Table 1. Primer sequences used in real time quantitative RT-PCR.

Gene	Forward	Reverse	GenBank Entry
Mouse and rat E12	ATACAGCGAAGGTGCCCACTT	AAAGGTGGCATAGGCATTCCG	AK017617
Mouse PIASy	CCACCAACCGCATTACTGTCA	TCACCCCAATCGTCTTCAACC	NM_021501
Mouse α-SMA	GCGTGAGATTGTCCGTGACAT	GCGTTCGTTTCCAATGGTGAT	NM_007392
Rat α-SMA	GGCATCCACGAAACCACCTAT	CCTTCTGCATCCTGTCAGCAA	NM_031004
Rat TGF-β	GCTGAACCAAGGAGACGGAAT	CGGTTCATGTCATGGATGGTG	NM_021578
Mouse and rat GAPDH	GCCTCACCCCATTTGATGTTA	GGCAAATTCAACGGCACAG	BC083149
Mouse Ubc9	GATGACTATCCGTCCTCACCACC	GGTGATAGCTGGCCTCCAGTCC	NM_011665.4
Rat Ubc9	AACCCTGATGGCACGATGA	CCCCTTCTTTCCAGGGATAGC	NM_013050.1

doi:10.1371/journal.pone.0041186.t001

Rats were housed under specific pathogen-free conditions. All animal experiments were performed in accordance with institutional guidelines, and the Review Board of Kyoto University granted ethical permission for this study (Permit Number: Med Kyo 09270). Thy1 GN was induced by a single intravenous injection of anti-rat Thy-1 monoclonal antibody (1 mg/kg) (Cedarlane Laboratories, Ontario, Canada) as described elsewhere [3]. These rats were sacrificed to obtain renal specimens, total glomerular RNA, and protein at days 3, 6 and 12 (n = 4 per group). Four rats were injected with vehicle only and sacrificed as controls. Rat glomeruli were isolated from renal cortex of rats using the differential sieving method [39,40]. The purity of the glomeruli was >90%.

Immunohistochemistry

Kidney halves were fixed in methyl Carnoy's solution and embedded in paraffin. Sections (2 μm) were stained with periodic acid-Schiff for routine histology. For the immunohistochemistry, cryopreserved kidney tissues were cut in 4-μm-thick sections, fixed in acetone for 20 min, and treated with 0.3% H_2O_2 in methanol for 15 minutes. Sections were blocked with the appropriate preimmune serum for 60 min at room temperature, followed with primary antibodies anti-PIASy antibody (clone PIA4, Sigma-Aldrich), anti-E12 antibody (Santa Cruz), anti-α-SMA antibody (clone 1A4, Sigma-Aldrich), and anti-SUMO1 antibody (Zymed Laboratories, CA, USA).

Reverse Transcription-Polymerase Chain Reaction

1 μg of total RNA was used to prepare complementary DNA (cDNA) with Superscript III reverse transcriptase (Invitrogen). 5 μl of cDNA was used as a template in the PCR reaction. PCR amplification was performed using Taq polymerase with primers. The oligonucleotide primers are listed in Table 2.

PCR was performed under the following conditions: 94°C for 2 minutes followed by 25 cycles at 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 minute, ending with a final extension at 72°C for10 minutes. The PCR products were run on 1% agarose gels and visualized by ethidium bromide staining.

Cell Proliferation Assay with Transient Transfections of siRNA

BrdU ELISA was performed according to the manufacturer's instructions. Briefly, 2500 mouse MCs/well were plated out in 96-well flat-bottomed microtiter plates in B medium/10% FCS. Six hours later, siRNA for PIASy, E12 and the control (Invitrogen) were transfected with Lipofectamine RNAi/MAX reagent (Invitrogen) according to the manufacturer's instructions. The proliferation of MCs was determined at 48 hours after the siRNA transfections using a colorimetric immunoassay, based on the measurement of BrdU incorporation during DNA synthesis (Amersham Biosciences). BrdU was added to the medium for the final 2 h of treatment. Cells were incubated for 30 min with diluted, peroxidase-conjugated anti-BrdU antibody. Absorbance was assessed at 370 nm with 492 nm as the reference wavelength utilizing a microplate ELISA reader. Appropriate control wells were used in each experiment.

Statistical Analysis

The data are expressed as the means ± SD. Comparison among more than two groups was performed by one-way analysis of variance followed by the post hoc analysis (Bonferroni/Dunn test) to evaluate statistical significance. All analyses were performed using StatView (SAS Institute, Cary, NC). Statistical significance was defined as $P<0.05$.

Table 2. Primer sequences used in RT-PCR.

Gene	Forward	Reverse	GenBank Entry	Product size (bp)
Mouse PIAS1	TCCTGCTGTAGATACAAGCTAC	TGCCAAAGATGGACGCTGTGTC	NM_019663	394
Mouse PIASX	GACTTTGCTTGGCAGAGACC	AAAGGGCACATCAAGGACAC	NM_008602	409
Mouse PIAS3	GTGGACATGCATCCTCCTCT	GCGTTCGTTTCCAATGGTGAT	NM_146135	405
Mouse PIASy	AGACCCTTAAGCCGGAGGTA	GTGGCCGAGGACAGATACAT	NM_021501	391

doi:10.1371/journal.pone.0041186.t002

Supporting Information

Figure S1 Sumoylation of E12 is predominantly enhanced by PIASy among PIAS family members. (**A**) 1 μg of total RNA from cultured mouse MCs or mouse testis was used to prepare complementary DNA (cDNA). PCR was done with oligonucleotide pairs for PIAS family members using 5 μl of cDNA. bp, base pairs. (B) 293T cells were cotransfected with 2 μg of plasmid expressing myc-E12 together with (+) or without (−), 2 μg of plasmid expressing HA-SUMO-1, and 2 μg of plasmid expressing GFP-PIAS1 (1), -PIAS3 (3), -PIASXα (Xα), -PIASXβ (Xβ) and -PIASy (y). Upper panel, Cell lysates were subjected to immunoblotting with anti-myc antibody. Middle and Lower panel, Cell lysates were immunoprecipitated (IP) with anti-myc antibody. The immunoprecipitates were subjected to SDS-PAGE and analyzed by Western blotting (WB) with anti-myc antibody. After ECL development, the filter shown in the middle panel was stripped and reproved with anti-HA antibody (lower panel).
(TIF)

Figure S2 PIASy promotes SUMO-1 and SUMO-3 modification of E12 in vivo. 293T cells were cotransfected with (+) or without (−) 2 μg of plasmid expressing myc-E12, 2 μg of plasmid expressing HA-SUMO-1 or HA-SUMO-3, and 2 μg of plasmid expressing flag-PIASy. Upper panel, Cell lysates were subjected to immunoblotting with anti-myc antibody. Middle and Lower panel, Cell lysates were immunoprecipitated (IP) with anti-myc antibody. The immunoprecipitates were subjected to SDS-PAGE and analyzed by Western blotting (WB) with anti-myc antibody. After ECL development, the filter shown in the middle panel was stripped and reproved with anti-HA antibody (lower panel).
(TIF)

Figure S3 Effect of PIASy on mutant E12 (K/R)-induced α-SMA gene expression. Mouse MCs (0.15×10^5) were plated in 24-well plates, and six hours later, cotransfected with 50 ng of the PIASy expression plasmid, 25 ng of the wild- type E12 (WT) or mutant E12 (K/R) expression plasmid, and 150 ng of the reporter construct. Luciferase activities in lysates prepared 36 hours post-transfection were measured. Luciferase activities were normalized to Renilla luciferase activities derived from cotransfected pRL-SV40-Luc. The relative activities with wild-type E12 or mutant E12 (K/R) alone were designated as 100% (lanes 1 and 2). The percentage of decrease by overexpressing PIASy was compared between wild-type E12 and mutant E12. Results are the mean ± SD of data by taking the average of triplicates.
(TIF)

Acknowledgments

We are grateful to Professor Shimotohno for help in sumoylation assay. We thank Ayumi Hosotani (Kyoto Universtity) for excellent technical assistance.

Author Contributions

Conceived and designed the experiments: TD H. Arai. Performed the experiments: KT H. Abe T. Matsubara TH TO. Analyzed the data: T. Matsubara MA AM AF TK NI. Contributed reagents/materials/analysis tools: KT H. Abe T. Matsubara TH TO T. Murakami. Wrote the paper: KT H. Abe T. Matsubara H. Arai TD.

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Endocrine Journal 2012, **59** (12), 1077-1084

ORIGINAL

Ezetimibe, an inhibitor of Niemann-Pick C1-like 1 protein, decreases cholesteryl ester transfer protein in type 2 diabetes mellitus

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Abstract. To address the effects of ezetimibe on high-density lipoprotein (HDL) metabolism, the HDL subclasses, cholesteryl ester transfer protein (CETP), and lecithin-cholesterol acyltransferase (LCAT) were measured in patients with type 2 diabetes mellitus (T2DM). Twenty-three hypercholesterolemic patients with T2DM were treated with 10 mg of ezetimibe daily for 12 weeks. Plasma total cholesterol (TC), low-density lipoprotein (LDL)-cholesterol (C), HDL-C, HDL_2-C, HDL_3-C, CETP mass, and LCAT activity were measured. HDL-C and HDL_2-C increased by 5% ($p<0.05$) and 12% ($p<0.01$), respectively, in response to ezetimibe. Of the 23 patients, 21 had decreased CETP mass, which led to an average reduction of 20% ($p<0.0001$). LCAT activity also decreased by 6% ($p<0.01$). A significant positive correlation was found in the changes from baseline between HDL_2-C and CETP mass, whereas a significant inverse relationship was observed between HDL_3-C and CETP mass. Furthermore, the change in HDL-C was positively correlated with the change in LCAT activity. In conclusion, ezetimibe may affect HDL metabolism and reverse cholesterol transport, especially CETP, in T2DM. These observations may provide some insights into how ezetimibe prevents atherosclerosis.

Key words: Ezetimibe, Type 2 diabetes, High-density lipoproteins, Lecithin-cholesterol acyltransferase, Cholesteryl ester transfer protein

EZETIMIBE is a drug that lowers plasma low-density lipoprotein (LDL)-cholesterol (C) and non-high-density lipoprotein (HDL)-C by inhibiting intestinal cholesterol absorption [1, 2]. It has been proposed that ezetimibe inhibits Niemann-Pick C1-like 1 (NPC1L1) protein [3], which is highly expressed in the brush border membrane of the enterocyte, where it facilitates intestinal cholesterol absorption. Furthermore, humans, but not mice, also express NPC1L1 in the liver as much as in the small intestine [3, 4], suggesting the essential role of hepatic NPC1L1 in hepatic and plasma lipid metabolism. In fact, ezetimibe ameliorates non-alcoholic steatohepatitis in humans [5].

Elevated LDL-C and decreased HDL-C levels are conventional risk factors for cardiovascular disease in patients with type 2 diabetes mellitus (T2DM). Ezetimibe has been shown to be effective in reducing plasma LDL-C and non-HDL-C levels in hypercholesterolemic patients with T2DM [6-8]. In addition, a meta-analysis of randomized, controlled trials in which diabetic patients accounted for 2-8% of the patients also showed that ezetimibe monotherapy significantly increased plasma HDL-C levels by 3.0% in patients with primary hypercholesterolemia [9], though the precise mechanism has not yet been determined.

Lecithin-cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) are key enzymes in the reverse cholesterol transport system and are involved in HDL metabolism [10, 11]. LCAT converts free cholesterol into cholesteryl esters (CEs), which are then sequestered into the core of lipoprotein particles, making the spherical HDL_3 particles that are converted into HDL_2 particles by the phospholipid transfer protein (PLTP)-activated fusion of smaller

Submitted Apr. 6, 2012; Accepted Jul. 10, 2012 as EJ12-0132
Released online in J-STAGE as advance publication Jul. 26, 2012

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HDL particles. CETP mediates neutral transport of CEs and triglycerides (TGs) between lipoproteins, resulting in the transfer of CEs in exchange for TGs from HDL to atherogenic lipoproteins such as LDL and very low-density lipoprotein (VLDL). TGs accumulating in HDL are then hydrolyzed by hepatic lipase (HL). Moreover, CETP transfers CEs between HDL particles and generates HDL_2 and pre-β HDL from HDL_3 particles.

We recently reported that pitavastatin decreases CETP mass and LCAT activity in hypercholesterolemic patients [12]. Furthermore, genome-wide association studies involving >100,000 individuals of European ancestry identified LCAT and CETP as significantly associated loci with plasma HDL-C [13]. In the present study, the effects of ezetimibe on the HDL subclasses, CETP mass, and LCAT activity were assessed in T2DM, and their relationships were examined.

Subjects and Methods

Hypercholesterolemic patients with T2DM (n = 23; men/women = 13/10, age = 59.1 ± 9.5 years) were enrolled in the study (Table 1). They received sulfonylureas (n=11), glinides (n=3), pioglitazone (n=3), biguanides (n=6), α-glucosidase inhibitors (n=2), and dipeptidyl peptidase-4 inhibitors (n=1). According to the plasma LDL-C levels as recommended by the guidelines of the Japan Atherosclerosis Society [14], patients with plasma LDL-C >120 mg/dL were the subjects of this study. None of the patients were taking any lipid-lowering drugs such as statins and fibrates. The patients were given 10 mg of ezetimibe daily for 12 weeks, while other drugs for other diseases, such as diabetes and hypertension, were left unchanged. The study was approved by the Ethics Committee of Jichi Medical University, and all patients gave their written, informed consent.

At baseline and after 12 weeks of treatment, blood samples were collected after a 12-h fasting period, and the following parameters were determined in plasma: total cholesterol (TC), TGs, HDL-C, HDL_2-C, HDL_3-C, LCAT activity, CETP mass, sitosterol, campesterol, cholestanol, lathosterol, high-sensitivity C-reactive protein (hs-CRP), glucose, and hemoglobin A1c (HbA1c).

Plasma TC, TGs, and HDL-C were measured by automated enzymatic assays. LDL-C levels were calculated by the Friedewald formula, since all patients had TG levels <400 mg/dL. HDL_2 and HDL_3 were isolated by density gradient ultracentrifugation, and the cholesterol levels in these lipoproteins were measured enzymatically. CETP mass was measured by ELISA assay (CETP ELIZA-DAIICHI, Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). LCAT activity was measured using dipalmitoyl lecithin as the substrate [15]. Sitosterol, campesterol, cholestanol, and lathosterol were measured by gas-liquid chromatography [16]. The hs-CRP level was determined using an

Table 1 Patients' characteristic at baseline and after 12 weeks of ezetimibe treatment

parameter	at baseline	after 12 weeks
Sex (Men/women)	10/13	-
Age	59.1 ± 9.5	-
TC (mg/dL)	232.7 ± 33.9	201.6 ± 28.1***
TG (mg/dL)	127.8 ± 54.3	118.65 ± 67.3
LDL-C (mg/dL)	152.8 ± 26.8	121.0 ± 20.0***
HDL-C (mg/dL)	53.7 ± 13.4	56.4 ± 17.1*
HDL_2-C (mg/dL)	33.1 ± 11.0	37.1 ± 14.4**
HDL_3-C (mg/dL)	19.8 ± 4.1	20.0 ± 3.3
LCAT (U)	116.3 ± 15.5	108.8 ± 14.0**
CETP (μg/mL)	2.5 ± 0.5	2.0 ± 0.5***
Sitosterol (μg/mL)	3.9 ± 1.5	2.0 ± 0.8***
Campesterol (μg/mL)	6.8 ± 2.8	3.2 ± 1.3***
Cholestanol (μg/mL)	3.0 ± 0.7	2.7 ± 0.5*
Lathosterol (μg/mL)	4.4 ± 2.4	4.9 ± 2.1
Glucose (mg/mL)	128.0 ± 27.4	136.6 ± 35.4
HbA1c (%)	7.1 ± 0.8	7.2 ± 0.9
Hs-CRP (ng/mL)	100.0 ± 277.8	58.4 ± 99.2

Results were expressed as mean ± S.D. ***$p < 0.0001$, *$p < 0.05$, **$p<0.01$ vs. baseline

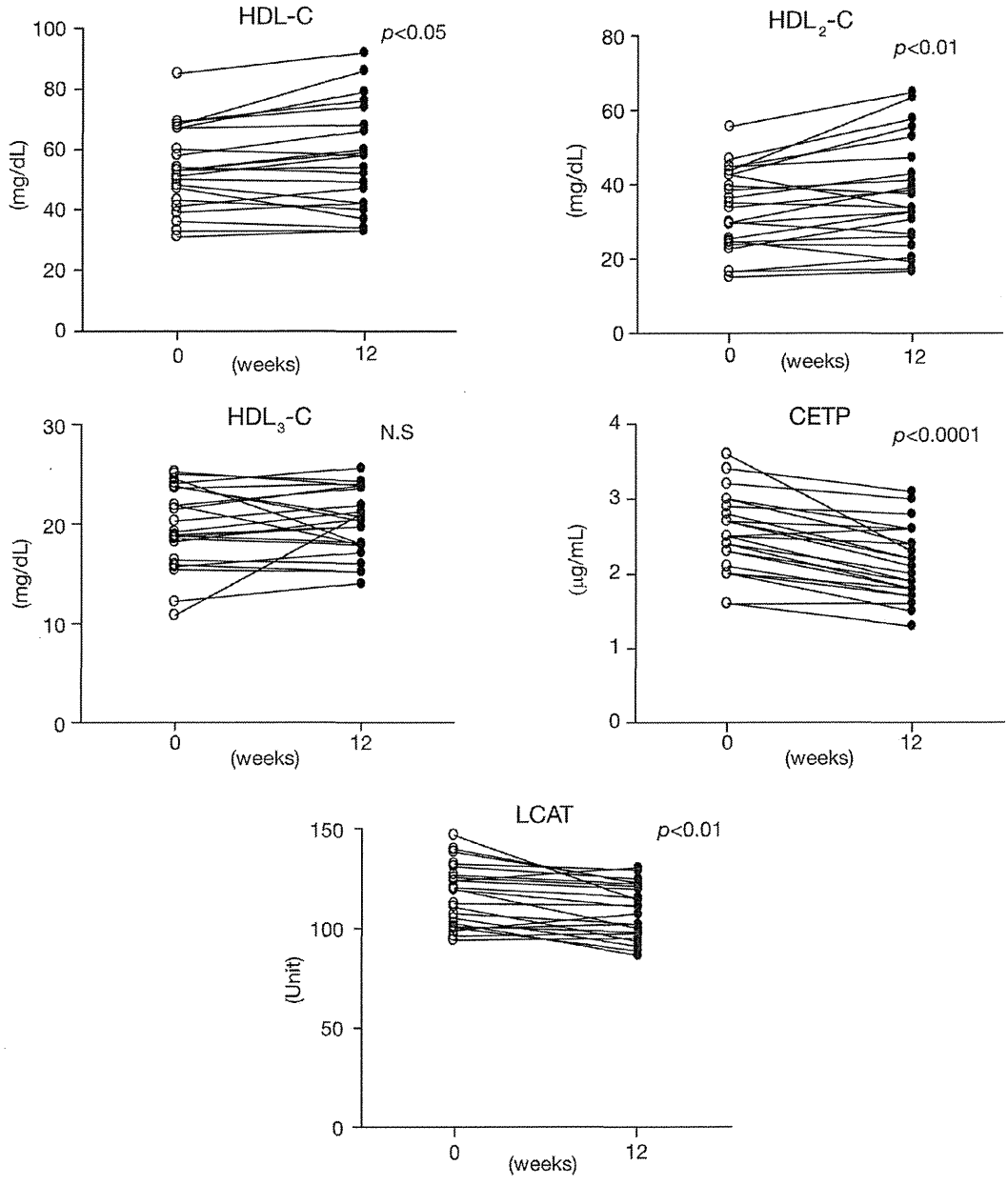

Fig. 1 HDL subclasses, CETP mass, and LCAT activity at baseline and at 12 weeks of ezetimibe treatment. Ezetimibe (10 mg/daily) was given for 12 weeks, and blood was collected before and after ezetimibe treatment. Plasma HDL-C, HDL_2-C, HDL_3-C, CETP mass, and LCAT activity were measured and compared before and after treatment. Data are expressed as means ± S.D. N.S: not significant.

ultra-high-sensitivity latex turbidimetric immunoassay (Bering Nephelometry, Tokyo, Japan).

Student's paired *t*-test was used for statistical analyses. Univariate Pearson's correlation coefficient analysis was performed to estimate the relationship between two variables. A p value of <0.05 was considered significant.

Results

Plasma TC and LDL-C levels decreased by 13% (p<0.0001) and 21% (p<0.0001), respectively, when patients were treated with ezetimibe for 12 weeks (Table 1, Fig. 1). Ezetimibe did not affect TG levels. HDL-C and HDL_2-C levels were marginally but significantly

increased after treatment. The cholesterol absorption markers, sitosterol, campesterol, and cholestanol, were significantly decreased by 49%, 53%, and 10%, respectively (Table 1). In contrast, no significant change was observed in lathosterol, a marker of cholesterol synthesis. There was no significant relationship in the changes from baseline between each cholesterol synthesis or absorption marker and LDL-C levels. The changes in HDL-C and HDL_2-C also did not have a significant relationship with the changes in any cholesterol absorption markers. The change in HDL_3-C had a significant positive correlation with the change in campesterol ($p<0.01$), but not sitosterol and cholestanol (data not shown).

After 12 weeks of treatment, 21 of the 23 patients had decreased CETP mass, which led to an average reduction of 20% ($p<0.0001$) (Table 1, Fig. 1). A significant positive correlation was found in the changes from baseline between HDL_2-C levels and CETP mass, whereas a significant inverse relationship was observed in the changes from baseline between HDL_3-C levels and CETP mass (Fig. 2). The change in HDL-C levels was not associated with that in CETP mass. LCAT activity was also decreased by 6% ($p<0.01$) with ezetimibe (Table 1, Fig. 1). A significant positive correlation was observed in the change from baseline between LCAT activity and HDL-C, but not HDL_2-C or HDL_3-C levels (Fig. 3). The change in LDL-C was not associated with the change in CETP mass or LCAT activity (data not shown).

Plasma hs-CRP, glucose, and HbA1c levels were not affected by ezetimibe therapy.

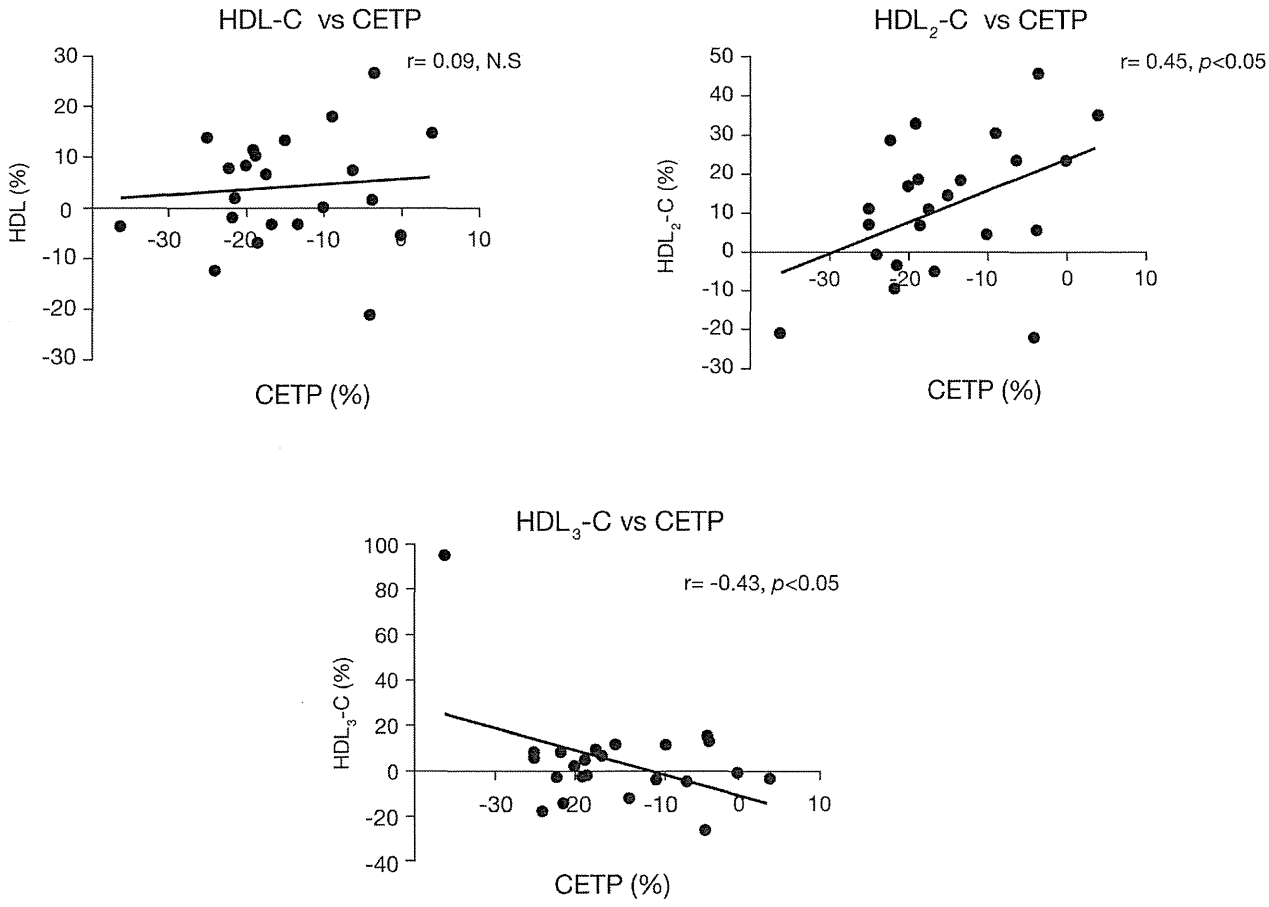

Fig. 2 Relationships between HDL subclasses and CETP mass. Ezetimibe (10 mg/daily) was given for 12 weeks, and blood was collected before and after ezetimibe treatment. The percent changes in HDL subclasses and CETP mass in response to ezetimibe treatment were calculated, and their relationships were examined. Data are expressed as means ± S.D. N.S: not significant.

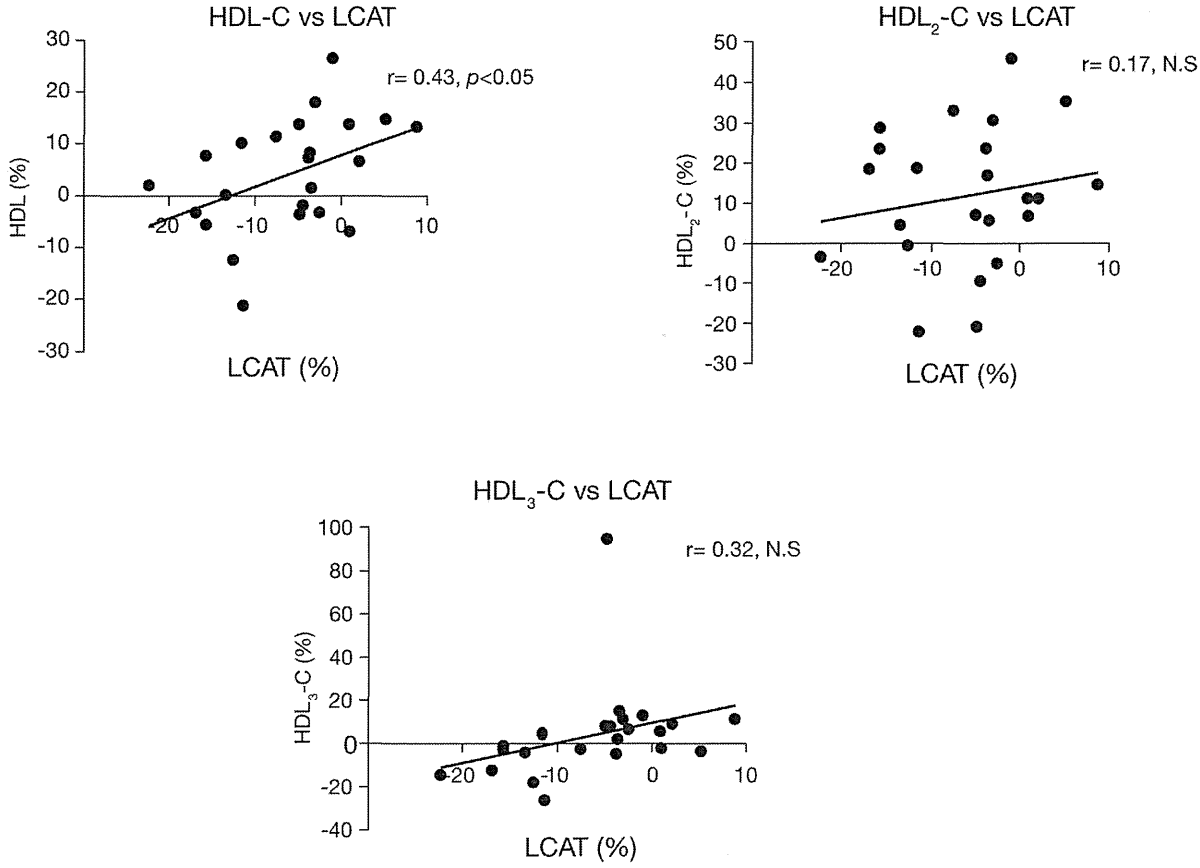

Fig. 3 Relationships between HDL subclasses and LCAT activity. Ezetimibe (10 mg/daily) was given for 12 weeks, and blood was collected before and after ezetimibe treatment. The percent changes in HDL subclasses and LCAT activity in response to ezetimibe treatment were calculated, and their relationships were compared. Data are expressed as means ± S.D. N.S: not significant.

Discussion

This is the first study to demonstrate the effects of ezetimibe on HDL subclasses, CETP mass, and LCAT activity in hypercholesterolemic patients with T2DM. Because these patients had not used any other drugs for hyperlipidemia, the observations in the present study simply reflect the effects of ezetimibe.

As expected, ezetimibe significantly reduced plasma TC and LDL-C levels. Markers for cholesterol absorption were also significantly decreased, but lathosterol, a marker of cholesterol synthesis, was not affected by ezetimibe therapy. In contrast to a study in which there was a positive correlation in the changes between a cholesterol absorption/synthesis marker and LDL-C in response to ezetimibe treatment [7], such a correlation was not observed in the present study. The reason for this discrepancy is unclear, but the results of the present study imply that the LDL-lowering effect of ezetimibe is not simply determined by the prevention of cholesterol absorption in T2DM. The reduction of CETP and LCAT by ezetimibe may also affect LDL metabolism, though a significant correlation was not found in the changes from baseline between CETP mass or LCAT activity and LDL-C.

In the present study, it was demonstrated that HDL-C and HDL_2-C, but not HDL_3-C, were significantly increased at 12 weeks of ezetimibe administration. Because HDL-C is 10-20% lower in T2DM [17], which is mainly the result of a decrease in HDL_2 and to some extent in HDL_3, ezetimibe is the drug to improve the decreased HDL levels in T2DM. Why ezetimibe modulates HDL metabolism remains unclear. It has been reported that plasma HDL-C levels are positively correlated with markers of cholesterol absorption in metabolic syndrome cases and healthy individuals [18, 19]. In the present study, the change in HDL_3-C was significantly associated with the change in campesterol.

However, the change in HDL_3-C was not associated with the changes in other cholesterol absorption markers such as sitosterol and cholestanol. Furthermore, the changes in HDL-C and HDL_2-C were not associated with changes in any cholesterol synthesis markers. Taken together with the observation that the changes in LDL-C was not associated with the changes in any cholesterol absorption markers, ezetimibe may be involved in plasma lipoprotein metabolism by means of not only intestinal but also hepatic lipid metabolism, because humans express NPC1L1, a target of ezetimibe, in the liver as much as in the small intestine.

Interestingly, the change in HDL_2-C levels was positively correlated with that in plasma CETP mass, whereas an inverse relationship was observed in the change between HDL_3-C levels and CETP mass (Fig. 2). Moreover, the change in HDL-C was positively correlated with the change in LCAT activity (Fig. 3). With regard to the relationship between CETP and HDL_2-C or HDL_3-C in the present study, these observations are contrary to the function of CETP, in which inhibition of CETP causes an imbalance between PLTP and CETP and results in the generation of larger HDL particles such as HDL_2, whereas smaller HDL particles such as HDL_3 are diminished [10]. Thus, these observations must be interpreted carefully. Indeed, the significant relationships in the changes between CETP and HDL_2-C or HDL_3-C disappeared after exclusion of one patient who had extremely high HDL_3-C elevations with ezetimibe. Moreover, in addition to HDL-C, the change in HDL_3-C came to have a significant positive correlation with the change in LCAT activity ($p<0.01$) after exclusion of the patient who had extremely high HDL_3-C elevations with ezetimibe. Because many other proteins, e.g., HL and PLTP, are also involved in reverse cholesterol transport and HDL metabolism [11, 17], ezetimibe may affect these proteins, which acted in concert in HDL_2 and HDL_3 remodeling in the present study.

It should be noted that 91% of the patients had decreased CETP mass by an average of 20% in response to ezetimibe. This may simply reflect the effects of lipid-lowering drugs, because statins also decrease CETP mass in hypercholesterolemic patients [12]. Alternatively, ezetimibe may regulate the CETP gene at transcriptional levels. The CETP gene has sterol regulatory elements (SREs) in the promoter where sterol regulatory element-binding proteins (SREBPs) bind and then activate the CETP gene [20, 21]. The CETP gene has been shown to be activated by SREBP-1a rather than SREBP-2 in the liver and human liposarcoma cells, an adipocytic cell line [20, 21]. Furthermore, ezetimibe upregulates hepatic SREBP-2 and downregulates hepatic SREBP-1c in wild-type mice fed a high-fat diet for 10 weeks [22]. Taken together, this suggests that ezetimibe suppresses CETP gene expression by modulating hepatic SREBPs. The mechanism by which ezetimibe decreased plasma LCAT activity in the present study is still unknown. This is simply secondary to the reduction of CETP by ezetimibe. Alternatively, ezetimibe may affect the gene expression levels of hepatic LCAT. Indeed, fibrates, which are drugs for the treatment of hyperlipidemia, have been shown to decrease hepatic LCAT mRNA at the transcriptional level, thereby reducing plasma LCAT activity [23]. Further study will be needed to determine whether ezetimibe decreases hepatic LCAT mRNA.

The role of ezetimibe in the prevention of atherosclerosis is still a matter of debate [24-26]. In the SEAS and ENHANCE trials, ezetimibe did not reduce the composite outcome of combined aortic valve events and ischemic events or carotid-artery intima-media thickness in patients with familial hypercholesterolemia [25, 26]. In contrast, reduction of plasma LDL-C levels by the combination of simvastatin and ezetimibe decreased the incidence of major atherosclerotic events in patients with advanced chronic disease in the SHARP trial [24]. Taken together with the fact that a CETP inhibitor has attracted attention as an anti-atherosclerotic agent [27, 28], ezetimibe may suppress atherosclerosis by means of its inhibitory effect on CETP.

In conclusion, ezetimibe modulates HDL metabolism and reverse cholesterol transport, especially CETP, in T2DM. These effects of ezetimibe, beyond the well-known effects on LDL, may provide some insights into how ezetimibe prevents atherosclerosis in humans.

Conflict of Interest

The authors have no further conflicts of interest to disclose.

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CARDIO VASCULAR DIABETOLOGY

ORIGINAL INVESTIGATION Open Access

High HbA1c levels correlate with reduced plaque regression during statin treatment in patients with stable coronary artery disease: Results of the coronary atherosclerosis study measuring effects of rosuvastatin using intravascular ultrasound in Japanese subjects (COSMOS)

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Abstract

Background: The incidence of cardiac events is higher in patients with diabetes than in people without diabetes. The Coronary Atherosclerosis Study Measuring Effects of Rosuvastatin Using Intravascular Ultrasound in Japanese Subjects (COSMOS) demonstrated significant plaque regression in Japanese patients with chronic coronary disease after 76 weeks of rosuvastatin (2.5 mg once daily, up-titrated to a maximum of 20 mg/day to achieve LDL cholesterol <80 mg/dl).

Methods: In this subanalysis of COSMOS, we examined the association between HbA1c and plaque regression in 40 patients with HbA1c ≥6.5% (high group) and 86 patients with HbA1c <6.5% (low group).

Results: In multivariate analyses, HbA1c and plaque volume at baseline were major determinants of plaque regression. LDL cholesterol decreased by 37% and 39% in the high and low groups, respectively, while HDL cholesterol increased by 16% and 22%, respectively. The reduction in plaque volume was significantly ($p = 0.04$) greater in the low group (from 71.0 ± 39.9 to 64.7 ± 34.7 mm^3) than in the high group (from 74.3 ± 34.2 to 71.4 ± 32.3 mm^3). Vessel volume increased in the high group but not in the low group (change from baseline: +4.2% vs −0.8%, $p = 0.02$). Change in plaque volume was significantly correlated with baseline HbA1c.

Conclusions: Despite similar improvements in lipid levels, plaque regression was less pronounced in patients with high HbA1c levels compared with those with low levels. Tight glucose control during statin therapy may enhance plaque regression in patients with stable coronary disease.

Trial registration: ClinicalTrials.gov, Identifier NCT00329160

Keywords: Atherosclerosis, Coronary artery disease, Intravascular ultrasound, HbA1c, Rosuvastatin

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Background

Intravascular ultrasound (IVUS) has been widely applied in recent clinical trials focusing on coronary atherosclerosis [1–6] and has provided new insights into the pathophysiology of atherosclerotic plaque progression and regression.

Plaque regression has been documented in several studies using IVUS to investigate the effects of lipid-lowering therapies, as well as those of anti-hypertensive drugs and anti-diabetic drugs [7–10]. The results of those studies indicate that plaque regression is influenced by several clinical factors, including lipid profiles, diabetic status and blood pressure. Interestingly, diabetic status appears to be one of the major determinants of plaque progression and/or regression [11]. Moreover, diabetes mellitus is an important residual risk factor for prevention of atherosclerotic disease following LDL cholesterol (LDL-C)-lowering therapy [12].

HbA1c has long been used as a marker for glycaemic control in patients with diabetes mellitus and in people with prediabetes [13]. Several epidemiological studies have shown a positive association between HbA1c and risk of cardiovascular disease [14,15]. However, it is unknown whether glucose control is associated with the change in plaque volume following interventions to treat dyslipidaemia, for example.

The Coronary Atherosclerosis Study Measuring Effects of Rosuvastatin Using Intravascular Ultrasound in Japanese Subjects (COSMOS, ClinicalTrials.gov Identifier: NCT 00329160) was a multicentre, open-label trial conducted in 37 institutions in Japan. The study examined the effects of rosuvastatin on plaque volume in 214 hypercholesterolaemic patients with stable coronary artery disease using IVUS [5,6]. In this trial, rosuvastatin decreased LDL-C and increased HDL cholesterol (HDL-C), which significantly reduced plaque volume by 5%. Considering the limited understanding of whether glucose control is associated with changes in plaque volume, we conducted a subanalysis of the COSMOS study to analyse the relationship between HbA1c and change in plaque volume following 76 weeks of treatment with rosuvastatin.

Methods

Study design

The study design and primary outcomes of the COSMOS study are reported in more detail elsewhere [5,6]. Briefly, the COSMOS study was a 76-week, open-label, multicentre study to evaluate the effects of rosuvastatin on coronary artery atheroma volume, as measured by IVUS, in patients with stable coronary artery disease. Eligible patients were started on 2.5 mg rosuvastatin once daily, which was up-titrated to a maximum of 20 mg/day to achieve a treatment goal of LDL-C <80 mg/dl.

Subjects attended follow-up visits every 4 weeks for 76 weeks after commencing treatment with rosuvastatin. IVUS and coronary angiography (CAG) were performed at baseline and at week 76. All subjects signed an informed consent form. This study was approved by institutional review boards or independent ethics committees at all participating centres.

Patients

A total of 126 patients completed the COSMOS study and were included in the study database used for this subanalysis. These patients met all of the following inclusion criteria: 20–75 years old; undergoing elective CAG or percutaneous coronary intervention (PCI); serum LDL-C ≥140 mg/dl or TC ≥220 mg/dl in untreated patients, or LDL-C ≥100 mg/dl or TC ≥180 mg/dl in patients already treated with lipid-lowering agents; at least one significant stenosis of ≥75% as a candidate for PCI; and at least one untreated non-culprit target lesion with ≤50% stenosis that could be imaged by IVUS. Exclusion criteria included the following: acute myocardial infarction within 72 h of the start of the study; heart failure of New York Heart Association class III or IV; secondary hyperlipidaemia; treatment with cyclosporine on haemodialysis; left main coronary artery disease with >50% stenosis; uncontrolled hypertension (diastolic blood pressure ≥110 mmHg or systolic blood pressure ≥200 mmHg for all measurements during the screening period); uncontrolled diabetes (HbA1c ≥9.5%); active liver disease or liver dysfunction with ≥2.5× the upper limit of normal (ULN) level for alanine aminotransferase, aspartate aminotransferase or alkaline phosphatase, or total bilirubin ≥3.0 mg/dl; creatinine clearance <30 ml/min or serum creatinine >2.0 mg/dl; and serum creatine kinase >3× the ULN. To ensure the patient population resembled that of actual clinical practice, we did not exclude patients who were taking lipid-lowering drugs at study entry.

IVUS procedure

IVUS was used to examine plaque volume, lumen volume and vessel volume at baseline and after 76 weeks of treatment. After administering 100–300 μg of intracoronary nitroglycerine, the catheter was advanced into the target vessel and the transducer was positioned as distal to the target lesion as possible. The operator had a motor driving pullback system that progressively withdrew the transducer at a speed of 0.5 mm/second. A Clearview®, Galaxy™ or Galaxy2™ ultrasound system with the Atlantis™ SR Pro 2 40 MHz imaging catheter (Boston Scientific, Natick, MA, USA) was used for both the baseline and follow-up examinations. The images were optimised visually by manipulating the system settings. IVUS images were recorded on super-VHS videotapes or Digital Video Disk plus Re-Writable disks.

IVUS imaging analysis

Plaque volume was assessed by volumetric analysis using the echoPlaque2 system (Indec Systems Inc., Santa Clara, CA, USA). Baseline and follow-up IVUS images were reviewed side-by-side on a display, and the target segment was selected. The target segment to be monitored was determined in a non-PCI site (>5 mm proximal or distal to the PCI site) with a reproducible feature such as a side branch and its bifurcation, calcifications, or stent edges. A series of cross-sectional images taken every 0.09 mm were measured by manual on-screen planimetry. IVUS tracing was performed in accordance with the standards of the American College of Cardiology and the European Society of Cardiology [16]. Manual planimetry was used to trace the leading edges of the luminal and external elastic membrane (EEM) borders. The images were logged and analysed by two experienced technicians in a central laboratory who were blinded to the patient's profile, imaging date and baseline/follow-up labels. The accuracy and reproducibility of this method has been established previously [16].

IVUS measurements

The IVUS parameters analysed in this study were percent change in plaque volume of the target lesion from baseline to follow-up at week 76, percent changes in lumen volume and vessel volume; and changes in plaque area, lumen area and vessel area at the plaque segment with a maximum baseline plaque area.

Percent change in total atheroma volume (TAV) was calculated as follows: percent change in TAV = [TAV(follow-up) − TAV(baseline)]/[TAV(baseline) × 100]. TAV was calculated as the sum of the difference between EEM and luminal area across all evaluated frame images: $TAV = \sum(EEM_{CSA} - lumen_{CSA})$, where CSA = cross-section area. All IVUS measurements were performed at a central laboratory.

Laboratory tests

All laboratory measurements were performed at a central clinical laboratory (SRL, Inc., Tokyo, Japan). HbA1c (%) is given as National Glycohemoglobin Standardization Program (NGSP) equivalent values (%), which were calculated using the following formula [17]: HbA1c (%) = HbA1c (Japan Diabetes Society value; %) + 0.4%. LDL-C was calculated using Friedewald's formula [18].

Statistical analysis

We used the original COSMOS study database for this subanalysis [5]. To identify the factors associated with the percent change in plaque volume, univariate analysis was performed with 70 baseline characteristics and laboratory profiles. Eight factors were significantly associated with the percent change in plaque volume. Multivariate regression analysis was performed using the variables shown to be significant in univariate analyses with stepwise model selection using $p < 0.05$ to retain variables in the model.

We next divided the subjects into two groups according to HbA1c (low, <6.5%; high, ≥6.5%) based on the American Diabetes Association criteria [19]. Continuous variables were compared between the two groups using two-sample *t*-tests, while comparisons between baseline and follow-up were made using one-sample *t*-tests. Categorical variables were compared between the two groups using χ^2 or Fisher's exact tests. Finally, the general linear model was used to examine the relationship between change in plaque volume and HbA1c. The two-sided significance level was set at 5%.

Analyses were performed using SAS software, version 9.1.3 (SAS Institute Inc., Cary, NC, USA). Efficacy data are reported as means ± SD.

Results

As previously reported, 126 patients completed the 76-week study and changes in lipid levels and plaque volume following rosuvastatin treatment were successfully measured [5]. The mean rosuvastatin dose at follow-up IVUS was 16.9 ± 5.3 mg/day, and 92/126 patients (72.2%) were on the maximum rosuvastatin dose (20 mg/day). In these patients (mean ± SD; age 62.6 ± 7.7 years, BMI 25.0 ± 3.3 kg/m^2, 76.2% males, 37.3% had diabetes), the percent changes in LDL-C and HDL-C were −38.6 ± 16.9% and +19.8 ± 22.9% (both, $p < 0.0001$). The percent change in plaque volume was −5.1 ± 14.1% ($p < 0.0001$). HbA1c increased slightly but not significantly from 5.92 ± 0.98% at baseline to 6.25 ± 1.00% at follow-up ($p = 0.3205$).

Determinants of plaque progression

The results of univariate analyses to identify factors associated with change in plaque volume are shown in Table 1. BMI, HbA1c and the use of sulfonylureas were positively associated with plaque progression. In contrast, the use of

Table 1 Univariate analyses for the determinants of plaque progression

Factor	β	95% CI	p Value
BMI	0.766	0.017, 1.514	0.045
Use of ACE inhibitors	−6.655	−12.604, −0.706	0.0286
Use of sulfonylureas	6.672	0.100, 13.244	0.0467
HbA1c	2.941	0.452, 5.429	0.0209
Evaluated plaque length	−0.997	−1.698, −0.296	0.0057
Plaque volume	−0.112	−0.174, −0.049	0.0006
Vessel volume	−0.047	−0.080, −0.013	0.0067
Plaque area	−0.918	−1.598, −0.238	0.0086

BMI, body mass index.
Parameters with p values <0.05 by univariate analysis are listed.

ACE inhibitors, plaque length and plaque volume were negatively associated with plaque progression. The presence of diabetes mellitus was not significantly associated with change in plaque volume. Multivariate analysis (Table 2) revealed that HbA1c and plaque volume at baseline were independent determinants of the change in plaque volume.

Characteristics of the high and low HbA1c groups

The 126 patients were divided into those with HbA1c <6.5% (n = 80; low) and those with HbA1c ≥6.5% (n = 46; high), with mean HbA1c levels at baseline of 5.35 ± 0.32 and 7.14 ± 0.80%, respectively ($p < 0.0001$). The baseline characteristics of the two groups were generally similar except for the prevalence of diabetes mellitus, which was higher in the high HbA1c group than in the low HbA1c group, as would be expected. As a result, more patients in the high HbA1c group had received treatment with oral antidiabetic drugs/insulin. Although the location of the lesion was significantly different between the two groups, this factor was not associated with the change in plaque volume. Other characteristics were not significantly different between the two groups (Table 3).

Lipid profiles

LDL-C levels decreased from 140.4 to 81.8 mg/dl (by 39.2%) and from 139.7 to 85.3 mg/dl (by 37.3%) in the high and low HbA1c groups, respectively (Table 4). LDL-C levels at baseline and follow-up and the percent change during the observation period were generally comparable between the two groups. HDL-C levels increased in both groups, although the magnitude of increase was slightly, but significantly, greater in patients with HbA1c <6.5% at baseline. VLDL-C decreased by 4.1% in the low HbA1c group, but increased by 18.1% in the high HbA1c group.

IVUS parameters

There were no significant differences in the baseline IVUS parameters (plaque volume, lumen volume and vessel volume) between the two groups of patients (Table 5). Plaque volume decreased from 71.0 ± 39.9 to 64.7 ± 34.7 mm^3 (by 6.8%) in the low HbA1c group ($p < 0.0001$ vs baseline) and from 74.3 ± 34.2 to 71.4 ± 32.3 mm^3 (by 1.3%) in the high HbA1c group. As a result, the percent change in plaque volume was significantly greater in the low HbA1c group than in the high HbA1c group ($p = 0.0410$). Similarly, vessel volume decreased by 0.8% in the low HbA1c group but increased in the high HbA1c group (by 4.2%), resulting in a significant difference between the two groups in terms of percent change in vessel volume ($p = 0.02$). In contrast, the change in lumen volume was not significantly different between the two groups ($p = 0.08$). Changes in percent plaque area and percent

Table 2 Multivariate analysis for the determinants of plaque progression

Factor	β	95% CI	p Value
HbA1c	2.683	0.292, 5.074	0.0282
Plaque volume	−0.107	−0.169, −0.046	0.0008

Parameters with p values <0.05 by multivariate analysis on parameters in Table 1 are listed.

Table 3 Baseline characteristics of patients stratified according to HbA1c at baseline

Characteristic	HbA1c <6.5% (n = 86)	HbA1c ≥6.5% (n = 40)	p Value
Males	65 (75.58%)	31 (77.50%)	1.0000[a]
Age (years)	62.5 ± 7.9	62.8 ± 7.4	0.8390[b]
Body weight (kg)	64.81 ± 10.63	67.65 ± 14.51	0.2176[b]
BMI (kg/m^2)	24.68 ± 2.71	25.67 ± 4.23	0.1186[b]
Lesion length	11.095 ± 3.398	10.213 ± 3.548	0.1835[b]
Lesion location			
Proximal	15 (17.44%)	18 (45.00%)	
Distal	33 (38.37%)	7 (17.50%)	
Other	38 (44.19%)	15 (37.50%)	
Target vessel			
RCA	35 (40.70%)	16 (40.00%)	
LAD	25 (29.07%)	13 (32.50%)	
LCX	26 (30.23%)	10 (25.00%)	
LMT	0 (0.00%)	1 (2.50%)	
LLT before enrolment	62 (72.09%)	30 (75.00%)	0.8308[a]
Hypertension	65 (75.58%)	31 (77.50%)	1.0000[a]
Smoking	22 (25.58%)	14 (35.00%)	0.2954[a]
Diabetes mellitus	9 (10.47%)	38 (95.00%)	<0.0001[a]
Family history of CAD	19 (22.09%)	7 (17.50%)	0.6411[a]
Concomitant therapy			
Ca channel blocker	46 (53.49%)	26 (65.00%)	0.2507[a]
Nitrate	57 (66.28%)	24 (60.00%)	0.5512[a]
ACE inhibitor	16 (18.60%)	11 (27.50%)	0.3507[a]
ARB	39 (45.35%)	17 (42.50%)	0.8481[a]
β-blocker	22 (25.58%)	7 (17.50%)	0.3697[a]
Thiazolidinedione	0 (0.00%)	9 (22.50%)	<0.0001[a]
Sulfonylurea	3 (3.49%)	18 (45.00%)	<0.0001[a]
α-glucosidase inhibitor	4 (4.65%)	17 (42.50%)	<0.0001[a]
Insulin	1 (1.16%)	10 (25.00%)	<0.0001[a]
Ticlopidine	83 (96.51%)	38 (95.00%)	0.6522[a]
Clopidogrel	5 (5.81%)	2 (5.00%)	1.0000[a]
Aspirin	86 (100.00%)	40 (100.00%)	1.0000[a]

Data are means ± SD or *n* (%).
[a] χ^2 or Fisher's exact test; [b]two-sample t-tests.
RCA, right coronary artery; LAD, left anterior descending; LCX, left circumflex; LMT, left main trunk; LLT, lipid-lowering therapy; CAD, coronary heart disease; HDL-C, HDL-cholesterol; IVUS, intravascular ultrasound; ARB: angiotensin receptor blocker.

Table 4 Changes in laboratory data in patients stratified according to HbA1c at baseline

	HbA1c <6.5% (n = 86)				HbA1c ≥6.5% (n = 40)			
	Baseline	Follow-up	Actual change	% change	Baseline	Follow-up	Actual change	% change
TC (mg/dl)	214.6 ± 34.5	157.1 ± 20	−57.6 ± 36.9	−25.3 ± 13.9	211.3 ± 35.4	159.3 ± 31.5	−52 ± 34.9	−23.5 ± 15
TG (mg/dl)	145.6 ± 90.3	121 ± 56.2	−24.6 ± 71.1	−8.9 ± 34.7	152.4 ± 75.9	150.4 ± 76.6*	−2.0 ± 76.2	4.0 ± 44.6
HDL-C (mg/dl)	47.9 ± 10.7	57.0 ± 11.6	9.2 ± 9.0	21.5 ± 22.3	45.4 ± 11.1	51.3 ± 11†	6.0 ± 10.2	16.0 ± 24
LDL-C (mg/dl)	140.4 ± 32.3	81.8 ± 15.7	−58.7 ± 33.1	−39.2 ± 16.6	139.7 ± 30.1	85.3 ± 23.9	−54.4 ± 31.2	−37.3 ± 17.7
VLDL-C (mg/dl)	25.4 ± 16.7	19.6 ± 10.4	−5.8 ± 14.2	−4.1 ± 61.8	26.7 ± 16.9	26.5 ± 15†	−0.2 ± 16.9	18.1 ± 76.9
non-HDL-C (mg/dl)	166.7 ± 33.9	100 ± 16.9	−66.7 ± 33.5	−38.2 ± 13.9	166 ± 33.1	108 ± 28.5	−58 ± 33	−33.6 ± 17.0
small dense LDL	0.36 ± 0.04	0.35 ± 0.03	−0.01 ± 0.04	−2.77 ± 10.97	0.36 ± 0.04	0.35 ± 0.03	−0.01 ± 0.05	−2.31 ± 11.48
RLP-C (mg/dl)	5.7 ± 5.6	3.6 ± 1.4	−2.1 ± 5.2	−17.3 ± 38.2	5.7 ± 3.7	4.9 ± 2.9†	−0.8 ± 3.9	−4 ± 51.3
LDL-C/HDL-C	3.1 ± 1.0	1.5 ± 0.4	−1.6 ± 0.8	−49.1 ± 14.1	3.2 ± 0.9	1.7 ± 0.5†	−1.5 ± 0.9	−44.3 ± 16.8
hs-CRP (ng/ml)	2836 ± 7045	821 ± 1331	−2014 ± 7111	−16.2 ± 104.7	4494 ± 9277	1172 ± 1934	−3322 ± 9158	91.9 ± 489.7

Data are means ± SD.
*p < 0.05 and †p < 0.01 vs patients with HbA1c <6.5%.
HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; VLDL-C, VLDL-cholesterol; hs-CRP, high-sensitive C-reactive protein.

vessel area were significantly greater in the low HbA1c group than in the high HbA1c group (both, $p < 0.05$).

Correlation between HbA1c and IVUS parameters

There was a weak but statistically significant correlation between baseline HbA1c and change in plaque volume ($r = 0.206$, $p = 0.02$) (Figure 1a). Because the low HbA1c group contained some patients with diabetes but good glycaemic control, we subdivided both groups of patients according to the presence/absence of diabetes to further examine the role of glycaemic control in diabetic patients. Interestingly, the correlation between baseline HbA1c and change in plaque volume was still apparent in diabetic patients, although this correlation was not statistically significant ($r = 0.263$, $p = 0.07$, Figure 1b). The same correlation was not apparent in non-diabetic patients ($r = 0.062$, $p = 0.58$, Figure 1c).

Discussion

In this subanalysis of the COSMOS study, we found that baseline HbA1c was significantly associated with change in plaque volume. Notably, no significant regression was observed in patients with high HbA1c at baseline. In contrast, significant plaque regression was observed in subjects with low HbA1c at baseline, even in those with diabetes, although the decreases in LDL-C levels were similar in both groups. This suggests that glycaemic control, in addition to LDL-C-lowering, is an important determinant of plaque progression or regression. Prior observational studies have revealed a positive association between the presence of diabetes mellitus and the incidence of cardiovascular disease [13,15]. Several recent trials using IVUS have also indicated that diabetes mellitus is a major determinant of plaque progression [11,20]. For example, Hiro et al. investigated the effect of aggressive LDL-C lowering on coronary atherosclerosis in 230 patients with acute coronary syndrome and found that

Table 5 Changes in IVUS parameters in patients stratified according to HbA1c at baseline

	HbA1c <6.5% (n = 86)				HbA1c ≥6.5% (n = 40)			
	Baseline	Follow-up	Actual change	% change	Baseline	Follow-up	Actual change	% change
Volume (mm^3)								
Plaque	71.0 ± 39.9	64.7 ± 34.7	−6.3 ± 12.3***	−6.8 ± 13.9***	74.3 ± 34.2	71.4 ± 32.3	−2.9 ± 11.4	−1.3 ± 13.8†
Lumen	80.3 ± 41.9	82.4 ± 39.8	2.1 ± 11.3	5.6 ± 15.3**	73.9 ± 36.2	80 ± 38.7	6.1 ± 15.6*	10.8 ± 15.8**
Vessel	151.4 ± 75.8	147.1 ± 69	−4.3 ± 18.8*	−0.8 ± 10.7	148.2 ± 65.2	151.4 ± 64.6	3.2 ± 20.5†	4.2 ± 13.1*†
Area (mm^2)								
Plaque	8.6 ± 3.7	6.4 ± 3.1	−2.2 ± 2.1***	−24.4 ± 20.5	9.5 ± 3.1	7.9 ± 2.7†	−1.7 ± 1.9***	−16.4 ± 17.9†
Lumen	6.0 ± 2.8	6.9 ± 3.2	1.0 ± 1.6***	20.2 ± 29.5	6.2 ± 2.4	7.4 ± 3	1.2 ± 1.6***	21.8 ± 26.4
Vessel	14.6 ± 5.7	13.4 ± 5.2	−1.2 ± 2.3***	−7.6 ± 14.4	15.7 ± 4.6	15.3 ± 4.5†	−0.5 ± 2.2	−2.0 ± 14.6†

Data are means ± SD.
*p < 0.05, **p < 0.01, ***p < 0.0001 vs baseline; †p < 0.05 vs patients with HbA1c <6.5%.
IVUS, intravascular ultrasound.

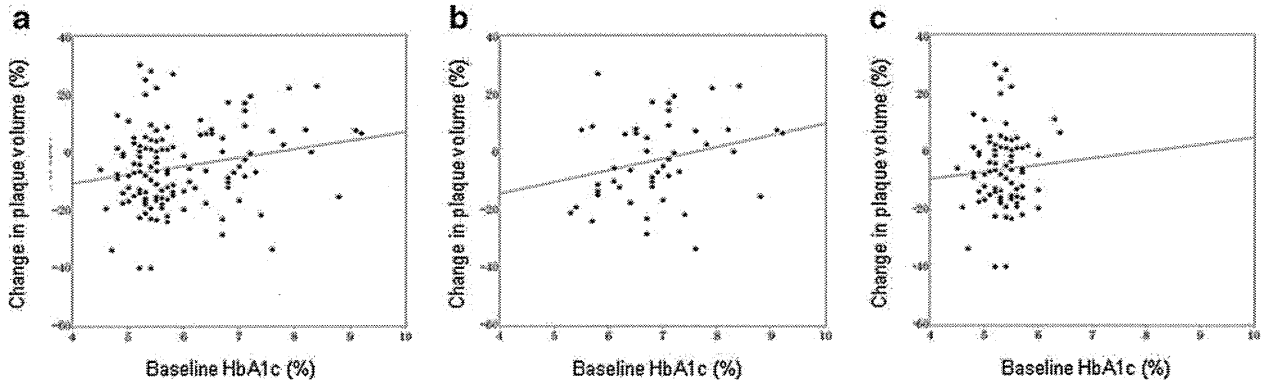

Figure 1 Associations between HbA1c and plaque volume change. Associations in all patients (**a**), in patients with diabetes mellitus (**b**), and in non-diabetic patients (**c**).

statin-induced regression of coronary plaque volume was weaker in diabetic patients than in non-diabetic patients [11]. Interestingly, they also found that percent change in plaque volume was significantly correlated with LDL-C in patients with diabetes, but not in non-diabetic patients. Nicholls et al. performed a pooled analysis of five IVUS trials involving 2,237 subjects and compared arterial remodelling, extent of coronary atherosclerosis and disease progression between patients with diabetes and those without [20]. They found that diabetic patients exhibited greater percent and total atheroma volumes, with more rapid progression of plaque volume and inadequate compensatory remodelling. They also found that percent atheroma volume was more strongly associated with HbA1c than with fasting glucose, although this difference became non-significant after adjustment for patient background. Similarly, Berry et al. observed significant associations between fasting glucose, HbA1c and the presence of diabetes mellitus, and the severity or progression of coronary atherosclerosis in 426 patients who underwent IVUS [21]. Similar to these earlier studies, we observed a significant association between baseline HbA1c and change in plaque volume.

The reduced plaque regression in patients with high HbA1c suggests that hyperglycaemia or diabetes mellitus may be involved in a unique pathogenic mechanism underlying plaque formation in these patients. Indeed, hyperglycaemia could accelerate the development of atherosclerosis through enhanced production of advanced glycation end products, oxidative stress and vascular inflammation, which may contribute to diabetes-specific atherosclerosis [22,23]. This is supported by the results of histological studies and imaging studies, which have revealed that several features are more pronounced in diabetic patients, including more extensive macrophage infiltration, significantly larger lipid cores, and more abundant dense-calcium or fibrocalcific tissue [24,25]. More recently, Parathath et al. demonstrated that diabetes modified plaque macrophage characteristics and thus hindered plaque regression [26].

As the relationship between the change in plaque volume and HbA1c was mainly observed in diabetic patients, and not in non-diabetic patients, HbA1c seems to be an important determinant of plaque regression in diabetic patients. Thus, targeting glycaemic control may aid plaque regression. Indeed several clinical trials have demonstrated significant plaque regression using oral antidiabetic drugs. For example, the Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation (PERISCOPE) trial compared the effects of treatment with pioglitazone or glimepiride for 18 months on plaque volume in 543 patients with coronary disease and type 2 diabetes [8]. The investigators found that the least squares mean percent atheroma volume decreased from baseline in patients treated with pioglitazone but increased in patients treated with glimepiride (−0.16 vs +0.73%, respectively, $p = 0.002$). HbA1c levels were $7.4 \pm 1.0\%$ in both groups at baseline, with greater decreases in patients treated with pioglitazone compared with those treated with glimepiride (−0.55 vs −0.36%, $p = 0.03$). Pioglitazone, but not glimepiride, was also associated with improvements in lipid levels, including HDL-C and triglycerides, which likely contributed to plaque regression in this group. These data suggest that interventions that improve glycaemic control alone may be insufficient to prevent increases in atheroma volume. Instead, improvements in multiple factors may be necessary to achieve clinically meaningful plaque regression. Clearly, further data from this and other studies are needed to examine the relative contributions of improved control of glucose and lipid levels to plaque regression.

HbA1c, which is an established diagnostic marker for diabetes mellitus [19], has been reported to be a significant predictor of cardiovascular events in diabetic patients [14,15]. In fact, a recent meta-analysis demonstrated that

intensive glucose control could reduce the occurrence of cardiovascular events, although longer observation periods than originally expected may be required to examine this association [27].

Quevedo et al. reported a high prevalence of vessel shrinkage in diabetic patients, which was associated with insulin requirement, HbA1c, apolipoprotein B and hypertension in their study using serial IVUS [28]. Nicholls et al. reported inadequate compensatory remodelling in diabetic patients [20]. However, unlike these earlier observations, in the present study, the percent change in vessel volume during the follow-up period indicated positive remodelling in patients with high HbA1c and negative remodelling in patients with low HbA1c. Reddy et al. also observed greater positive remodelling in diabetic patients than in non-diabetic patients [29]. Meanwhile, Chhatriwalla et al. [30] found that lower levels of LDL-C and systolic blood pressure were associated with negative remodelling of the elastic membrane in patients with established coronary artery disease. Differences in patient characteristics and IVUS methodologies may partly explain these differences in vascular remodelling between these studies. Multifactorial treatment strategies targeting not just LDL-C, but also other risk factors, including glycaemic control and blood pressure, may be important to achieve negative remodelling, and slow the progression of coronary atherosclerosis.

We observed a slight increase in HbA1c from 5.92 to 6.25% during the study, although this change was not significant. Some studies have reported that rosuvastatin and other statins increase markers of insulin resistance, such as homeostasis model assessment of insulin resistance (HOMA-IR) and fasting insulin levels [31–33]. It is possible that rosuvastatin reduces insulin sensitivity, resulting in a slight deterioration in glycaemic control. However, this effect of rosuvastatin is not consistent among studies [34–36]. Therefore, we think a more likely explanation is that the change in HbA1c reflects the natural progression of insulin resistance or worsening glycaemic control in a cohort of patients, in which 37.3% had diabetes at baseline. It must also be noted that the earlier studies were much shorter (total study length of 1–3 months) than our study, possibly too short to reliably attribute the changes in HbA1c to rosuvastatin itself. Unfortunately, as we did not measure fasting glucose or fasting insulin, we were unable to determine HOMA-IR as a direct marker for insulin resistance. We should also consider that, although HbA1c is strongly associated with fasting and post-prandial plasma glucose, the use of HbA1c rather than specific glucose parameters may mask possible associations between plasma glucose and both fasting/post-prandial glucose excursions and plaque progression.

It is also important to consider that other factors not assessed here may partly explain some of the observed associations. For example, in a cohort of Korean individuals with normal glucose tolerance or type 2 diabetes, serum levels of the adipokine omentin-1 were independently associated with arterial stiffness and carotid plaque, even after adjusting for other cardiovascular risk factors [37].

In the present study, there were some marked differences in the changes in lipid levels between the two groups. For example, VLDL-C decreased by 4.1% in the low HbA1c group, but increased by 18.1% in the high HbA1c group. The reason for this difference and its clinical relevance are unclear, because VLDL-C was not associated with plaque progression in univariate analyses, and was not included in the multivariate model. Further studies may be required to understand these results and their possible implications.

This subanalysis of the COSMOS study was a post-marketing study designed to investigate the effects of intensive LDL-C lowering with rosuvastatin on coronary atherosclerosis measured using IVUS and its safety; as such, the lack of glucose measurements and the small sample size limit further interpretations of the current results. It is also possible that other factors not assessed here, including insulin and adipokine concentrations, insulin resistance, and other markers of glycaemic control may partly explain the associations observed. Nevertheless, the association between HbA1c and change in plaque volume demonstrates the importance of good glucose control and the unique atherogenic processes in diabetes. The results also support a prospective interventional trial to better understand the impact of optimal glycaemic control on plaque regression.

Conclusions

This subanalysis of the COSMOS study revealed a significant association between baseline HbA1c and change in plaque volume. In patients with high HbA1c, no significant plaque regression was observed, whereas marked plaque regression was observed in patients with low HbA1c, even in those with diabetes, although the decreases in LDL-C levels were similar in both groups. These findings, together with earlier results, suggest that glycaemic control, along with LDL-C, is an important determinant of plaque progression and regression in patients with stable coronary artery disease.

Appendix

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Abbreviations

ARB: Angiotensin receptor blocker; CAD: Coronary artery disease; CAG: Coronary angiography; COSMOS: Coronary Atherosclerosis Study Measuring Effects of Rosuvastatin Using Intravascular Ultrasound in Japanese Subjects; CSA: Cross-section area; EEM: External elastic membrane; hs-CRP: High-sensitive C-reactive protein; IVUS: Intravascular ultrasound; LAD: Left anterior descending artery; LCX: Left circumflex artery; LLT: Lipid-lowering therapy; LMT: Left main coronary artery; NGSP: National Glycohemoglobin Standardization Program; PCI: Percutaneous coronary intervention; RCA: Right coronary artery; TAV: Total atheroma volume; ULN: Upper limit of normal.

Competing interests

H. Daida has received consulting fees and support for travel to meetings from AstraZeneca K.K. and Shionogi & Co., Ltd; acted as a consultant for GlaxoSmithKline K.K, Kowa Pharmaceutical Co., Ltd., Sanofi-Aventis K.K. and Schering-Plough Corp; received grants from Astellas Pharma Inc., AstraZeneca K.K, Bayer Yakuhun Ltd., Dainippon Sumitomo Pharma Co., Ltd., Kissei Pharmaceutical Co., Ltd., Kowa Pharmaceutical Co., Ltd., Kyowa Medex Co., Ltd., MSD K.K, Novartis Pharma K.K, Nippon Boehringer Ingelheim Co., Ltd., Otsuka Pharmaceutical Co., Ltd., Public Health Research Foundation, Roche Diagnostics K.K, Sanofi-Aventis K.K, Sanwa Kagaku Kenkyusho Co., Ltd., Schering-Plough Corp., Shionogi & Co., Ltd., Takeda Pharmaceutical Co., Ltd., and The Waksman Foundation of Japan, Inc.; and served on the speakers bureaus of Astellas Pharma Inc., Bayer Yakuhin Ltd., Dainippon Sumitomo Pharma Co., Ltd., Kissei Pharmaceutical Co., Ltd., Kowa Pharmaceutical Co., Ltd., MSD K.K, Novartis Pharma K.K, Nippon Boehringer Ingleheim Co., Ltd., Sanofi-Aventis K.K, Sanwa Kagaku Kenkyusho Co., Ltd., Schering-Plough Corp. and Takeda Pharmaceutical Co., Ltd. The other authors report no conflicts of interest.

Author contributions

HD drafted the manuscript; TT, TH and MY conceived of and designed the study; AY and SS contributed to the analysis of the data; TY collected data; and MM coordinated and managed the study. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by AstraZeneca and Shionogi Co., Ltd. The study was reported to Ministry of Health, Labour and Welfare, Japan and conformed to the Good Clinical Practice and the Good Post-Marketing Study Practice guidelines.

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Received: 13 April 2012 Accepted: 11 July 2012
Published: 25 July 2012

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