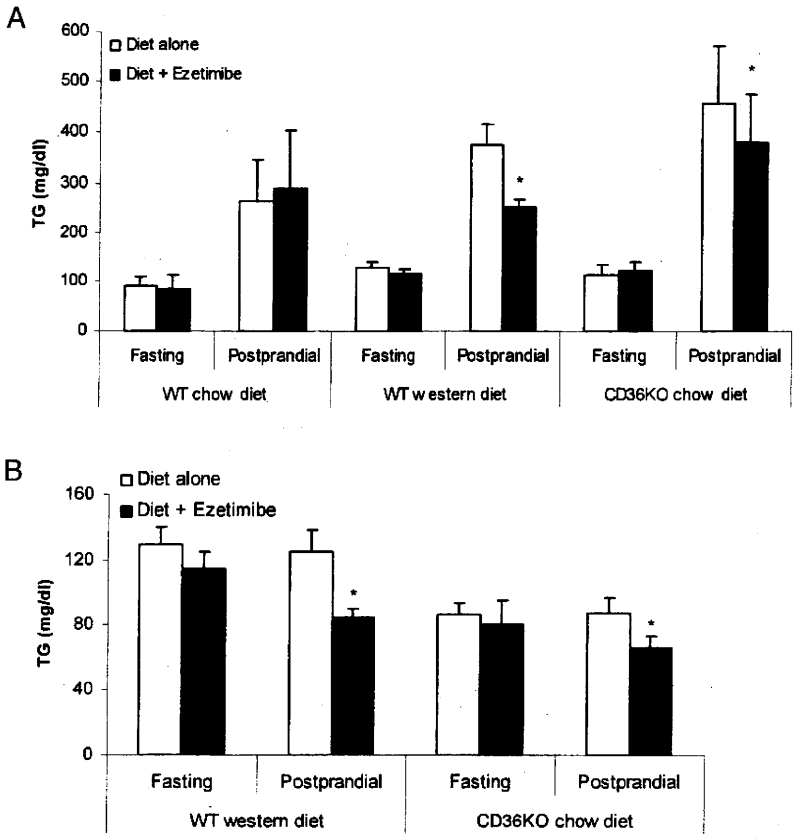

Fig. 1. Ezetimibe Reduces Postprandial Hypertriglyceridemia in Both CD36KO and WT Mice.

CD36KO mice fed a standard chow diet and WT mice fed a western diet, respectively, showed significantly higher plasma TG levels than WT fed a standard chow diet in the postprandial state (white bars) in non-treated groups. Administration of ezetimibe (black bars) decreased plasma TG concentrations at postprandial state in both CD36KO and WT fed a western diet but not in WT mice fed a standard chow diet (A). Ezetimibe also reduced the postprandial concentration of total cholesterol in plasma of both study groups (B). (*$p<0.05$)

177448): 5'-gaagaagcagcatcagggac-3' and 5'-gtgtgggatt-agggggactt-3', ApoB (NM_009693): 5'-tgggattccatct-gccatctcgag-3' and 5'-gtagagatccatcacaggacaatg-3', Apobec1 (NM_031159): 5'-accacaacggatcagcgaaa-3' and 5'-tcatgatctggatagtcacaccg-3', ACF (NM_001081074): 5'-agccagaatcctgcaatcc-3' and 5'-agcata-cctcttcgcttcatcc-3', ACSL1(NM_007981): 5'-tgacctc-tccatgcagtcag-3' and 5'-agcctatgcactcagcgagt-3', HMGCR (NM_008255): 5'-ctggaattatgagtgccccaaa-3' and 5'-acgactgtactgaagacaaagc-3', ACAT2 (NM_009338): 5'-tgtcacagaacagggcagag-3' and 5'-tgacagttcc-tgtcccatca-3' MTTP (NM_008642): 5'-catgtcagccatcct-gtttg-3' and 5'-ctcgcgataccacagactga-3', and GAPDH (NM_008084): 5'-actccactcacggcaaattc-3' and 5'-tctc-catggtggtgaagaca-3'.

Statistical Analysis

The values were expressed as the means ± S.D. Statistical significance was assessed by Student's t-test for paired values and set at $p<0.05$.

Results

Ezetimibe Reduces Postprandial Hypertriglyceridemia in Both CD36KO and Wild Type Mice

CD36KO mice fed a standard chow diet and WT mice fed a western diet showed significantly higher plasma TG levels than WT fed a standard chow diet in the postprandial state without ezetimibe treatment (CD36KO 457 ± 114 mg/dL, WT western diet 376 ± 41 mg/dL vs WT chow diet 267 ± 81 mg/dL. $n=25$). Administration of ezetimibe decreased plasma

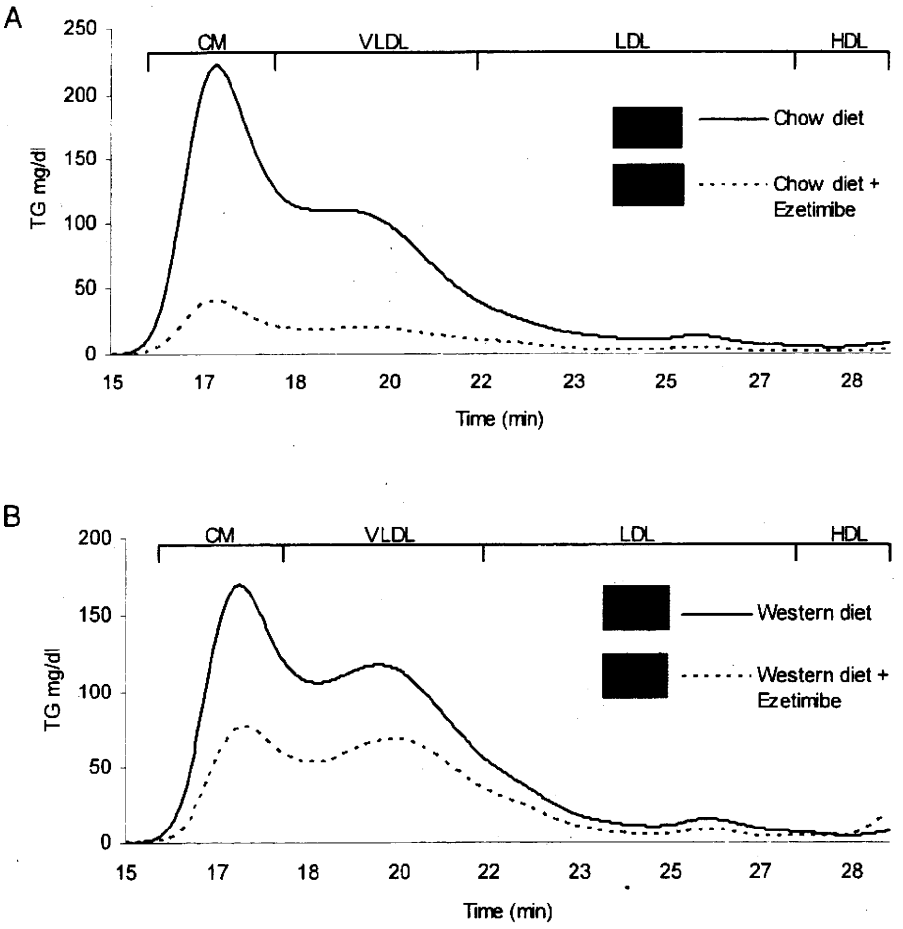

Fig. 2. Ezetimibe Reduces Postprandial CM- and VLDL-sized Particles As Well As ApoB48 Mass in Plasma of WT and CD36KO Mice.

Plasma lipoprotein profile was analyzed by HPLC. Ezetimibe (dotted line) reduced the average postprandial TG levels in both CD36KO (A) and WT mice (B) in CM- and VLDL-sized subfractions, which corresponded to CM remnants. Moreover, ezetimibe decreased the ApoB48 mass in plasma in both groups (representative sample). These results support the idea that ezetimibe might have some modulating effect on intestinal CM production.

TG concentrations in the postprandial state in both CD36KO and WT fed a western diet but not in WT mice fed a standard chow diet (**Fig. 1A**) as well as plasma total cholesterol concentrations in plasma in both study groups (**Fig. 1B**). The selective decrease in both postprandial TG levels suggests that the ezetimibe action on plasma TG concentrations is enhanced by a postprandial MetS environment, since both affected groups are indeed animal models of postprandial hyperlipidemia.

Ezetimibe Reduces Postprandial CM and VLDL-Sized Particles as Well as ApoB48 Mass in Plasma of WT and CD36KO Mice

Plasma lipoprotein profile was analyzed by HPLC using five samples for each group. The highest peak corresponded to CM- and VLDL-sized fractions in both ezetimibe-treated and non-treated mice in both groups. We found that ezetimibe reduced postprandial TG levels in both WT and CD36KO mice mainly in CM- and VLDL-sized subfractions, which corresponded to CM remnants (**Fig. 2A** and **2B**). Moreover, ezetimibe decreased the ApoB48 mass in plasma in both groups. These results support the idea that ezetimibe might have some modulating effect on intestinal CM production. Thus, we further investigated lipoproteins in the intestinal lymph, the intestinal absorption of tritium-labeled FAs, and intestinal mRNA expression of a variety of genes involved in CM synthesis in both strains of mice in the postpran-

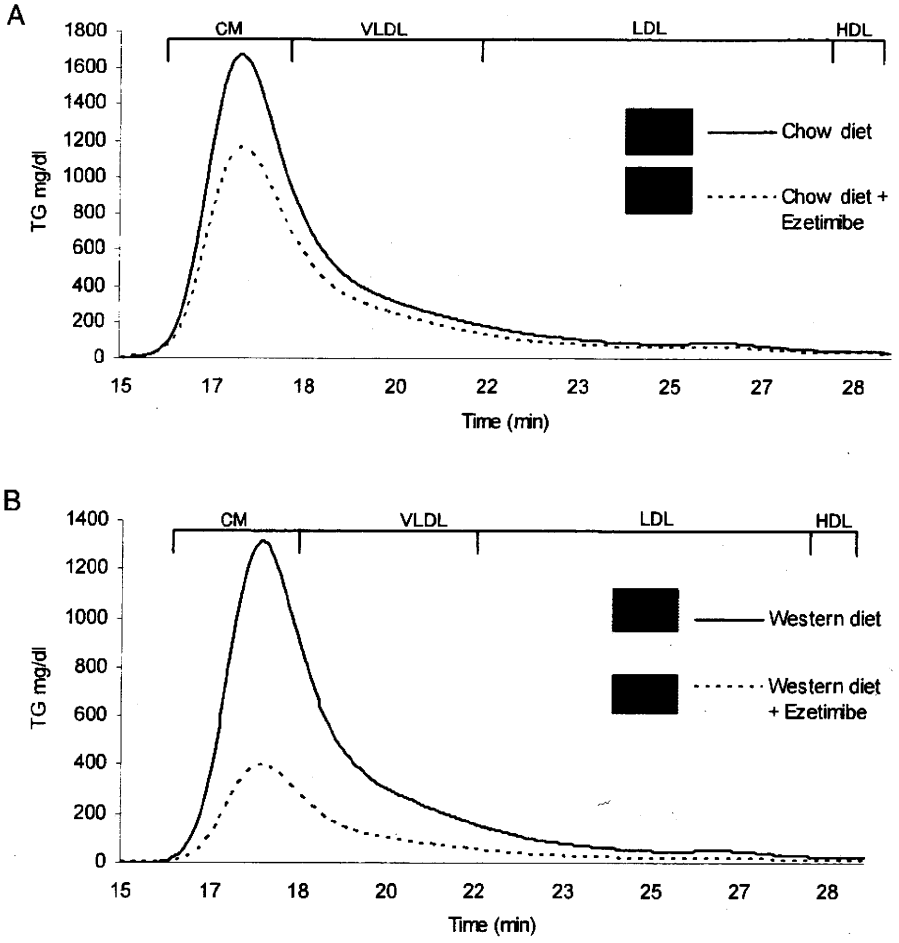

Fig. 3. Ezetimibe Reduces Postprandial TG and ApoB48 Mass in Intestinal Lymph of WT and CD36KO Mice.

HPLC analysis showed that ezetimibe (dotted lines) reduced significantly the average postprandial TG concentration in the intestinal lymph of CD36KO (A) and WT (B) mice in the postprandial state; this reduction was accompanied by a decrease in ApoB48 mass. Ezetimibe decreased the CM peak in both groups, suggesting that it might act by lowering the production of intestine-derived lipoproteins in the postprandial state.

dial state with and without ezetimibe treatment.

Ezetimibe Reduces Postprandial TG and ApoB48 Mass in Intestinal Lymph of WT and CD36KO Mice

Ezetimibe reduced the postprandial TG concentration significantly in intestinal lymph of both study groups in the postprandial state; this reduction was accompanied by a decrease in apoB48 mass in lymph. The highest peak in TG levels corresponded to the CM fraction in treated and non-treated mice in both groups. Ezetimibe decreased the CM peak, suggesting that it might act by lowering the production of intestine-derived lipoproteins in the postprandial state in both groups of mice (**Fig. 3A** and **3B**).

Ezetimibe Reduces the Intestinal Absorption of Radio-Labeled Triolein

To investigate the possible mechanisms by which ezetimibe reduced the intestinal TG secretion, we evaluated intestinal FA absorption. Ezetimibe-treated and non-treated mice from both strains were loaded with 17 μL/g olive oil containing 3 μCi of [9,10-^{3}H(N)] triolein. At 3 h after oral fat loading, ezetimibe significantly reduced ^{3}H radioactivity in the plasma of both strains (**Fig. 4A** and **4B**), establishing that there is a reduction in intestinal FA absorption associated with the administration of ezetimibe.

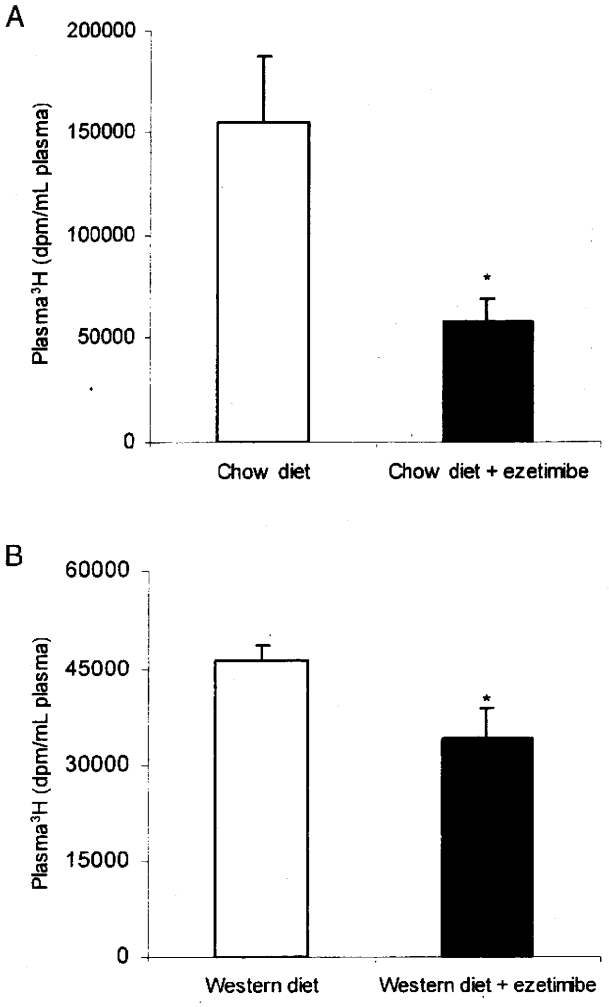

Fig. 4. Ezetimibe Reduces the Intestinal Absorption of Labeled Triolein.

Treated and non-treated mice from CD36KO (A) and WT mice (B) were loaded with 17 μL/g body weight of olive oil containing 3 μCi of [9,10-^{3}H(N)] triolein. Three hours after oral fat loading, ezetimibe reduced significantly ^{3}H radioactivity in plasma of both groups. ($^*p < 0.05$)

Effect of Ezetimibe on the Transcriptional Regulation of Genes Involved in Fatty Acid Transport, TG Formation and CM Assembly in the Intestinal Cells in the Postprandial State

To determine the molecular mechanisms involved in the attenuation of PHTG by ezetimibe, qRT-PCR using total mRNA isolated from the small intestines was performed, and the expression of genes associated with FA transport, TG formation and CM assembly in the intestine of both strains treated and non-treated with ezetimibe was examined.

In CD36KO mice, the mRNA levels of fatty acid transport protein 4 (FATP4), the only FATP in the intestine, were significantly reduced by the administration of ezetimibe, whereas the mRNA levels of fatty acid binding protein 1 (FABP1) and FABP-2, which are also associated with the transport of long-chain FAs, were not changed significantly in the treated groups. The mRNA expression of stearoyl-coenzyme A desaturase 1 (SCD1), diacyl glycerol acyl transferase 1 (DGAT1), DGAT-2, and monoacyl glycerol acyl transferase 2 (MGAT2), all involved in the intracellular formation of TG in intestinal epithelial cells, did not change significantly in the presence of ezetimibe. Interestingly, ApoB mRNA was found to be decreased in mice treated with ezetimibe; this reduction might be associated with a decrease in the expression of apobec-1 mRNA, one of the important factors and components of the protein complex involved in the mRNA edition of ApoB. The expression of microsome triglyceride transfer protein (MTP), which has an important role in CM assembly in intestinal cells, did not change significantly in the presence of ezetimibe. These results suggest that reduction in the hypertriglyceridemic response of ezetimibe in CD36KO mice might be associated with a decrease in cholesterol absorption, fatty acid transport and apo B48 synthesis, resulting in the attenuated formation of CM by a reduction of apoB48 mRNA (**Fig. 5A**).

In WT mice fed a western diet, the mRNA levels of FATP4 and FABP2 were found to be reduced by the administration of ezetimibe, while FABP1 and CD36 were unaffected by this treatment; we also found that SCD1, DGAT1 and DGAT2 were decreased in treated mice. Moreover, in this group, we also found that apoB mRNA was decreased, and this reduction might be associated with a decrease in ACF (apobec-1 complementary factor), a component of the apoB mRNA editing complex. These results suggest that ezetimibe reduces PHTG in WT mice by decreasing fatty acid transport, TG formation and CM assembly in intestinal epithelial cells (**Fig. 5B**).

We also identified an upregulation of fatty acid synthase (FAS), and acetyl-Coenzyme A acetyltransferase 2 (ACAT2) in both groups, which might be due to compensatory responses to the reduction of fatty acid transport and CM production.

Discussion

In the present study, we have investigated the inhibitory effect of ezetimibe on PHTG in MetS using two different animal models: WT mice fed a western, high fat, high cholesterol diet; and CD36KO mice, which is considered as a model of PHTG and a mono-

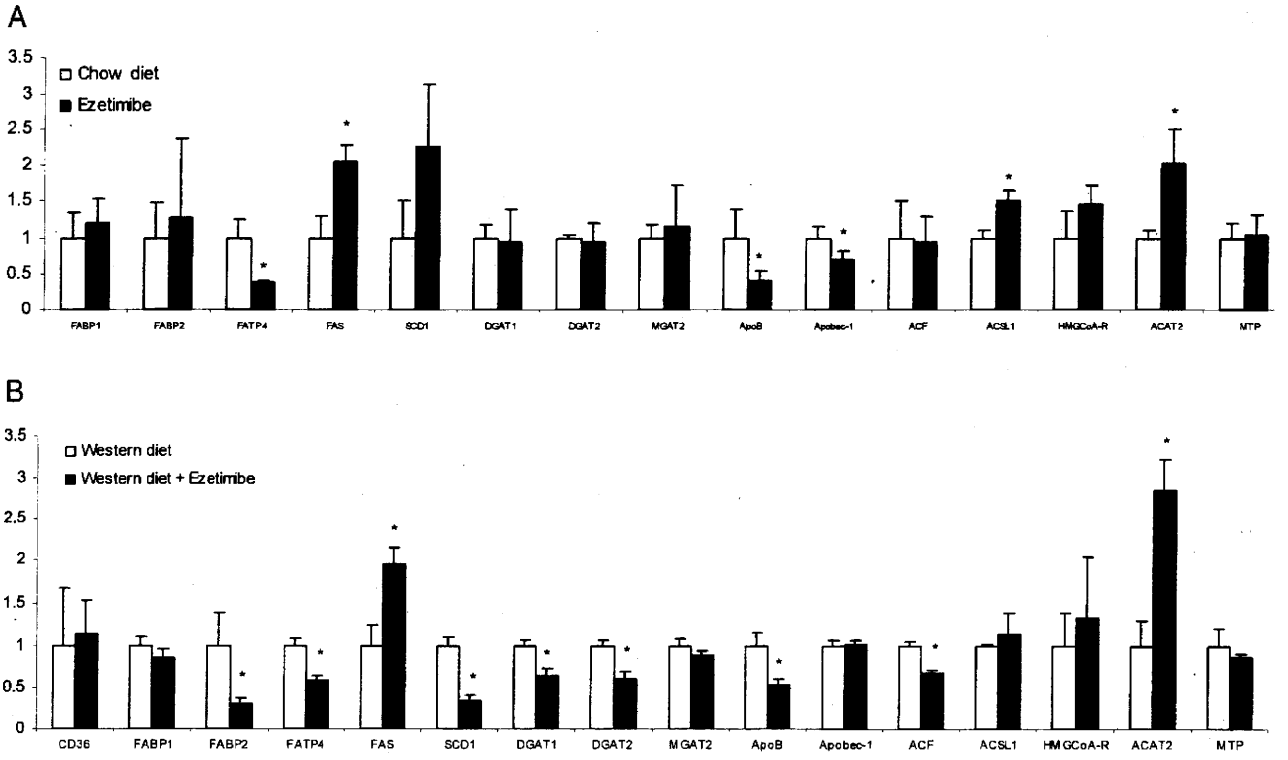

Fig. 5. Effect of Ezetimibe on the Transcriptional Regulation of Genes Involved in Fatty Acid Transport, TG Formation and CM Assembly in the Intestinal Cells in the Postprandial State.

We performed qRT-PCR using total mRNA isolated from intestines, and examined the expression of genes associated with FA transport, TG formation and CM assembly for CD36KO (A) and found that ezetimibe administration significantly inhibited the expression of FATP4, apoB and apobec1. In WT mice (B) ezetimibe decreased the expression of FATP4, FABP2, DGAT1, DGAT2, SCD1, apoB and ACF significantly. There was also the upregulation of FAS and ACAT2, which could correspond to a compensatory response. In both cases, ezetimibe decreased the expression of genes involved in FA metabolism and CM production.

genic model of MetS[11]. We have elucidated the possible molecular mechanisms responsible for the reduced production of ApoB-48-containing lipoproteins in intestinal epithelial cells. Because of the lack of hepatic NPC1L1 expression in mice[20], the usage of mice has an advantage to understanding the physiological mechanisms of lipid metabolism in the small intestines as a main target of ezetimibe, contrary to human subjects in which NPC1L1 is believed to be expressed in both small intestines and the liver. Ezetimibe is a strong inhibitor of cholesterol absorption via NPC1L1, and thus cholesterol incorporation into the CM synthesized in the small intestines is reduced by ezetimibe treatment. Therefore, the reduction of cholesterol content in CM and CM remnants may result in a decreased cholesterol pool in the liver, leading to the enhancement of hepatic LDL receptor. Thus, ezetimibe treatment may enhance the catabolism of LDL via hepatic LDL receptor, resulting in the reduction of LDL-cholesterol and possibly CM remnants. Furthermore, reduced cholesterol absorption may lead to the loss of the substrate for CM formation and thereby to attenuation of CM synthesis in the small intestines.

We found that, in both groups, WT mice fed a high fat diet and CD36KO mice fed a chow diet, ezetimibe did not reduce plasma cholesterol concentrations significantly in the fasting state (**Fig. 1B**); however, there remained a small, non-significant tendency for the cholesterol content in plasma to fall in both groups. This might be associated with increased endogenous production of cholesterol in both the intestine and liver in both models, possibly through an increased expression of HMG-CoA synthase, which should be further considered.

We also found that in WT mice fed a chow diet, ezetimibe did not decrease postprandial TG levels (**Fig. 1A**); however, when WT mice were fed a high-fat, high-cholesterol diet, ezetimibe reduced the PHTG to normal levels. This might suggest that ezetimibe could reduce postprandial triglyceride levels in

conditions of CM overproduction.

In a previous publication, our group found that CD36KO mice have an increased TG response to acute fat loads in both plasma and lymph[11]. In the current study, we found that CD36KO showed a higher TG concentration than WT mice even in a high fat loading state in intestinal lymph (**Fig. 3**); this might suggest the hypothesis that CD36KO mice would have a larger CM than WT mice. However, since the CM fraction in our HPLC method was included in the void volume, we could not determine the specific size of individual particles in this fraction, which is considered a whole group, and therefore, we were not able to confirm whether there was really a difference in particle size between these two groups.

The reduced absorption of long-chain FAs observed in this study was in part associated with an inhibitory effect on FATP4 in CD36KO mice as well as the reduction of both FATP4 and FABP2 intestinal expression in WT mice. FATP4 is the only FATP expressed in the intestines[21], is located in the ER of the intestinal cells and has demonstrated acyl-CoA synthetase activity, which decreases the intracellular concentration of FAs, and would indirectly increase FA uptake when the extracellular concentration is high enough, as in the postprandial state[22]. FATP4 has also been associated with obesity and the insulin-resistant state[23]. Labonté *et al.*[24] reported a reduction of the FATP4 amount in the intestines of both WT mice receiving ezetimibe and NPC1L1 knockout mice compared to WT controls. Although we did not measure the amount of FATP4 protein by Western blotting, we found a decrease in the mRNA content in both treated groups, suggesting inhibition of the regulation of FATP4 at the transcriptional level, which would lead to a decreased amount of FATP4. Taken together, these findings suggest a close relationship between the presence of active NPC1L1 and the uptake, intracellular transport and esterification of long-chain FAs.

In the current study, we also found that WT mice fed a western diet under ezetimibe treatment showed a reduced expression of DGAT1 and DGAT2, two proteins involved in TG synthesis, located in the ER[25], as well as a decreased expression of SCD1, which is an important lipogenic factor associated with dietary saturated fat-related obesity[26]. SCD1 has been reported to colocalize and interact with DGAT2[27], suggesting a mechanism of the incorporation of endogenously synthesized FAs into TG. Therefore, ezetimibe might also decrease PHTG in WT mice fed a western diet by reducing the formation of TG in intestinal cells.

Interestingly, in CD36KO mice, ezetimibe administration inhibited only FATP4 expression in the steps prior to CM assembly to reduce PHTG, but not FABP2, nor any of the proteins involved in TG production, as in WT mice, which might suggest that FATP4 could play an essential role in FA metabolism in the CD36KO model, different from WT mice, which also supports the idea that intestinal lipid metabolism in CD36KO mice is different from in WT mice.

On the other hand, we found that ezetimibe administration reduced ApoB mRNA in both treated groups, and moreover, ezetimibe decreased the mRNA levels of apobec1 in CD36KO mice and Apobec1 complementary factor (ACF) in WT mice. Whether ezetimibe decreased ApoB48 mass in lymph only by inhibiting the transcription or by enhancing the post-transcriptional degradation of ApoB is not known yet, and further examination will be required to gain a better understanding of intestinal ApoB metabolism.

Apobec1 is the catalytic subunit of the ApoB editing complex; in the absence of apobec1, there is no ApoB mRNA edition; apobec1 KO mice lack ApoB48, and the only ApoB found in this model is ApoB100[28]. In our study, ezetimibe decreased apobec1 mRNA significantly in CD36KO mice; however, we did not find any traces of ApoB100 in the intestinal lymph collected; therefore, we presume that ApoB mRNA edition was not so low as to make the enterocytes produce ApoB100-containing lipoproteins, but decreased enough to reduce the production of ApoB48 which, in addition to the presence of low TG as a substrate, led to reduced CM production.

Apobec1 complementary factor (ACF), the RNA-binding subunit of the editing complex, interacts with both apobec1 and ApoB mRNA, positioning the ApoB mRNA structure in the optimal configuration to expose the C residue to apobec1, and it has been proposed to be responsible for the specificity of the reaction[29], and a stabilizer for apobec1[30]. It has been proposed that ACF plays a pivotal role independent of apobec1, since attempts to generate ACF KO mice were not successful beyond the blastocyst state, and siRNA knockdown of ACF in rat and human cells induced an increase in apoptosis. In heterozygote ACF KO mice, ACF protein was found to be decreased in the small intestines; however, intestinal ApoB mRNA edition was not compromised[31]. From this evidence, we could not draw the conclusion that the lowering effect of ezetimibe on the expression of ACF would be actually relevant to ApoB mRNA edition and the production of CM in WT mice.

We have summarized in **Fig. 6** the possible

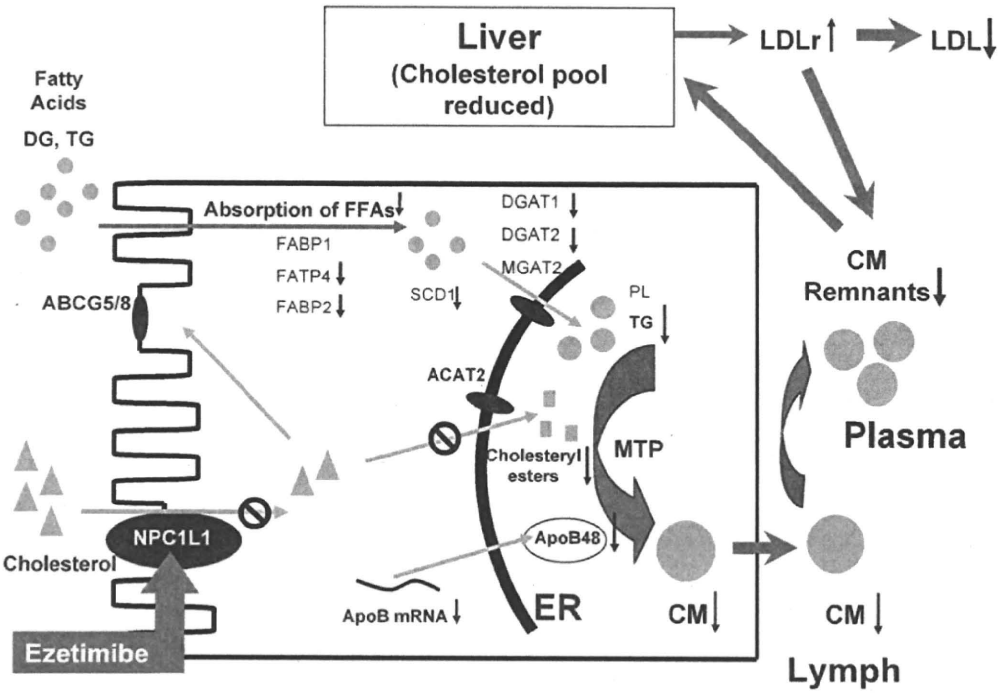

Fig. 6. Possible Mechanisms for the Inhibitory Effect of Ezetimibe on Postprandial Hypertriglyceridemia.

Administration of ezetimibe alone reduces PHTG by inhibiting cholesterol absorption and the expression of genes involved in the uptake, intracellular trafficking and metabolism of long-chain FAs (FATP4 in both WT and CD36KO mice and FABP2 in WT mice only), as well as by decreasing the formation of TG (SCD1, DGAT1 and DGAT2 in WT mice) and the expression of apoB (both WT and CD36KO mice), necessary for the production of ApoB48-containing lipoproteins in the small intestine. Furthermore, reduced cholesterol influx to the liver may lead to the up-regulation of hepatic LDL receptor, resulting in the enhanced catabolism of LDL and CM remnants.

mechanisms for the inhibitory effect of ezetimibe treatment on postprandial hypertriglyceridemia. The administration of ezetimibe alone reduces PHTG by inhibiting cholesterol absorption and the expression of genes involved in the uptake, intracellular trafficking and the metabolism of long-chain FAs (FATP4 in both WT and CD36KO mice and FABP2 in WT mice only), as well as by decreasing the formation of TG (SCD1, DGAT1 and DGAT2 in WT mice) and the expression of ApoB (both WT and CD36KO mice) necessary for the production of ApoB48-containing lipoproteins in the small intestine. Furthermore, reduced cholesterol influx to the liver may lead to the up-regulation of hepatic LDL receptor, resulting in the enhanced catabolism of LDL and CM remnants.

In conclusion, ezetimibe alone reduces PHTG in mouse models of MetS by inhibiting cholesterol absorption and uptake, intracellular trafficking and the metabolism of long-chain FAs, as well as decreasing the formation of TG and the expression of apoB, necessary for the production of apoB48-containing lipoproteins in the small intestine. Thus, ezetimibe strongly attenuates the intestinal production of CM, resulting in the inhibition of PHTG, which may eventually lead to the reduction of atherosclerosis in both animal models and humans.

Acknowledgments

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DUAL-TASK WALK IS A RELIABLE PREDICTOR OF FALLS IN ROBUST ELDERLY ADULTS

To the Editor: Falls are relatively common in elderly people, with approximately 30% of individuals aged 65 and older falling at least once a year and approximately half experiencing repeated falls.[1] In daily-life situations, locomotion occurs under complicated circumstances with cognitive attention focused on a particular task, such as watching traffic or reading street signs, rather than performing the specific motor task of walking. A seminal study demonstrating that the characteristic "stops walking when talking" could serve as a predictor of falls introduced a novel method for fall prediction based on dual-task (DT) performance.[2] Recently, a number of studies have evaluated DT walking in elderly people, but one found that reliable conclusions based on DT results for fall prediction cannot be made because of the lack of standardization in DT paradigms.[3] The aim of the current study was therefore to examine prospectively whether two kinds of DT walking (cognitive task (CT) and manual task (MT)) could predict the risk of falls in a community-dwelling elderly population according to physical function.

The study population consisted of 1,038 community-dwelling elderly Japanese people aged 65 and older (401 men, 637 women, mean age 77 ± 8) in 2009. Six items of physical function were assessed: single-task (ST) 10-m walking time, DT (CT and MT) 10-m walking time, Timed Up and Go (TUG) Test,[4] functional reach, and five-chair stand test (Table 1). In CT walking, participants walked 15 m at the most comfortable speed while counting numbers aloud in reverse order starting at 100. In MT walking, participants walked 15 m at the most comfortable speed while carrying a ball (7 cm in diameter, 150 g in weight) on a tray (17 cm in diameter, 50 g in weight). The DT cost (CT and MT) was then calculated as follows:

$$\text{DT cost}[\%] = 100 \times (\text{DT walking time} - \text{ST walking time})/((\text{ST walking time} + \text{DT walking time})/2)$$

Information on the incidence of falls during the following year was collected from participants in a monthly

Table 1. Characteristics of 1,038 Individuals Aged 65 to 97 According to Quartiles of Timed Up and Go Test Results (Seconds)

	Mean ± Standard Deviation							
	Fastest (≤ 8.3) (n = 230)		Faster (8.4–11.0) (n = 258)		Slower (11.1–14.9) (n = 264)		Slowest (≥ 15) (n = 286)	
Characteristic	Faller, 46 (20.0%)	Nonfaller,	Faller, 47 (18.2%)	Nonfaller	Faller, 90 (34.1%)	Nonfaller	Faller, 126 (44.1%)	Nonfaller
Age	77.9 ± 7.9	78.4 ± 6.6	77.4 ± 7.3	78.2 ± 8.0	77.5 ± 8.1	78.2 ± 8.8	77.6 ± 9.3	77.3 ± 8.3
Height, cm	154.4 ± 8.4	153.3 ± 6.8	156.5 ± 9.5	154.7 ± 9.4	157.6 ± 8.3	156.3 ± 11.1	153.6 ± 10.2	154.2 ± 9.6
Body, kg	55.6 ± 11.0	53.6 ± 8.3	50.1 ± 22.9	48.9 ± 16.8	51.7 ± 14.7	53.3 ± 9.3	50.4 ± 17.1	49.7 ± 26.1
Locomotive function, seconds*	9.6 ± 2.0	9.2 ± 2.0	10.5 ± 1.9	10.5 ± 2.5	11.4 ± 2.7	11.2 ± 3.6	17.5 ± 7.1	16.8 ± 7.3
Balance function, cm†	27.1 ± 5.5	25.0 ± 5.4	24.3 ± 7.2	22.6 ± 6.4	21.4 ± 7.9	21.6 ± 7.6	16.6 ± 7.0	18.6 ± 7.0
Muscle power, seconds‡	7.7 ± 1.7	7.5 ± 1.9	9.7 ± 2.8	9.9 ± 2.4	12.8 ± 4.7	11.4 ± 3.5§	17.4 ± 9.8	14.9 ± 5.9§
Cognitive task costs, %	18.7 ± 29.7	16.4 ± 25.5	21.8 ± 23.6	10.6 ± 19.1§	20.2 ± 17.2	20.1 ± 22.2	20.8 ± 20.9	23.1 ± 23.6
Manual task costs, %	8.5 ± 15.8	0.2 ± 11.0§	2.2 ± 14.0	5.8 ± 14.7	12.8 ± 14.0	14.5 ± 16.5	14.5 ± 19.7	16.3 ± 20.7

*Time to complete single-task 10-m walk.
†Distance of functional reach.
‡Time to complete five-chair stand.
§Independent variable that remained in the final step of the regression model.

telephone interview. A fall was defined as any event that led to unplanned, unexpected contact with a supporting surface during walking.

For analysis, the TUG test results were divided into quartiles (fastest, faster, slower, and slowest). A multivariate analysis using logistic regression with a stepwise-forward method was performed to investigate which of the five measures of physical function (ST walking time, CT cost, MT cost, functional reach, and five-chair stand test) was independently associated with falls.

In the fastest group (n = 230), the regression analysis indicated that the MT cost (odds ratio (OR) = 1.068, 95% confidence interval (CI) = 1.04–1.10, $P<.001$) was an independent predictor of falling that remained in the final step of the regression model. In the faster group (n = 258), the regression analysis indicated that the CT cost (OR = 1.03, 95% CI = 1.01–1.04, $P<.001$) was an independent predictor of falling. In the slower (n = 264) and slowest groups (n = 286), the five-chair stand test (slower group OR = 1.11, 95% CI = 1.03–1.19, $P<.001$; slowest group OR = 1.05, CI = 1.01–1.09, $P=.045$) was found to be an independent predictor of falling.

In conclusion, this study demonstrated that DT cost is an independent and prospective predictor of falls in elderly adults with higher functional capacity (faster and fastest groups), although DT cost did not predict falls in elderly adults with lower functional capacity (slower and slowest groups). Thus, the finding that DT walking is a reliable predictor of falls is limited to the robust elderly population.

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TAURINE DIURETIC AND RENAL-REVITALIZING EFFECTS IN NONAGENARIANS

To the Editor: Congestive heart failure (CHF) is the most ominous cause of edema in older adults living in extended-care nursing homes. Despite no obvious CHF, edema resistant even to diuretic doses that cause hypotension, especially in fragile nonagenarians, often develops, and an alternative was sought.

Long-term oral taurine (OT 3 g/d) ameliorates CHF,[1] so it was desired to determine whether OT (1.0 g three times per day) relieves edema without causing hypotension in nonagenarians. Forty-nine residents of an extended-care nursing home (20 taking antihypertensive therapy) who developed edema (score ≥ 2, Appendix A) despite hospital-prescribed diuretics or excessive hypotension precluding effective diuretic usage were enrolled from March 1, 2007, to March 31, 2010.

The remarkable effects of OT on edema were apparent within the first month of treatment (Figure 1A); decreases in body weight occurred with some delay. Required doses of diuretics decreased after institution of OT in the majority of residents. Serum albumin levels increased in 32 hypoalbuminemic residents (Figure 1B).

Significant increases were observed in estimated glomerular filtration rate (eGFR) expressed as a percentage of baseline values from 6 months to 2.25 years of treatment in residents with chronic kidney disease (CKD) Stage 3 or greater (Figure 1C, lower panel); the effects of OT were distinctly greater in residents with CKD Stage 3 or greater than in those with CKD Stage 2 or less (two-way analysis of variance $P<.001$), with differences reaching significance in the third year (Figure 1C upper panel; Bonferroni***). The hyperuricemia ($\geq$ 8.6 mg/dL) observed in eight residents became normal in 6 to 9 months (Figure 1D).

Factors other than CHF play a significant pathogenic role in edema in older extended-care nursing home residents

Figure 1. (A) Effects of taurine are strongest on edema, significantly decreasing body weight. (B) Taurine increases albumin levels in patients with <3.8 g/dL at baseline. (C) Effects of taurine on renal function: Lower panel: taurine significantly increases estimated glomerular filtration rate (eGFR) in patients with chronic kidney disease (CKD) Stage 3 or greater when normalized to baseline values by the sixth month of treatment, and continues to improve significantly for up to 2.25 years. Upper panel: greater improvement of eGFR in residents with CKD Stage 3 or greater compared that in those with CKD Stage 2 of less (two-way analysis of variance $P<.001$) reaches significance after 3 years of treatment (Bonferroni***). (D) Taurine decreases hyperuricemia greater than 8.6 mg/dL to normal levels in 3 to 6 months. ANOVA = analysis of variance; SEM = standard error of the mean.

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Original article

Prevalence of the metabolic syndrome in elderly and middle-aged Japanese

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ABSTRACT

Background/Purpose: Diagnosis and management of the metabolic syndrome (MetS) are beneficial for successful aging. In spite of several criteria for MetS, there is little information on cardiometabolic risk clustering in elderly Japanese. The purpose of this study was, therefore, to determine the relationship between age-associated changes in obesity and metabolic components in the Japanese.
Methods: We analyzed data from the nationwide survey conducted in 2000. Using Adult Treatment Panel III (ATP III) and Japanese diagnostic criteria for MetS, we analyzed 2366 people aged from 40 to 79 years (men, 1425 and women, 941) from the total participants.
Results: The prevalence of MetS was almost three fold higher by modified ATP III, International Diabetes Federation, and Japanese criteria, in elderly women than in middle-aged women, whereas no difference was found between middle-aged and elderly men by the three criteria. A marked increase in the prevalence of MetS was found by modified ATP III and International Diabetes Federation criteria compared with that by the Japanese criteria in women. Among the risk factors, the prevalence of central obesity and dyslipidemia increased only in women and that of high fasting glucose and high blood pressure increased in both genders with aging. Among the MetS subjects who fulfilled the modified ATP III criteria, more clustering of risk was observed in elderly than in middle-aged subjects, especially in women. Blood pressure increased and triglyceride decreased in both genders, and non-high-density-lipoprotein cholesterol decreased in elderly men. The prevalence of dyslipidemia decreased in elderly men.
Conclusion: Aging is an important factor that affects the metabolic abnormality, and aging of the population would lead to increase in the prevalence of MetS. Therefore, the development of better approaches to the prevention and management of MetS is necessary for successful aging in our society.

1. Introduction

In the developed countries, life expectancy has shown a continuous increase in the last decades, especially in Japan, along with an increase in age-associated diseases and disabilities.

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Because of the westernization of our lifestyle, this aging population will endure more chronic medical conditions, such as cardiovascular disease, dyslipidemia, diabetes mellitus, chronic kidney disease, and the metabolic syndrome (MetS). MetS is a constellation of multiple risk factors, such as central obesity, dyslipidemia, elevated glucose, and elevated blood pressure, in which insulin resistance is the main underlying disorder.[1] Because the degree of insulin resistance increases with age, elderly are at a higher risk to develop cardiometabolic disorders.[2] At the same time, elderly with MetS are supposed to have a higher risk of cardiovascular disease.[3] In elderly, the diagnosis of MetS is also related to a more pronounced cognitive decline and, thus, disability.[4] Therefore, the identification and treatment of patients with MetS would be an important approach to reduce morbidity and impairment in elderly.

In 2000, we conducted lipid survey in various districts in Japan.[5] In this survey, we found that the level of triglyceride increased in middle-aged men along with the increased body mass index (BMI) compared with the data in 1990.[6] However, the BMI did not change in the elderly population in spite of a small increase in triglyceride levels. Although MetS is a risk factor for cardiovascular disease in middle-aged and elderly people and, therefore, a public health problem, it is still unknown whether the same diagnostic criteria can be applied to both groups.

In the last few years, several expert groups have attempted to set forth simple diagnostic criteria to be used in clinical practice to identify patients with MetS. The committee of International Diabetes Federation (IDF) adopted waist circumference as the surrogate marker for central obesity as an essential component of this syndrome,[7] whereas the National Cholesterol Education Program Adult Treatment Panel III (ATP III) criteria required no single factor for diagnosis, but instead, required the presence of at least three out of five components for the diagnosis.[1] In Japan, the committee has established the diagnostic criteria under the same principle as the IDF criteria, except the cutoff for high glucose as 110 mg/dL instead of 100 mg/dL.[8] The cutoff of waist circumference for central obesity was adopted as 85 cm or greater in men and 90 cm or greater in women in Japanese criteria, although the Asian cutoff of waist circumference is 90 cm or greater in men and 80 cm or greater in women. Recently, several groups have shown that the Asian cutoff for the waist circumference is better than that of the Japanese.[9–12] Furthermore, Hata et al.[13] have shown that MetS defined by the Japanese criteria, with the modification of a waist circumference of 90 cm or greater in men and 80 cm or greater in women, is a better predictor of each ischemic stroke subtype in the Japanese population. Therefore, modification of the Japanese criteria for MetS might be necessary in the future.

The purpose of this study was to examine the prevalence of MetS in the Japanese elderly population and to compare the prevalence of MetS and comorbidities with those in the middle-aged population. We also compared the prevalence of MetS by modified ATP III, IDF, and Japanese diagnostic criteria.

2. Methods

2.1. Designs and data collection

The Research Group on Serum Lipid Level Survey 2000 in Japan asked the members of 36 institutes from various areas around Japan to join this survey. The project was designed to produce representative data of serum lipid levels in the civilian Japanese population. The subjects were people receiving annual health examinations in general community, companies, and schools, and not those visiting hospitals. Among the total number of 12,839 participants, we measured the waist circumferences of 3264 people aged 20–79 years (men, 1917 and women, 1357). In this study, we examined the prevalence of MetS in subjects aged 40–79 years (men, 1425 and women, 941) and compared the prevalence of MetS along with each metabolic abnormality according to the Japanese and ATP III criteria. The Ethics Committee in Kyoto University School of Medicine approved this study. Oral informed consent was obtained from all the participants.

2.2. Laboratory methods

All serum and plasma samples were obtained in the fasting state. All lipid and other analyses were conducted on venous blood samples within 1 week of collection at Bio Medical Laboratories (BML) (Saitama, Japan). Serum cholesterol and triglyceride levels were measured by enzymatic assay. High-density-lipoprotein (HDL) and low-density-lipoprotein cholesterols were measured enzymatically by a kit from Daiichi Kagaku Co. Ltd. (Tokyo, Japan). The results of lipid analyses in the four surveys were indirectly standardized according to the criteria of the Centers for Disease Control and Prevention (CDC) Lipid Standardization Program.[14] Thus, the cholesterol levels in these five surveys appear to be comparable. Plasma glucose was determined enzymatically, and hemoglobin A1c (HbA1c) was determined by a kit from Kyowa Medex Co. Ltd. (Tokyo, Japan). Serum insulin was determined by immunoradiometric assay (Abbott Diagnostics Division, Abbot Park, IL). Waist circumference at the umbilical level was measured in the late exhalation phase in standing position.

2.3. Definition of MetS

According to the definition released by ATP III, published in 2008, we analyzed the prevalence of MetS. We modified the criteria by using the Asian cutoff of waist circumference (90 cm for men and 80 cm for women). Other differences are fasting glucose greater than or equal to 100 mg/dL and HDL cholesterol less than 50 mg/dL in women. MetS of modified ATP III criteria was defined as the presence of at least three abnormalities among central obesity, hypertriglyceridemia, low HDL cholesterolemia, high blood pressure, and fasting high glucose. We also analyzed using the Japanese diagnostic criteria of the MetS in 2005, defining MetS as the presence of two or more abnormalities in the presence of central obesity (waist circumference: 85 cm or more in men and 90 cm or more in women). Three abnormalities are as follows: (1) triglycerides greater than or equal to 150 mg/dL and/or HDL cholesterol less than 40 mg/dL or under treatment for this type of dyslipidemia; (2) systolic blood pressure greater than or equal to 130 mmHg and/or diastolic blood pressure greater than or equal to 85 mmHg, or under treatment for high blood pressure; (3) fasting glucose greater than or equal to 110 mg/dL or under treatment for diabetes (Table 1). Furthermore, we used modified IDF criteria for comparison. People treated with lipid-lowering drugs who had normal triglyceride and HDL cholesterol in this study were excluded, because we could not obtain data whether they were treated for hypercholesterolemia or hypertriglyceridemia.

2.4. Data analysis

The results were expressed as mean value ± standard deviation. Differences in means were evaluated by unpaired t test, Mann–Whitney test, or analysis of variance, when appropriate. The categorical variables were compared by chi-square test. The analysis was performed by the Statistical Package for Social Sciences (ver. 11.5; SPSS Japan Inc., Tokyo, Japan). A p value of 0.05 or less was considered to indicate a statistically significant difference.

Table 1
Comparison among Japanese, modified IDF, and modified ATP III criteria for metabolic syndrome

Definition of metabolic syndrome	Japanese (1) + any 2 or more of (2)–(4)	Modified ATP III for Asians 3 or more of (1)–(5)	Modified IDF for Asians (1) + any 2 or more of (2)–(5)
Components			
Central obesity (waist circumference)	(1) ≥85 cm (men), ≥90 cm (women)	(1) ≥90 cm (men), ≥80 cm (women)	(1) ≥90 cm (men), ≥80 cm (women)
High blood pressure	(2) ≥130/85 mmHg and/or antihypertensive medication	(2) ≥130/85 mmHg and/or antihypertensive medication	(2) ≥130/85 mmHg and/or antihypertensive medication
Fasting high glucose	(3) ≥110 mg/dL and/or antidiabetic medication	(3) ≥100 mg/dL and/or antidiabetic medication	(3) ≥100 mg/dL and/or antidiabetic medication
Dyslipidemia	(4) Triglyceride ≥150 mg/dL and/or HDL-C <40 mg/dL	(4) Triglyceride ≥150 mg/dL	(4) Triglyceride ≥150 mg/dL
		(5) HDL-C <40 mg/dL (men), <50 mg/dL (women)	(5) HDL-C <40 mg/dL (men), <50 mg/dL (women)

IDF = International Diabetes Federation; ATP III = Adult Treatment Panel III; HDL-C = high-density-lipoprotein cholesterol.

3. Results

Table 2 shows the prevalence of MetS in Japanese middle-aged and elderly men and women according to the Japanese and modified ATP III and IDF criteria. According to the Japanese criteria, the prevalence of MetS was higher in both elderly men and women (13.3% *vs.* 18.9% in men and 1.5% *vs.* 4.8% in women). In women, the prevalence of MetS was almost three fold higher in the elderly than that in the middle-aged group ($p < 0.01$ by chi-square test). When we apply the modified ATP III and IDF criteria, the prevalence of MetS was also increased in women of each group ($p < 0.01$ in ATP III and IDF criteria by chi-square test), and the three fold increase in MetS in elderly women was consistent among the three criteria. The increase of MetS prevalence in women by modified ATP III and IDF criteria compared with that by the Japanese criteria was also statistically significant ($p < 0.01$). Intriguingly, when we used modified IDF criteria, the prevalence of MetS in middle-aged and elderly men was similar to that using the Japanese criteria. However, the prevalence of MetS in women by modified IDF criteria was similar to that by modified ATP III criteria.

To assess the effect of aging on each metabolic component, we compared the prevalence of central obesity, dyslipidemia, high blood pressure, high fasting glucose, and MetS in each age group according to the Japanese and modified ATP III criteria. In men, the prevalence of MetS was similar in each age group; yet, more people satisfied the modified ATP III criteria than the Japanese criteria (Table 3). In women, the prevalence of MetS was about 5% in the elderly, and almost no subjects were diagnosed with MetS less than 65 years old by the Japanese criteria. According to the modified ATP III criteria, the prevalence of MetS in women also increased in their 60s and was almost the same as that of men older than 65 years. We found a big difference in the prevalence of central obesity diagnosed by Japanese and Asian criteria for waist circumference in both genders. Thus, it is critical which cutoff is used to diagnose MetS.

The prevalence of central obesity in men was almost constant according to the Japanese or Asian criteria of waist circumference, although the prevalence seemed to be decreased in their 70s. The prevalence of central obesity in women increased toward menopause and remained almost the same after their 50s. However, when we used the Asian criteria, the prevalence of central obesity in women further increased in their 70s. The prevalence of dyslipidemia was almost constant in men among each age group and increased toward menopause in women. However, the prevalence reached a plateau after 55 years of age. As expected, the prevalence of dyslipidemia was higher in women according to the ATP III criteria than that by the Japanese criteria. The prevalence of high blood pressure increased with age both in men and in women. Intriguingly, the prevalence of high blood pressure did not show further increase after 60 years of age in both genders. The prevalence of high fasting glucose increased after 50 years of age in men and after 65 years of age in women. Thus, the prevalence of MetS and related components are associated with age, especially with menopause in women. We also compared the number of the MetS traits by dividing the cohort into three groups: from 40 to 49 years (young middle age); from 50 to 64 years (old middle age); and from 65 to 79 years (elderly). As shown in Figure 1, the prevalence of the subjects with indicated numbers of MetS components according to the modified ATP III criteria is quite similar in men of all age groups. However, in older women, the number of MetS components increased, which is consistent with the data in Table 2.

Next, we compared the demographic characteristics of men and women diagnosed with MetS by the modified ATP III criteria. As shown in Table 4, age and total, HDL, and low-density-lipoprotein cholesterol were higher, and waist circumference, triglyceride, diastolic blood pressure, remnant-like particle (RLP) cholesterol, and fasting glucose were lower in women than in men.

We then compared the demographic characteristics of elderly and middle-aged men and women with MetS by modified ATP III criteria. As shown in Table 5, systolic blood pressure was higher in older-middle-aged and elderly than in younger-middle-aged group, and diastolic blood pressure was higher in older middle-aged group in both genders. HDL cholesterol was lower in younger-middle-aged men and non-HDL cholesterol was lower in elderly men. Triglyceride was lower in elderly men and women. Insulin levels decreased according to age in men. There were no statistical differences in the other components.

Table 2
Prevalence of metabolic syndrome in middle-aged and elderly Japanese

%	Male (1425)		*p*	Female (941)		*p*
	40–64 yr (1266)	65–79 yr (159)		40–64 yr (732)	65–79 yr (209)	
Japanese criteria	13.3	18.9	NS	1.5	4.8	<0.01
Modified Adult Treatment Panel III	19.0	21.4	NS	9.0	26.8	<0.01
Modified International Diabetes Federation	14.0	14.8	NS	8.2	23.9	<0.01

The numbers in parentheses indicate the number of subjects in each group. *p* Value, 40–64 yr *vs.* 65–79 yr.
NS = not significant.

Table 3
Prevalence of metabolic syndrome and metabolic components according to the Japanese and modified ATP III criteria in each age group in the Japanese population (%)

Criteria and metabolic components	Sex															
	Male (age group, yr), %								Female (age group, yr), %							
	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79
n	291	289	359	191	136	67	62	30	171	123	185	108	145	93	75	41
Japanese criteria																
Central obesity	49.1	56.4	54.0	57.1	55.1	61.2	48.4	43.3	5.3	11.4	16.8	16.7	11.7	16.1	14.7	17.1
Hypertriglyceridemia	33.7	43.6	31.2	28.8	33.1	25.4	25.8	13.3	6.4	7.3	15.1	22.2	20.7	20.4	18.7	14.6
Low HDL cholesterolemia	11.3	14.2	12.3	13.1	12.5	9.0	9.7	13.3	1.8	4.1	2.2	2.8	2.8	6.5	2.7	4.9
Dyslipidemia	36.4	46.0	34.5	33.5	36.0	31.3	30.6	20.0	7.6	8.9	15.1	22.2	22.1	21.5	20.0	19.5
High blood pressure	16.2	20.8	28.7	27.2	49.3	41.8	43.5	40.0	8.8	8.9	17.8	19.4	49.7	49.5	40.0	43.9
High fasting glucose	11.3	15.6	19.5	23.6	22.8	22.4	25.8	16.7	1.8	4.9	3.8	10.2	9.7	15.1	16.0	14.6
Metabolic syndrome	9.6	15.2	14.2	12.0	16.2	22.4	17.7	13.3	0.0	0.8	2.2	3.7	1.4	5.4	4.0	4.9
Modified ATP III criteria																
Central obesity	23.7	34.6	30.1	26.7	30.9	38.8	22.6	23.3	18.7	32.5	31.4	47.2	36.6	48.4	60.0	63.4
Hypertriglyceridemia	33.7	43.6	31.2	28.8	33.1	25.4	25.8	13.3	6.4	7.3	15.1	22.2	20.7	20.4	18.7	14.6
Low HDL cholesterolemia	11.3	14.2	12.3	13.1	12.5	9.0	9.7	13.3	8.2	15.4	15.7	12.0	17.9	23.7	24.0	19.5
Dyslipidemia	36.4	46.0	34.5	33.5	36.0	31.3	30.6	20.0	12.9	17.9	22.7	26.9	29.7	33.3	32.0	31.7
High blood pressure	16.2	20.8	28.7	27.2	49.3	41.8	43.5	40.0	8.8	8.9	17.8	19.4	49.7	49.5	40.0	43.9
High fasting glucose	34.7	38.4	43.2	44.0	39.7	41.8	40.3	36.7	8.8	15.4	14.6	18.5	24.8	29.0	33.3	26.8
Metabolic syndrome	14.1	22.8	19.2	16.2	25.0	22.4	19.4	23.3	1.8	5.7	9.2	13.9	16.6	26.9	30.7	19.5

ATP III = Adult Treatment Panel III; HDL = high-density lipoprotein.

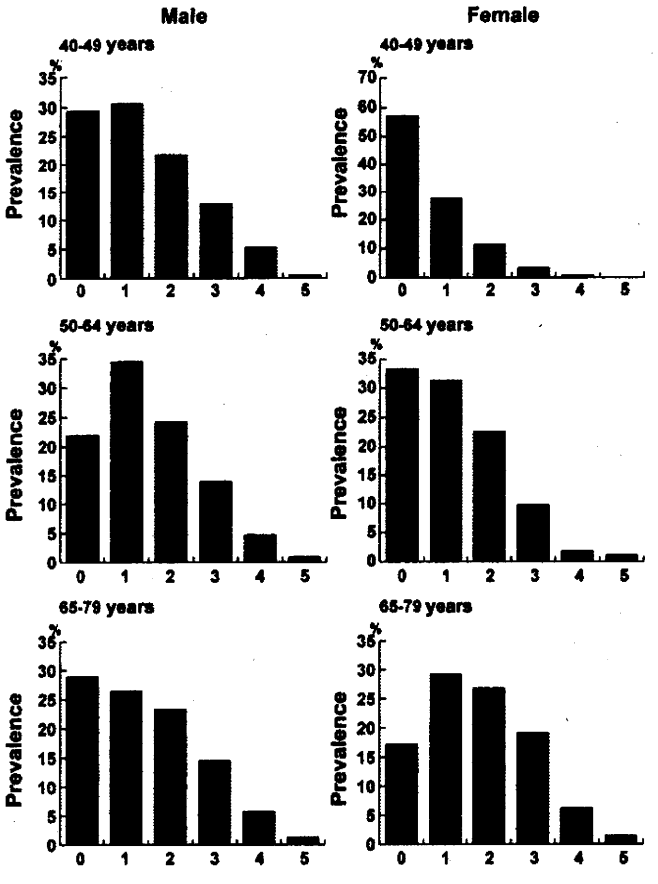

Fig. 1. Prevalence of the subjects with indicated numbers of metabolic syndrome components according to modified Adult Treatment Panel III criteria (central obesity, hypertriglyceridemia, low high-density-lipoprotein cholesterolemia, high blood pressure, high fasting glucose) in each age group of both genders.

Finally, we compared the prevalence of central obesity and other components among the people who satisfied the modified ATP III criteria according to their age in both genders. As shown in Table 6, the prevalence of hypertriglyceridemia and dyslipidemia in men was lower in the elderly, whereas the prevalence of high blood pressure increased in older-middle-aged and elderly men. In women, there was a tendency that the prevalence of hypertriglyceridemia was lower in elderly and that of high blood pressure increased according to age.

4. Discussion

In this study, we compared the prevalence of MetS in the Japanese middle-aged and elderly population by the Japanese and

Table 4
Demographic characteristics of men and women with metabolic syndrome

	Male ($n = 467$)		Female ($n = 201$)		*p*
	Mean	SD	Mean	SD	
Age, yr	53.4	8.8	61.9	9.4	<0.01
Body mass index	25.9	3.0	26.3	2.9	0.27
Waist circumference, cm	92.1	6.7	86.2	8.0	<0.01
Systolic blood pressure, mmHg	136	18	135	18	0.69
Diastolic blood pressure, mmHg	84	12.6	81	9.9	0.02
T-Cho, mg/dL	214	35.4	224	33.6	0.01
TG, mg/dL	197	155, 268	159	115, 194	<0.01
HDL-C, mg/dL	45.7	12.2	51.7	11.0	<0.01
Low-density-lipoprotein cholesterol, mg/dL	126	34.0	140	30.0	<0.01
Non-HDL-C, mg/dL	168	35.8	173	32.5	0.24
RLP-C, mg/dL	6.6	4.2, 10.7	4.3	3.2, 8.5	0.02
HbA1c, %	5.3	0.77	5.4	0.82	0.15
FBS, mg/dL	108	26	100	18.4	0.01
Insulin, μU/mL	8.3	4.7	8.0	4.4	0.52

TG and RLP-C are expressed as median (interquartile range). The difference was analyzed by unpaired *t* test except for TG and RLP-C. Mann–Whitney test was used for TG and RLP-C.
SD = standard deviation; HDL-C = high-density-lipoprotein cholesterol; T-cho = total cholesterol; TG = triglyceride; RLP-C = remnant-like particle cholesterol; HbA1c= hemoglobin A1c; FBS = fasting blood sugar.

Table 5
Demographic data of subjects with metabolic syndrome in each age group

	Sex	40–49 yr (M, 195; F, 27)		50–64 yr (M, 223; F, 102)		65–79 yr (M, 49; F, 72)		p
		Mean	SD	Mean	SD	Mean	SD	
Body mass index	M	26.1	3.0	25.9	3.1	25.5	2.5	0.55
	F	26.7	2.1	26.3	3.1	26.1	2.7	0.78
Waist circumference, cm	M	92.2	7.0	91.9	6.8	92.2	5.8	0.96
	F	87.9	8.7	85.6	9.1	86.3	6.7	0.65
Systolic blood pressure, mmHg	M	130	17.6	138	18.1	143	17.7	<0.01
	F	125	18.0	140	19.4	132	14.6	0.01
Diastolic blood pressure, mmHg	M	82	13.1	86	12.5	82	9.4	0.02
	F	80	11.2	84	10.9	79	7.8	0.04
T-Cho, mg/dL	M	219	34.9	212	35.6	203	33.6	0.06
	F	213	28.8	230	33.2	220	34.3	0.13
TG, mg/dL	M	272	154	234	242	171	71	0.03
	F	169	79.7	189	89.6	144	57.1	0.01
HDL-C, mg/dL	M	43.1	8.3	47.2	13.7	47.9	14.9	0.02
	F	48.3	9.6	52.9	11.5	51.4	10.7	0.38
Low-density-lipoprotein cholesterol, mg/dL	M	128	34.8	126	34.2	121	31.6	0.62
	F	131	26.2	142	28.9	140	31.1	0.51
Non-HDL-C, mg/dL	M	176	34.8	165	36.4	155	32.3	0.01
	F	165	33.5	178	31.0	169	33.6	0.26
HbA1c, %	M	5.1	0.7	5.4	0.9	5.4	0.6	0.14
	F	5.3	0.7	5.3	1.0	5.5	0.7	0.63
FBS, mg/dL	M	112	24.3	113	25.2	108	21.5	0.58
	F	100	13.6	106	22.5	106	19.7	0.61
Insulin, mU/mL	M	9.4	5.9	7.8	3.8	7.2	3.4	0.01
	F	7.9	2.0	8.1	3.6	8.0	5.6	0.98

p Value was analyzed by analysis of variance.
M = male; F = female; HDL-C = high-density-lipoprotein cholesterol; T-cho = total cholesterol; TG = triglyceride; RLP-C = remnant-like particle cholesterol; HbA1c= hemoglobin A1c; FBS = fasting blood sugar.

modified ATP III and IDF criteria. We showed that the prevalence of MetS was almost three fold higher by all the three criteria in elderly women than in middle-aged women, whereas there was almost no difference between middle-aged and elderly men. Consistent with our findings that the prevalence of MetS increased in elderly women compared with that in middle-aged population, other studies have also shown that the prevalence of MetS increases with increasing age.[15] Ford et al.[15] reported that the prevalence of MetS in subjects older than 60 years is approximately 40% in the Third Report of the National Cholesterol Education Program Expert Panel, in which they used the cutoff of 110 mg/dL for high fasting glucose and their criteria of waist circumference for central obesity. The prevalence of MetS in middle-aged population is approximately 25% in both genders, which is different from the result in our cohort. In Japan, Ishizaka et al.[16] and Aizawa et al.[17] have shown that the prevalence of MetS in men is approximately 20% in both middle-aged and elderly populations, whereas that in women is approximately 5% and 10% in middle-aged and elderly populations, respectively, although they used the original ATP III criteria and BMI instead of waist circumference. Tanaka et al.[18] also showed that the prevalence of MetS in Okinawa, a group of islands located in southwest of Japan, is approximately 30% in middle-aged and elderly men and 10% and 20% in middle-aged and elderly women, respectively, when they use ATP III criteria with the Japanese cutoff of waist circumference. Thus, the higher prevalence of MetS in middle-aged and elderly men than that in women is consistent in Japanese cohorts, although the prevalence is different with each diagnostic criterion.

Table 6
Prevalence of each metabolic abnormality in each age group in the subjects with metabolic syndrome

	Sex	Age group (yr)			p
		40–49	50–64	65–79	
Central obesity	M	74.5	72.1	82.4	0.47
	F	92.3	82.8	89.3	0.48
Hypertriglyceridemia	M	92.5	72.9	58.8	<0.01
	F	61.5	69	48.2	0.08
Low HDL cholesterolemia	M	39.6	34.3	32.4	0.61
	F	69.2	46.6	55.4	0.29
Dyslipidemia	M	94.3	77.9	73.5	<0.01
	F	76.9	79.3	71.4	0.62
High blood pressure	M	50.9	70.0	85.3	<0.01
	F	46.2	67.2	76.8	0.09
High fasting glucose	M	79.3	82.1	79.4	0.75
	F	46.2	65.5	64.3	0.41

The difference was analyzed by chi-square test.
M = male; F = female.

Among the metabolic components, the prevalence of central obesity and dyslipidemia increased with aging only in women, and that of high fasting glucose and high blood pressure increased in both genders (Table 3). The prevalence of dyslipidemia decreased in elderly men. Thus, middle-aged men tend to be more dyslipidemic, whereas elderly population tends to have a higher prevalence of central obesity, impaired glucose metabolism, and high blood pressure. Among the subjects diagnosed with MetS by modified ATP III criteria, systolic blood pressure increased with aging in both genders, whereas triglyceride and insulin decreased with aging in both genders, and insulin levels decreased with aging only in men (Table 5). The increased prevalence of high blood pressure in older middle-aged and elderly population is also confirmed in Table 6, although the *p* value was not statistically significant in women. Thus, blood pressure seems to have the strongest association with aging in both genders. The prevalence of central obesity did not increase with aging in the female subjects with MetS (Tables 5 and 6), whereas the prevalence of central obesity increased in female general population, as shown in Table 3. Thus, central obesity in women seems to be affected by aging, which is consistent with the results of other studies in Japan.[16,18] In men, the insulin levels decreased in the elderly in spite of the fact that FBS and HbA1c were not changed among the three groups, suggesting impaired insulin secretion in elderly men with MetS.

In this study, we used the Japanese and modified ATP III and IDF definitions to determine MetS. However, there was a large difference in the prevalence of MetS among the three definitions. This difference

is because of the fact that the Japanese definition requires the central obesity for its diagnosis as in the modified IDF criteria and has more stringent criteria for high fasting glucose for both genders and for HDL cholesterol for women. As shown in this study, the prevalence of MetS in elderly women by the Japanese criteria was very low. This is the reason why we used various analyses using modified ATP III criteria in this cohort. However, the Japanese guideline for MetS was established to identify patients with central obesity, who can reduce the risks by weight loss, whereas the ATP III criteria try to identify patients with multiple risk factors. Therefore, the Japanese criteria should be used to identify obese patients who can have a benefit by weight loss in middle-aged and elderly populations. However, in terms of risk prediction, there have been several reports discussing the cutoff levels of MetS components. Hata et al.[13] have shown significant associations between MetS defined by various criteria and the risk of ischemic stroke in the Hisayama study. In the study, they found that MetS was an independent risk factor for ischemic stroke when they used the modified Japanese criteria with Asian definition of central obesity. Another study from the same group showed that the optimal cutoff level of waist circumference to predict cardiovascular disease was 90 cm in men and 80 cm in women,[19] as we used in modified ATP III definition in this study. Sone et al.[11] also proposed to use the Asian cutoff for waist circumference to define central obesity from the data of Japan Diabetes Complication Study. In terms of the appropriate cutoff level of HDL cholesterol for the definition of MetS in Japanese women, not so many analyses have been done. In our study, the prevalence of low HDL cholesterolemia with the cutoff of 40 mg/dL was less than 5% and was approximately 20% with the cutoff of 50 mg/dL in elderly women. We previously showed that central obesity was significantly associated with low HDL cholesterolemia only when we used the cutoff of 50 mg/dL for women.[5] Therefore, further study is necessary to determine the appropriate cutoff level of HDL cholesterol in women.

In summary, we have shown the prevalence of MetS in Japanese elderly and middle-aged population using Japanese and modified ATP III and IDF criteria, and found the effect of aging on the prevalence only in women with either criterion. We also showed the effect of aging on each metabolic component in this cohort. Thus, aging is an important factor that affects the metabolic abnormality, and aging of the population would lead to the increase in the prevalence of MetS. Therefore, the development of better approaches to the prevention and management of MetS is necessary for successful aging in our society.

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Original Article

More Intensive Lipid Lowering is Associated with Regression of Coronary Atherosclerosis in Diabetic Patients with Acute Coronary Syndrome - Sub-Analysis of JAPAN-ACS Study

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Aim: We have shown that aggressive lipid lowering by pitavastatin and atorvastatin results in marked regression of atherosclerotic coronary lesions after acute coronary syndrome (ACS). The purpose of this study was to address the association of lipid levels after statin therapy with regression of atherosclerotic coronary lesions and major cardiovascular events in patients after ACS.
Methods: JAPAN-ACS is a prospective, randomized open-label study performed at 33 centers in Japan. Patients with ACS undergoing intravascular ultrasound (IVUS)-guided percutaneous coronary intervention (PCI) were randomly assigned to receive either 4 mg/day pitavastatin or 20 mg/day atorvastatin within 72 hours after PCI. IVUS image was obtained in 251 patients, including 73 diabetic patients. Lipid profiles at the end of the study were divided into quartiles and the association with the percent change in non-culprit coronary plaque volume (PV) was assessed in total and diabetic patients. We also studied whether baseline and follow-up levels of HDL-cholesterol are associated with restenosis after PCI.
Results: Decreasing LDL-cholesterol, non-HDL-cholesterol, LDL-C/HDL-C ratio, apolipoprotein B quartiles were associated with a progressively smaller plaque burden in total and diabetic patients. In diabetic patients, further reduction of these parameters was associated with a significantly greater reduction in PV. We also found that patients with lower HDL-cholesterol had a significantly higher incidence of target lesion revascularization.
Conclusions: Early intensive statin therapy in patients after ACS results in remarkable regression of coronary PV. Diabetic patients can have a benefit with more intensive therapy to achieve a lower target level in Japanese.

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Key words; Acute coronary syndrome, Plaque, Statin, Intravascular ultrasound, Diabetes mellitus

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Introduction

Accumulating evidence indicates that statins can reduce both cardiovascular morbidity and mortality in primary and secondary prevention, including patients

with acute coronary syndrome (ACS)[1-3]. Lowering LDL-cholesterol to even lower levels is associated with a further reduction in cardiovascular risk, as shown in the Treatment to New Target (TNT) Study[4] and in the Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) Study of secondary prevention[5]. These studies support the hypothesis that a lower LDL-cholesterol level can induce a greater risk reduction, at least in secondary prevention.

Moreover, many studies with surrogate endpoints show improvement of atherosclerosis by aggressively lowering LDL-cholesterol. Studies using intravascular ultrasound (IVUS) imaging demonstrate that statins attenuate the progression of atherosclerosis or even enable regression of atheromatous plaque[6, 7]. An IVUS study of patients with ACS also demonstrated that atorvastatin can reduce non-culprit coronary plaque in Japanese[8]; however, this was a relatively small trial conducted at a single center. Therefore, a larger multicenter study is expected to address the further roles of statins in patients with ACS.

We previously reported the results of the JAPAN-ACS (Japan Assessment of Pitavastatin and Atorvastatin in Acute Coronary Syndrome) study to address the role of statins in patients with ACS[9]. The JAPAN-ACS was performed as a prospective, randomized open-label parallel-group study with a blind endpoint evaluation at 33 centers, to comparatively examine the effect of 8- to 12-month treatment with pitavastatin and atorvastatin on the degree of coronary plaque regression in non-culprit lesions of the culprit vessel treated by PCI in patients with ACS. This study demonstrated the non-inferiority of pitavastatin 4 mg/day to atorvastatin 20 mg/day, with approximately 17% regression of the plaque volume (PV), suggesting that the effect of inducing plaque regression can be generalized to other statins with similar LDL-lowering effects with atorvastatin. However, in this study, diabetic patients showed less regression of coronary atheroma than non-diabetic patients in spite of similar LDL-cholesterol reduction by statins. In the sub-analysis of JAPAN-ACS we showed significant correlations between the percent change in PV and percent change of the LDL-cholesterol level or follow-up LDL-cholesterol level in diabetic patients[10]; however, a question remains whether there is an appropriate target lipid level to obtain the maximum effect on plaque regression. Therefore, in this sub-analysis of JAPAN-ACS we examined the association of lipid levels after statin therapy with the regression of atherosclerotic coronary lesions in diabetic and total patients after ACS. This analysis was performed in the entire patient population, using the full analysis set of the JAPAN-ACS study, as the regressive effect of the two statins was shown to be equivalent.

In this study we also asked whether baseline and follow-up levels of HDL-cholesterol are associated with restenosis or other cardiovascular events after PCI to show the effect of low HDL-cholesterolemia on coronary events, because previous studies have shown that low levels of HDL-cholesterol can predict major cardiovascular events[11-13].

Methods

Study Design

The present study is a post-hoc sub-analysis of the JAPAN-ACS (Japan Assessment of Pitavastatin and Atorvastatin in Acute Coronary Syndrome) study. JAPAN-ACS is a prospective, randomized open-label parallel group study with blind endpoint evaluation at 33 centers to examine the effect of 8–12 months treatment with pitavastatin versus atorvastatin in coronary plaque regression in non-percutaneous coronary intervention (PCI) sites of the culprit vessel in patients with ACS. The details of the study design have been reported previously[9, 14]. In brief, ACS patients selected in this study were over 20 years of age with hypercholesterolemia and had undergone successful PCI under IVUS guidance. They were found to have coronary plaques (more than 500 μm in thickness, or percent plaque area $\geq$20%) in the culprit vessel at least 5 mm from the PCI- treated lesions. ACS was defined as unstable angina pectoris, non-ST-elevation myocardial infarction (MI) or ST-elevation MI. The diagnosis of ACS was made based on the fulfillment of at least two of the following three criteria: 1) evidence of coronary ischemia on ECG, 2) increase ($\geq$2 times) in the serum creatinine phosphokinase (CK) or CK-MB levels, and/or troponin-T positivity, 3) presence of symptoms suggestive of ACS. Diabetes mellitus and other complications were diagnosed by the attending physicians. This study was conducted according to the 'Declaration of Helsinki', and with the approval of the institutional review boards of all 33 participating institutions. Written informed consent to participate was obtained from all of the patients enrolled.

Intravascular Ultrasound Procedure and Examination

Details of the intravascular ultrasound (IVUS) procedure and examination are documented elsewhere[9]. In brief, following IVUS-guided PCI for the culprit lesion in the patients with ACS, a final IVUS examination for analysis was performed in the culprit vessel. The IVUS catheter Atlantis SR Pro2 (Boston