Journal of Diabetes and Metabolism
ISSN: 2155-6156

Nakagami et al. J Diabetes Metab 2011, 2:6
DOI: 10.4172/2155-6156.1000139

Research Article Open Access

Assessment of Cholesterol Absorption and Synthesis in Japanese Patients with Type-2 Diabetes and Lipid-Lowering Effect of Ezetimibe

Tomoko Nakagami*, Yayoi Yamamoto, Sayaka Fukushima, Junko Oya, Yasuhiko Iwamoto and Yasuko Uchigata

Department of Internal Medicine, Diabetes Center, Tokyo Women's Medical University School of Medicine, 8-1, Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

Abstract

Aims: Cholesterol absorption is reported as an independent risk factor for cardiovascular disease. However, factors related to cholesterol absorption have not been fully examined in patients with type-2 diabetes (T2DM). The aim of this study was to assess cholesterol absorption/synthesis markers and the effect of an inhibitor of cholesterol absorption, ezetimibe, in patients with T2DM with hyper-low-density lipoprotein cholesterolemia.

Methods: We included 59 patients treated with statins (S group) and 121 patients who were not receiving any lipid-lowering treatments (N group). Levels of cholesterol absorption and synthesis markers were compared between the 2 groups and between subjects in the N group with and without microvascular complications. The lipid-lowering effect of ezetimibe treatment (10 mg/day for 12 weeks) was examined in 70 patients with high levels of low-density lipoprotein-cholesterol (LDL-C). These patients were divided into the monotherapy (M) group (n = 57; ezetimibe treatment only) and the combination therapy (C) group (n = 13; ezetimibe and statin treatment).

Results: The levels of cholesterol absorption and synthesis markers were higher and lower, respectively, in patients in the S group than in the N group (both $p < 0.05$). In the N group, the cholesterol-absorption marker levels were higher in patients with microvascular complications than in those without ($p < 0.05$). Ezetimibe decreased total cholesterol and LDL-C levels by 13% and 21% and 11% and 16% in patients in the M group and C group, respectively (all $p < 0.05$). In patients of the M group, ezetimibe decreased the levels of remnant-like particle-cholesterol, high-sensitivity c-reactive protein, and triglycerides (TG; only in cases with TG ≥ 150 mg/dL) by 16%, 5%, and 21%, respectively, and increased high-density lipoprotein-cholesterol by 6.8% (all p 0.05).

Conclusions: Ezetimibe may be a useful therapeutic option to prevent micro- and macrovascular complications for dyslipidemia in patients with T2DM.

Keywords: Cholesterol absorption; Cholesterol synthesis; Cholesterol absorption inhibitor; Type-2 diabetes; Microvascular complications

Abbreviations: A1C: Hemoglobin A_{1C}; BMI: Body Mass Index; CPR: C-Peptide Reactivity; CVD: Cardiovascular Diseases; DM: Diabetes; FBG: Fasting Blood Glucose; HDL-C: High-Density Lipoprotein-Cholesterol; hs-CRP: High-Sensitivity C-Reactive Protein; JDCS: Japan Diabetes Complications Study; LDL-C: Low-density Lipoprotein-Cholesterol; NGSP: National Glycosylated Standard Program; NPC1L1: Niemann-Pick C1-Like 1; RLP-C: Remnant-Like Particle-Cholesterol; TC: Total Cholesterol; TG: Triglycerides; T2DM: Type-2 Diabetes Mellitus

Introduction

A westernized diet has led to an increased cholesterol intake and high morbidity associated with dyslipidemia among the modern Japanese population [1,2]. The Japan Diabetes Complication Study has shown that low-density lipoprotein-cholesterol (LDL-C) is an established risk factor for cardiovascular diseases (CVD), and the risk of CVD increased markedly when abnormal glucose levels and dyslipidemia coexist [3]. Various large-scale clinical trials have shown the beneficial effects of LDL-C-lowering therapy for preventing coronary heart diseases [4,5], and the Japan Atherosclerosis Society has documented a target level for LDL-C in its guidelines for the prevention of atherosclerotic CVD [6]. The guidelines recommend changes in lifestyle by diet and exercise as the first line of therapy in the primary prevention of CVD. However, maintaining changes in lifestyle is difficult, and in some patients, LDL-C levels are not sufficiently decreased [7].

Statins are 3-hydroxy-3-methyl-glutaryl-Coenzyme A (HMG-CoA) reductase inhibitors, which inhibit hepatic cholesterol synthesis and are used as main lipid-lowering agents [8]. However, drugs that alter cholesterol absorption have recently attracted attention after the approval of ezetimibe, which is a selectively inhibits cholesterol absorption from the small intestine [9]. In addition, Niemann-Pick C1-like 1 (NPC1L1), which plays an important role in cholesterol absorption, has been cloned [10]. Limited data has shown that *NPC1L1* gene expression was greater in patients with type-2 diabetes mellitus (T2DM) than in non-DM patients [11]. Because it is controversial whether cholesterol absorption is higher in patients with T2DM than in non-DM patients [11,12] and factors related to cholesterol absorption have not been fully examined in T2DM, we assessed the relationship between cholesterol absorption markers and the clinical characteristics of patients with T2DM. Furthermore, the lipid-lowering effect of ezetimibe [9], a selective cholesterol absorption inhibitor, was evaluated in patients with hyper-LDL-cholesterolemia whose LDL-C

***Corresponding author:** Tomoko Nakagami, Department of Internal Medicine, Diabetes Center, Tokyo Women's Medical University School of Medicine, 8-1, Shinjuku-ku, Tokyo 162-8666, Japan, Tel: 81-3-3353-8111; Fax: 81-3-3358-1941; E-mail: nakagami@dmc.twmu.ac.jp

Received May 25, 2011; **Accepted** September 10, 2011; **Published** September 14, 2011

Citation: Nakagami T, Yamamoto Y, Fukushima S, Oya J, Iwamoto Y, et al. (2011) Assessment of Cholesterol Absorption and Synthesis in Japanese Patients with Type-2 Diabetes and Lipid-Lowering Effect of Ezetimibe. J Diabetes Metab 2:139. doi:10.4172/2155-6156.1000139

levels had not reached the target level [6] despite diet or administration of statins.

Patients and Methods

The flow diagram of this study is shown in Figure 1.

Study subjects

The study comprised 180 patients (123 men) with T2DM who had been followed at the Diabetes Center of Tokyo Women's Medical University Hospital for 4 weeks or longer at the time of enrollment (February 2008 to April 2009) and who gave a written informed consent to participate in the study. All patients had hemoglobin-A_{1C} levels (A1C) <10.4% at baseline with a variation of <2.0% during the prior three months. Patients who had hepatic or renal dysfunction, familial hypercholesterolemia, drug-induced/secondary hyperlipidemia, or who were pregnant or nursing were excluded from the study. All patients were classified into two groups: patients not receiving any lipid-lowering treatments (n = 121; no treatment (N) group) and patients treated by statins alone (n = 59; statin-treatment (S) group).

Ezetimibe was administrated at a dose of 10 mg once daily during 12 weeks in 70 patients whose LDL-C levels had not reached the target level

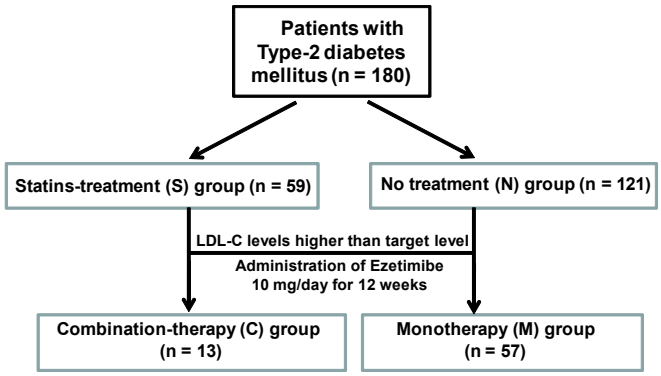

Figure 1: Flow diagram of this study.

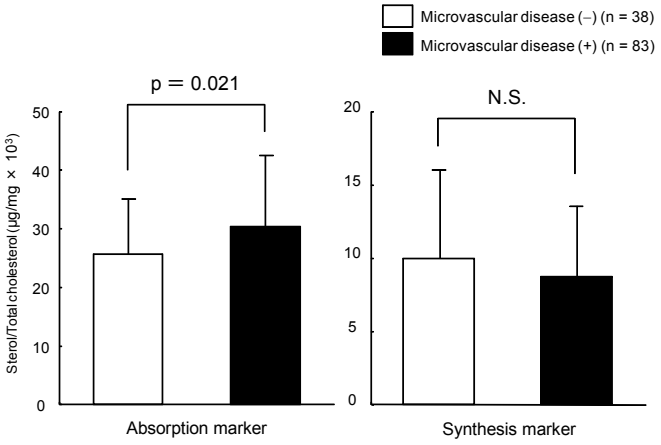

Figure 2: Bar graphs illustrating the differences in the levels of cholesterol absorption and synthesis markers in patients with and without microvascular complications. The black boxes indicate patients with microvascular complications while the white boxes indicate those without microvascular complications.

established by the guidelines of the Japan Atherosclerosis Society [6]: <120 (100) mg/dL in patients without (with) a past history of coronary heart disease. In cases where ezetimibe treatment was added to statin treatment (S group), the patients were classified as the combination therapy (C) group. In cases where ezetimibe was administered to those not receiving any lipid-lowering therapy (N group), the patients were classified as the mono-therapy (M) group. The concomitant use of antihypertensives, antidiabetics, and antiplatelet drugs were allowed in the study, but starting any other new drugs, discontinuing any drug, or changing dosages were not allowed during the study period.

Blood sampling and analytical methods

Fasting blood samples were obtained from the patients and measurements of all study parameters were performed in a laboratory in Tokyo Women's University Hospital. The laboratory data (assays) included total cholesterol (TC; cholesterol dehydrogenase assay), triglycerides (TG; enzymatic method), high-density lipoprotein cholesterol (HDL-C; direct method), LDL-C (direct method), and remnant-like particle-cholesterol (RLP-C; immunoadsorption). For cholesterol absorption markers, sitosterol and campesterol, which are vegetable sterols not synthesized in the body, as well as cholestanol, which is generated during bile acid synthesis, excreted in the bile, and reabsorbed, were measured. Lathosterol was measured as a cholesterol synthesis marker because it is a precursor of cholesterol that is generated by the rate-limiting step of cholesterol biosynthesis. These cholesterol absorption/synthesis markers were measured in serum by gas chromatography [13] and reported after dividing by TC because TC and cholesterol absorption/synthesis markers have a strong relationship [14]. Fasting blood glucose (FBG; hexokinase UV method), A1C (latex agglutination assay), and C-Peptide Reactivity (CPR; chemiluminescent enzyme immunoassay) were measured in order to assess glucose metabolism, while high-sensitivity C-reactive protein (hs-CRP; nephelometry) was measured as a marker of inflammation. As for A1C, the National Glycosylated Standard Program aligned value was reported.

Age, body mass index (BMI), waist circumference, blood pressure, duration of DM, complications, drugs for concomitant diseases, and risk factors for coronary heart diseases (history of coronary artery disease, stroke, and arteriosclerosis obliterans, hypertension, smoking status, and the family history of coronary artery disease) were investigated.

The cholesterol absorption and synthesis markers were compared between the S and N groups and between those with and without microvascular complications in the N group. The linear trend was examined between the clinical characteristics and the quartiles of the cholesterol absorption markers. The lipids were compared before and 12 weeks after the administration of ezetimibe.

This study was approved by the ethical committee of Tokyo Women's Medical University School of Medicine and was registered in advance with the University Hospital Medical Information Network (U-MIN; U-MIN No.: 000002070, receipt No.: R000002498).

Statistical analysis

Chi-squared tests and paired *t*-tests were used to compare proportions and means between the two groups. Analysis of variance (ANOVA) was used to compare means, and a logistic regression model was used to compare proportions across strata. A p-value < 0.05 was considered statistically significant. All statistical analyses were

performed using SPSS for Windows, version 16.0 (SPSS, Inc., Chicago, IL, USA).

Results

Table 1 shows the clinical data for the patients enrolled in the N and S groups. Patients in the S group, in comparison to those in the N group, were significantly older and more obese and had higher proportions of users of buiguanides/thiazolidinediones, insulin and antihypertensive drugs and patients with past history of coronary artery disease. Patients in the S group had significantly lower levels of the synthesis markers and higher levels of the absorption markers than those in the N group.

In the N group, LDL-C and HDL-C levels were increased, and the cholesterol synthesis markers and CPR levels were decreased with an increase of cholesterol absorption marker levels (Table 2). There was no trend in the relationship between the cholesterol absorption markers and the clinical characteristics (Table 2). However, the cholesterol absorption marker levels were significantly higher in patients with microvascular complications than in those without (30.4 ± 12.4 vs. 25.2 ± 9.1 μg/mg $*10^3$, p = 0.021; Figure 2), and this trend did not change after adjusting for sex, duration of DM, A1C, and systolic blood pressure at baseline (p = 0.034). Although the opposite relationship was seen between the levels of cholesterol synthesis markers and the presence/absence of microvascular complications, there was no statistical significance.

Lipid-lowering effect of ezetimibe

Table 3 shows the results of the administration of ezetimibe in the patients with hyper-LDL cholesterolemia. TC was significantly decreased by the administration of ezetimibe, and the relative decrease was -13% in all treated patients, -12.5% in patients of the M group, and -11.0% in patients of the C group, respectively. Similarly, LDL-C levels were significantly decreased, and the relative decrease was -19.7% in all treated patients, -20.7% in patients of the M group, and -15.5% in patients of the C group, respectively. The favorable changes of HDL-C, RLP-C, hs-CRP, and TG were observed both in all treated patients and in patients of the M group. The relative change in all treated patients and patients of the M group was 5.9% and 6.8% for HDL-C, -13.4% and -16.0% for RLP-C, -5.8% and -5.3% for hs-CRP, and -23.1% and -21.2% for TG (only in the patients with TG ≥ 150 mg/dL), respectively. The similar tendency was shown in the changes of the C group, but they were not statistically significant. Ezetimibe treatment significantly decreased the levels of the cholesterol absorption marker levels and increased cholesterol synthesis marker levels in all treated patients.

During the study period, there was neither a significant change in glucose and CPR levels nor adverse events that were attributable to ezetimibe.

	No treatment group	Statins-treatment group	p-value
Number	121	59	
Men (%)	73.6	57.6	0.04
Age (years)	60.9 ± 11.4	67.0 ± 9.9	<0.0001
Duration of diabetes (years)	12.7 ± 9.2	16.6 ± 8.8	0.007
Body Mass Index (kg/m^2)	25.0 ± 4.9	26.7 ± 4.0	0.029
Waist circumference (cm)	89.1 ± 10.8	93.0 ± 9.8	0.019
Systolic Blood Pressure (mmHg)	133.6 ± 12.2	135.2 ± 15.8	N.S
Diastolic Blood Pressure (mmHg)	74.9 ± 10.0	71.9 ± 12.5	N.S
Total cholesterol (mg/dL)	221.2 ± 32.9	204.8 ± 28.9	0.001
Triglycerides (mg/dL)	136.0 ± 152.9	166.3 ± 145.3	N.S
HDL cholesterol (mg/dL)	55.7 ± 14.9	54.6 ± 13.3	N.S
LDL cholesterol (mg/dL)	137.2 ± 26.6	115.9 ± 22.9	<0.001
RLP cholesterol (mg/dL)	6.7 ± 16.4	7.0 ± 9.2	N.S
Cholesterol absorption marker/total cholesterol ($μg/mg*10^3$)	27.4 ± 9.3	33.5 ± 18.3	<0.05
Cholesterol synthesis maker/total cholesterol ($μg/mg*10^3$)	9.1 ± 5.1	5.8 ± 3.5	<0.001
A1C (%)	7.4 ± 1.0	7.6 ± 1.1	N.S
Fasting blood glucose (mg/dL)	138.6 ± 43.1	139.1 ± 46.3	N.S
CPR (ng/mL)	1.7 ± 1.0	2.1 ± 1.7	N.S
Concomitant drugs (%)			
Sulfonylurea drugs	40.5	47.5	N.S
α-glucosidase inhibitors	24.0	32.2	N.S
Biguanides/Thiazolidinediones	31.4	49.2	0.023
Insulin	33.1	52.5	0.015
Antihypertensive drugs	38.0	76.3	<0.001
Antiplatelet agents	17.6	36.2	0.008
Risk factors (%)			
Smoker	19.0	15.3	N.S
Family history of coronary artery disease	3.3	5.1	N.S
History of coronary artery disease	4.2	18.6	0.004
History of stroke	7.5	5.1	N.S
History of peripheral artery disease	0.8	1.7	N.S

CPR: C-peptide reactivity. CPR (reference value): 1.2 to 2.0 ng/mL. Data are expressed as mean ± SD. HDL, high-density lipoprotein; LDL, low-density lipoprotein; RLP, remnant-like particle; A1C, hemoglobin A_{1C}.

Table 1: Clinical data of study patients.

	Quartiles of cholesterol absorption marker/total cholesterol (μg/mg*10³)				
	Q1	Q2	Q3	Q4	P-value
	(<4.6)	(4.6-5.6)	(5.6-7.2)	(≥7.3)	
Number of subjects	30	30	30	31	
Men (%)	20 (70.0)	25 (83.3)	25 (83.3)	18 (58.1)	N.S.
Age (years)	60.4 ± 10.1	61.7 ± 11.8	61.0 ± 12.1	60.6 ± 12.0	N.S.
Duration of diabetes (years)	10.9 (7.9)	12.9 (10.1)	13.5 (9.2)	12.8 (9.5)	N.S.
Body Mass Index (kg/m²)	25.2 ± 4.1	25.1 ± 5.7	25.6 ± 5.1	24.2 ± 4.7	N.S.
Waist circumference (cm)	90.7 ± 9.7	89.8 ± 10.4	89.3 ± 11.8	86.5 ± 11.1	N.S.
Systolic BP (mmHg)	135.1 ± 11.3	133.2 ± 12.5	130.3 ± 11.4	135.7 ± 13.3	N.S.
Diastolic BP (mmHg)	76.7 ± 10.5	74.3 ± 11.4	74.3 ± 9.2	74.8 ± 9.3	N.S.
Triglycerides (mg/dL)	157.1 ± 137.6	108.1 ± 64.1	170.1 ± 256.5	109.5 ± 67.7	N.S.
HDL cholesterol (mg/dL)	50.4 ± 10.4	55.7 ± 16.8	54.2 ± 13.6	62.4 ± 15.8	0.003
LDL cholesterol (mg/dL)	128.6 ± 31.3	131.5 ± 18.1	143.4 ± 27.3	145.0 ± 25.4	0.004
RLP cholesterol (mg/dL)	6.2 ± 7.0	4.5 ± 2.6	5.3 ± 2.4	5.2 ± 3.2	N.S.
Cholesterol synthesis marker/total cholesterol (μg/mg*10³)	2.4 ± 1.7	2.1 ± 1.4	2.0 ± 1.3	1.6 ± 0.9	0.014
A1C (%)	7.5 ± 1.2	7.4 ± 1.1	7.4 ± 1.0	7.2 ± 0.9	N.S.
Fasting blood glucose (mg/dL)	144.0 ± 32.4	147.7 ± 58.0	135.5 ± 44.1	127.5 ± 32.0	N.S.
CPR (ng/mL)	2.0 ± 1.0	1.8 ± 1.1	1.7 ± 1.1	1.3 ± 0.8	0.009
hs-CRP (ng/mL)	1098.2 ± 1560.4	1506.4 ± 1936.4	971.8 ± 1140.0	877.4 ± 1799.3	N.S.
History of coronary artery disease (%)	1 (3.3)	2 (6.7)	1 (3.3)	1 (3.3)	N.S.
History of stroke (%)	4 (13.3)	0 (0.0)	2 (6.7)	3 (10.0)	N.S.
Hypertension (%)	12 (40.0)	10 (33.3)	8 (26.7)	12 (38.7)	N.S.
Smoking (%)	7 (23.3)	4 (13.3)	4 (13.3)	8 (25.8)	N.S.

CPR: C-peptide reactivity. CPR (reference value): 1.2 to 2.0 ng/mL, hs-CRP: high-sensitivity C-reactive protein

Table 2: Clinical data according to quartiles of cholesterol absorption markers in 121 type-2 diabetic patients without any lipid lowering therapy.

Discussion

We have shown that the lipid-lowering treatment of statins reduced cholesterol synthesis but increased cholesterol absorption in patients with T2DM. Moreover, among the patients not receiving treatment for dyslipidemia, cholesterol absorption was significantly higher in the cases with microvascular complications than in those without the complications. The administration of a cholesterol absorption inhibitor, ezetimibe, significantly reduced LDL-C, RLP-C, and hs-CPR levels and increased HDL-C levels in patients with hyper LDL-cholesterolemia. Thus, ezetimibe may be a useful therapeutic option for dyslipidemia by additionally preventing vascular complications in patients with T2DM.

Several large-scale clinical trials have verified the usefulness of statins for inhibiting cholesterol synthesis in the liver and reducing LDL-C levels and preventing CVD [4,5]. Recently, ezetimibe, which is a cholesterol transporter inhibitor and an alternative approach for reducing LDL-C levels, has been approved. This has attracted attention as a new option for the treatment of dyslipidemia in consideration of the currently increased number of patients with dyslipidemia due to an increased dietary fat intake in Japan [1,2,15]. Accumulated data have recently highlighted cholesterol absorption as an important CVD risk factor [16-18]. A subanalysis of the 4S study has shown that CVD events were not decreased by the administration of statins in the study group of subjects with high cholesterol absorption [16]. The DEBATE study showed that CVD events were more common in patients with elevated cholesterol absorption than in those without, although LDL-C levels were the same in both groups [17]. Moreover, the Framingham Offspring Study [18] has shown that cholesterol absorption and synthesis markers were better predictors of CVD than traditional lipid risk factors. Cholesterol synthesis is reduced by the administration of statins, while cholesterol absorption shows a compensatory increase in patients on statin therapy [19,20]. These findings were concordant with our findings in T2DM.

The positive relationship between cholesterol absorption and LDL-C levels that was shown in our study has already been reported in nondiabetic patients with dyslipidemia [14,20]. The positive relationship between cholesterol absorption and HDL-C levels in our study was somewhat unexpected because cholesterol absorption seems to be a positive risk factor of CVD. The reason is not known, but a similar finding was previously reported in the prospective DEBETE Study [16]. In our study, the endogenous insulin levels (fasting CPR) decreased with increased levels of the cholesterol absorption marker, although glucose indicators and the duration of DM did not have any trend. The reason for this is not known. However, there is a possibility that the cholesterol absorption accelerates the secretion of chylomicron from small intestine, and leads to over transportation of cholesterol, TG and free fatty acids. It is reported that the excess free fatty acids promotes impairment of endogenous insulin secretion in pancreatic β-cell (= pancreatic β-cell lipotoxisity) [21]. Moreover, the levels of the cholesterol absorption marker were significantly higher in cases with microvascular complications than in those without. These findings might suggest that patients who have worse β-cell function with higher absorption marker levels were more likely to have microvascular complications, although the underlying mechanism is not known. A study from Japan showed that the coadministration of ezetimibe enhanced the proteinuria-lowering effects of pitavastatin in nondiabetic chronic kidney disease patients partly through a cholesterol-independent manner [22]. Both micro- and macro-vascular complications may share a common causal mechanism: advanced glycation end-products [23], inflammation processes, and/or oxidative stress [24]. Thus, the relationship between cholesterol absorption and microvascular diseases should be further investigated in the future. Previously, a study conducted in Finnish patients with T2DM showed that cholesterol absorption had an inverse relationship with insulin concentrations [25]. Our subjects were leaner and had a far longer duration of DM than the subjects in the Finnish study.

Our study showed that LDL-C levels were reduced by approximately 20%, and hypertriglyceridemia was significantly improved by the 12-week administration of ezetimibe. These findings are in accordance with previous reports [26,27]. Moreover, ezetimibe decreased RLP-C levels, which reflect remnants in the blood. Remnants, as well as TC, are recognized as risk factors for arteriosclerosis because they directly enter into the vascular endothelium and are taken up by macrophages without degeneration, leading to arteriosclerosis by inducing foam cell formation [28]. It has been reported that patients who are possibly insulin resistant have postprandial hyperlipidemia with prolonged triglyceride metabolism and high remnant levels [29]. In addition, it has been reported that RLP-C levels are elevated in patients with T2DM [30]. Ezetimibe suppressed chylomicron secretion from the small intestine and improved postprandial hyperlipidemia [31,32]. Thus, the improvement of postprandial hyperlipidemia by ezetimibe may relate to decreased levels of RLP-C in patients with T2DM. Kugiyama et al. reported that RLP-C was a risk factor for coronary events [33]. Thus, the decrease of RLP-C due to ezetimibe may be associated with the lowering of hs-CRP found in this study. In this study, the absorption marker levels were reduced and the synthesis marker levels were increased after the administration of ezetimibe. These results are also in accordance with a previous report [20].

		Baseline	Week 12	% change	p value
All treated patients (n = 70)					
Total cholesterol	(mg/dL)	228.8 ± 29.0	198.0 ± 26.1	-13.0 ± 10.1	<0.001
Triglycerides	(mg/dL)	136.4 ± 110.3	119.1 ± 89.4	-5.0 ± 46.2	N.S.
TG ≥ 150 mg/dL (n = 20)	(mg/dL)	249.7 ± 145.1	182.2 ± 100.6	-23.1 ± 28.9	0.005
HDL cholesterol	(mg/dL)	54.1 ± 12.3	56.9 ± 12.6	5.9 ± 11.0	<0.001
HDL cholesterol <40 mg/dL (n = 5)	(mg/dL)	32.8 ± 5.9	36.0 ± 6.0	10.5 ± 11.5	N.S.
LDL cholesterol	(mg/dL)	145.2 ± 21.3	115.6 ± 23.1	-19.7 ± 16.2	<0.001
RLP cholesterol	(mg/dL)	5.8 ± 5.4	4.4 ± 2.8	-13.4 ± 29.9	<0.001
RLP cholesterol ≥5.2 (n = 23)	(mg/dL)	10.1 ± 7.7	6.1 ± 4.1	-34.4 ± 25.7	<0.001
Cholesterol absorption marker/total cholesterol	(mg/mg*103)	7.1 ± 3.7	5.8 ± 2.1	-11.9 ± 26.4	<0.001
Cholesterol synthesis marker/total cholesterol	(mg/mg*103)	1.8 ± 1.2	3.1 ± 1.7	90.8 ± 59.4	<0.001
Fasting blood glucose	(mg/dL)	146.8 ± 49.2	147.6 ± 37.9	8.2 ± 39.0	N.S.
A1C	(%)	7.4 ± 0.8	7.5 ± 0.9	1.8 ± 6.2	N.S.
CPR	(ng/mL)	1.7 ± 1.1	1.7 ± 1.1	29.2 ± 106.6	N.S.
hs-CRP	(ng/mL)	1183.2 ± 1542.6	792.9 ± 866.6	-5.8 ± 57.0	0.003
Monotherapy (n=57)					
Total cholesterol	(mg/dL)	228.9 ±28.4	199.4 ± 25.3	-12.5 ± 9.5	<0.001
Triglycerides	(mg/dL)	135.4 ± 110.5	117.0 ± 94.0	-7.5 ± 47.8	N.S.
TG ≥ 150 mg/dL (n = 17)	(mg/dL)	241.9 ± 150.4	180.9 ± 107.3	-21.2 ± 30.8	0.02
HDL cholesterol	(mg/dL)	54.2 ± 12.6	57.6 ± 13.2	6.8 ± 10.4	<0.001
HDL cholesterol <40 mg/dL (n = 4)	(mg/dL)	32.8 ± 5.9	36.0 ± 6.0	10.5 ± 11.5	N.S.
LDL cholesterol	(mg/dL)	146.7 ± 21.2	115.5 ± 21.0	-20.7 ± 13.7	<0.001
RLP cholesterol	(mg/dL)	5.8 ± 5.4	4.3 ± 3.0	-16.0 ± 30.0	0.001
RLP cholesterol ≥5.2 (n = 20)	(mg/dL)	9.7 ± 7.7	6.0 ± 4.4	-34.5 ± 26.1	0.002
Cholesterol absorption marker/total cholesterol	(μg/mg*10^3)	6.7 ± 2.7	5.7 ± 1.8	-10.7 ± 25.1	<0.001
Cholesterol synthesis marker/total cholesterol	(μg/mg*103)	1.9 ± 1.2	3.3 ± 1.6	93.6 ± 61.7	<0.001
Fasting blood glucose	(mg/dL)	145.5 ± 47.0	149.3 ± 38.3	9.4 ± 38.8	N.S.
A1C	(%)	7.3 ± 0.8	7.4± 0.9	1.6 ± 8.6	N.S.
CPR	(ng/mL)	1.6 ± 1.1	1.7 ± 1.1	34.1 ± 110.7	N.S.
hs-CRP	(ng/mL)	1089.7 ± 1346.2	717.7 ± 705.0	-5.3 ± 56.9	0.011
Combination (Ezetimibe + Statins) therapy (n=13)					
Total cholesterol	(mg/dL)	222.0 ± 33.7	194.9 ± 29.6	-11.0 ± 16.5	0.029
Triglycerides	(mg/dL)	137.5 ± 105.6	127.8 ± 58.3	6.7 ± 33.2	N.S.
TG≥150 mg/dL (n=3)	(mg/dL)	293.7 ± 125.0	189.3 ± 62.7	-33.8 ± 12.3	N.S.
HDL cholesterol	(mg/dL)	53.7 ± 11.8	53.9 ± 9.4	2.0 ± 12.7	N.S.
HDL cholesterol <40 mg/dL (n=1)	(mg/dL)	32.8 ± 5.9	36.0 ± 6.0	10.5 ± 11.5	N.S.
LDL cholesterol	(mg/dL)	141.0 ± 20.2	114.2 ± 32.3	-15.5 ± 24.5	0.009
RLP cholesterol	(mg/dL)	5.6 ± 5.3	4.5 ± 2.0	-2.5 ± 28.1	N.S.
RLP cholesterol ≥5.2 (n=3)	(mg/dL)	12.5 ± 8.6	6.9 ± 2.1	-33.6 ± 27.4	N.S.
Cholesterol absorption marker/total cholesterol	(μg/mg*10^3)	9.1 ± 6.6	6.5 ± 3.0	-17.4 ± 32.4	<0.05
Cholesterol synthesis marker/total cholesterol	(μg/mg*10^3)	1.3 ± 1.0	2.2 ± 1.8	78.8 ± 48.4	<0.05
Fasting blood glucose	(mg/dL)	152.1 ± 60.0	140.3 ± 36.6	2.6 ± 40.9	N.S.
A1C	(%)	7.5 ± 0.8	7.6 ± 0.7	2.6 ± 5.4	N.S.
CPR	(ng/mL)	2.0 ± 1.3	1.7 ± 1.1	7.9 ± 87.1	N.S.
hs-CRP	(ng/mL)	1009.1 ± 1037.1	775.0 ± 692.8	-5.2 ± 62.1	N.S.

A1C, hemoglobin A_{1C}; CPR: C-peptide reactivity, hs-CRP: high-sensitivity C-reactive protein, HDL: high density lipoprotein, LDL: low density lipoprotein, RLP: remnant-like lipoprotein. CPR (reference value): 1.2 to 2.0 ng/mL.

Table 3: Effect of ezetimibe in 70 patients with hyper-LDL-cholesterolemia

One limitation of our study is that the number of patients was too small to show a statistically significant effect of ezetimibe in the statin combination therapy group. Alternatively, the low drug (ezetimibe) adherence in statin combination therapy group might have caused the insignificant changes on lipid profiles. Regrettably, we have not confirmed the drug adherence rate in this study. Nevertheless, the favorable change in lipids and inflammation markers was seen in the statin combination therapy group. Thus, a large-scale and replicable clinical trial needs to be done in the future in order to confirm the improvement suggested by our data.

In conclusion, ezetimibe may be a useful therapeutic option to prevent micro- and macrovascular complications for dyslipidemia in patients with T2DM.

Competing Interests

This study has not been published or submitted elsewhere, and no ethical problems or conflicts of interest are declared.

Acknowledgements

We thank Ms. Midori Hasegawa (Diabetes Center, Tokyo Women's Medical University School of Medicine) for her assistance with data management in this study.

References

1. Tsuji E (2007) Cholesterol intake of Japanese. Geriat Med 45: 1511-1515.
2. Kubo M, Kiyohara Y, Kato I, Tanizaki Y, Arima H, et al. (2003) Trends in the incidence, mortality, and survival rate of cardiovascular disease in Japanese community. Stroke 34: 2349-2354.
3. Sone H, Mizuno S, Ohashi Y, Akanuma Y, Yamada N, et al. (2004) Japan Diabetes Complications Study Group: Prevention and Therapeutic strategy of metabolic syndrome -Implications from the interim results of Japan Diabetes Complications Study (JDCS)-. Nippon Rinsho 62: 1150-1157.
4. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, et al. (1995) Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Eng J Med 333: 1301-1307.
5. Scandinavian Simvastatin Survival Study Group (1994) Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 344: 1383-1389.
6. Executive summary of Japan Atherosclerosis Society (2007) Guideline for Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases 4: 45-50.
7. Henkin Y, Garber DW, Osterlund LC, Darnell BE (1992) Saturated fats, cholesterol, and dietary compliance. Arch Inter Med 152: 1167-1174.
8. Endo A (1985) Drugs inhibiting HMG-CoA reductase. Pharmacol Ther 31: 257-267.
9. Harris M, Davis W, Brown WV (2003) Ezetimibe. Drugs Today (Barc) 39: 229-247.
10. Altmann SW, Davis HR Jr, Zhu LJ, Yao X, Hoos LM, et al. (2004) Niemann-Pick C1 Like 1 protein is critical for cholesterol absorption. Science 303: 1201-1204.
11. Lally S, Tan CY, Owens D, Tomkin GH (2006) Messenger RNA levels of genes involved in dysregulation of postprandial lipoproteins in type 2 diabetes: the role of Niemann-Pick C1-like 1, ATP-binding cassette, transporters G5 and G8, and of microsomal triglyceride transfer protein. Diabetorogia 49: 1008-1016.
12. Simonen P, Gylling H, Miettinen TA (2008) The validity of serum squalene and non-cholesterol sterols as surrogate markers of cholesterol synthesis and absorption in type 2 diabetes. Atherosclerosis 197: 883-888.
13. Miettinen TA, Ahrens EH Jr, Grundy SM (1965) Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral steroids. J Lipid Res 6: 411-424.
14. Kesaniemi YA, Miettinen TA (1987) Cholesterol absorption efficiency regulates plasma cholesterol level in the Finish population. Eur J Clin Invest 17: 391-395.
15. Health Service Bureau, Ministry of Health, Labour and Welfare (2007) Survey of national health and nutrition in 2007.
16. Miettinen TA, Gylling H, Strandberg T, Sarna S (1998) Baseline serum cholesterol as predictor of recurrent coronary events in subgroup of Scandinavian simvastatin survival study. BMJ 316: 1127-1130.
17. Strandberg TE, Tilvis RS, Pitkala KH, Miettinen TA (2006) Cholesterol and glucose metabolism and recurrent cardiovascular events among the elderly. J Am Coll Cardiol 48: 708-714.
18. Matthan NR, Pencine M, LaRocque JM, Jacquwa PF, D'Agostino RB, et al. (2009) Alterations in cholesterol absorption/synthesis markers characterize Framingham Offspring Study participants with CHD. J Lipid Res 50: 1927-1935.
19. Miettinen TA, Gylling H, Lindbohm N, Miettinen TE, Rajaratnam RA, et al. (2003) Serum noncholesterol sterols during inhibition of cholesterol synthesis by statins. J Lab Clin Med 141: 131-137.
20. Hiramitsu S, Ishiguro Y, Matsuyama H, Yamada K, Kato K, et al. (2010) The effects of Ezetimibe on surrogate markers of cholesterol absorption and synthesis in Japanese patients with dyslipidemia. J Atheroscler Thromb 17: 106-114.
21. Shimabukuro M, Koyama K, Chen G, Wang MY, Trieu F, et al. (1997) Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci U S A 94: 4637-4641.
22. Nakamura T, Sato E, Fujiwara N, Kawagoe Y, Ueda Y, et al. (2010) Co-administration of ezetimibe enhances proteinuria-lowering effects of pitavastatin in chronic kidney disease patients partly via a cholesterol-independent manner. Pharmacol Res 61: 58-61.
23. Goldin A, Beckman JA, Schmidt AM, Creager MA (2006) Advanced glycation endoproducts: sparkling the development of diabetic vascular injury. Circulation 114: 597-605.
24. Orasanu G, Plutzky J (2009) The pathologic continuum of diabetic microvascular disease. J Am Coll Cardiol 53 (5Suppl) S35-42.
25. Simonen P, Gylling H, Howard AN, Mittinen TA (2000) Introducing a new component of metabolic syndrome: low cholesterol absorption. Am J Clin Nutr 72: 82-88.
26. Saito Y, Yamada N, Nakaya N, Teramoto T, Oikawa S, et al. (2007) Phase Clinical study of Ezetimibe -Double-Blind Comparative Study with colestilan. Journal of Clinical Therapeutics and Medicine 23: 493-522.
27. Davidson MH, Ballantyne CM, Kerzner B, Melani L, Sagar PT, et al. (2004) Efficacy and safety of Ezetimibe coadministered with statins: randomized, placebo-controlled, blinded experience in 2383 patients with primary hypercholesterolemia. Int J Pract 58: 746-755.
28. Tomono S, Kawazu S, Kato N, Ono T, Ishii C, et al. (1994) Uptake of remnant like particles (RLP) from diabetic patients from mouse peritoneal macrophages. J Atheroscler Thromb 1: 98-102.
29. Ai M, Tanaka A, Ogita K, Sekinc M, Numano F, et al. (2001) Relationship between plasma insulin concentration and plasma remnant lipoprotein response to an oral fat load in patients with type 2 diabetes. J Am Coll Cardiol 38: 1628-1632.
30. Watanabe N, Taniguchi T, Taketoh H, Kitagawa Y, Namura H, et al. (1999) Elevated remnant-like lipoprotein particles in impaired glucose tolerance and type 2 diabetic patients. Diabetes Care 22: 152-156.
31. Tremblay AJ, Lamarche B, Hogue JC, Couture P (2009) Effects of ezetimibe and simvastatin on apolipoprotein B metabolism in males with mixed hyperlipidemia. J Lipid Res 50: 1463-1471.
32. Masuda D, Nakagawa-Toyama Y, Nakatani K, Inagaki M, Tsubakio-Yamamoto K, et al. (2009) Ezetimibe improves postprandial hyperlipidaemia in patients with type IIb hyperlipidaemia. Eur J Clin Invest 39: 689-698.
33. Kugiyama K, Doi H, Takazoe K, Kawano H, Soejima H, et al. (1999) Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. Circulation 99: 2858-2860.