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Enhanced Impact of Cholesterol Absorption Marker on New Atherosclerotic Lesion Progression After Coronary Intervention During Statin Therapy

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Aim: Clinical trials suggest that residual risks remain for coronary artery disease (CAD) during low-density lipoprotein cholesterol (LDL-C) lowering therapy. We aimed to investigate the role of exogenous lipids in the prognosis of CAD after percutaneous coronary intervention (PCI).

Methods: A total of 145 patients with CAD, who underwent elective PCI, and 82 non-CAD (control) patients were enrolled in this study. CAD patients underwent follow-up coronary angiography 6–9 months after PCI, and were classified into three groups: 1) patients who showed in-stent restenosis (ISR) in the original stented segment, 2) patients with other non-target coronary atherosclerotic lesions (de novo), and 3) patients with neither ISR nor a de novo lesion. Biochemical analyses were performed on fasting serum samples at the time of follow-up coronary angiography.

Results: Despite the controlled serum LDL-C levels, CAD patients with statin showed elevated cholesterol absorption marker campesterol/total cholesterol (TC), synthesis marker lathosterol/TC, campesterol/lathosterol ratio, and apolipoprotein B48 (apoB48) concentration compared with non-CAD patients. The high campesterol/TC, campesterol/lathosterol ratio, and apoB48 concentration were associated with de novo lesion progression after PCI. In stepwise multivariate logistic regression analysis, campesterol/TC and apoB48 concentrations were independent risk factors for de novo lesion progression in statin-treated CAD patients after PCI.

Conclusion: The increase of cholesterol absorption marker and apoB48 concentration may lead to the progression of de novo lesions, and these markers may represent a residual risk during statin treatment after PCI.

See editorial vol. 24: 120-122

Key words: Coronary artery disease, Cholesterol absorption, Exogenous cholesterol, Restenosis

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Introduction

The plasma level of low-density lipoprotein cholesterol (LDL-C) is the most valuable predictor and modulator for coronary artery disease (CAD). Statin treatment is a principal strategy, not only for hyper-

cholesterolemia but also for the primary and secondary prevention of CAD. In fact, intensive LDL-lowering treatment with statin has efficiently reduced coronary events and mortality^{1,2}. However, recent clinical trials suggest that residual risks remain for CAD during the statin treatment, irrespective of the potency of statin³. These findings stimulate enhanced interest in therapeutic target beyond the quantity of LDL-C.

Cholesterol homeostasis is regulated both by “endogenous” cholesterol, which is synthesized mostly in the liver, and “exogenous” cholesterol, which is derived from diet and absorbed in the small intestine. Dietary or exogenous cholesterol accounts for approxi-

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mately 30% of total cholesterol, and the remaining 70% is synthesized in the body, while the liver predominantly regulates the circulating cholesterol level. In catabolism, cholesterol is excreted into the bile, then re-absorbed in the intestine or excreted into feces. Statin is a strong inhibitor of cholesterol synthesis, and, as a compensatory action, cholesterol absorption is increased⁴⁾; such an effect seems to depend on the LDL-lowering potency of the statin⁵⁾. Cholesterol absorption also serves as a regulator of the serum LDL-C level⁶⁾. It has been reported that cholesterol absorption is associated with recurrent cardiovascular events⁷⁾, and the beneficial effect of statin was attenuated in patients with increased cholesterol absorption⁸⁻¹⁰⁾. In a recent study, the addition of ezetimibe, a selective inhibitor of cholesterol absorption, to statin, resulted in further reduction of cardiovascular events in patients with acute coronary syndrome¹¹⁾. Moreover, impaired cholesterol homeostasis, as expressed by low synthesis and high absorption marker concentrations, is a predictor of CAD as shown in the Framingham study⁹⁾. Thus, the change in the relative balance of circulation cholesterol may affect the prognosis of CAD, irrespective of plasma LDL-C levels. Although it is difficult to discriminate between exogenous and endogenous cholesterol, measurement of serum markers for cholesterol absorption (campesterol or sitosterol) and synthesis (lathosterol or desmosterol) has enabled estimation of the relative predominance of circulation exogenous and endogenous cholesterol. On the other hand, it has recently become possible to measure apolipoprotein B48 (apoB48) concentration, which is present only in intestinally derived exogenous lipoproteins, such as chylomicron and chylomicron remnants¹²⁾.

Aim

We aimed to investigate whether the serum markers for exogenous lipids affect and predict CAD progression after percutaneous coronary intervention (PCI).

Methods

Patients

This study was performed in accordance with the ethical principles of the Declaration of Helsinki and the Ethical Guidelines for Clinical Research, enforced by the Ministry of Health, Labour and Welfare of Japan, from July 31, 2008. The protocol was approved by the Institutional Review Board of Kobe University Graduate School of Medicine, Japan. All patients gave informed written consent before enrolment.

From January 2010 to May 2011, a total of 145 consecutive patients with CAD, who underwent elected PCI at Kobe University Hospital and satisfied inclusion criteria, were enrolled in this study. Within 6–9 months after the PCI, the CAD patients underwent coronary angiography (CAG) for the onset of ischemic symptoms or as a follow-up re-examination. In the CAD group, 103 patients were taking statin (CAD/statin+); 42 CAD patients were not taking statin (CAD/statin–). Patients who had arrhythmia, valvular disease, or cardiomyopathy, but not CAD were enrolled in the control group (non-CAD, $n=82$). In the non-CAD group, 40 patients were receiving statin (non-CAD/statin+) and 42 patients were not (non-CAD/statin–).

In addition, CAD patients were classified by means of follow-up coronary angiography, according to the following definition: patients who showed restenosis in the original stented segment (in-stent restenosis, ISR group); patients with occurrence of other non-target coronary atherosclerotic lesions (de novo lesion group); and patients with neither ISR nor de novo lesion (no lesion group). ISR and de novo lesions were defined as displaying luminal stenosis $\geq 75\%$ and demonstrating ischemia in the perfusion area of narrowed coronary by stress myocardial scintigram. CAD patients, who had been treated with a bare-metal stent or a drug-eluting stent, received dual antiplatelet therapy with aspirin and clopidogrel or ticlopidine. The diagnosis of hypertension (HT), diabetes mellitus (DM), dyslipidemia (DL), and metabolic syndrome (MetS) was defined as described previously¹³⁾.

Exclusion criteria were emergency admission, heart failure (New York Heart Association functional class 4), cancer in the past 5 years, pulmonary hypertension, serum triglyceride level >400 mg/dL, kidney failure (serum creatinine concentration >2.0 mg/dL or hemodialysis), and active inflammation (serum C-reactive protein concentration >1 mg/dL).

Biochemical Analyses

Serum samples were collected after overnight fast on admission for follow-up CAG. There was no difference in the time period from the prior PCI to the blood sampling among the groups. The samples were stored at -80°C until use; conventional biochemical analyses were performed using standard techniques. Serum concentration of remnant-like particle cholesterol (RLP-C), apoB48, and estimated glomerular filtration rate (eGFR) were analyzed as described previously¹³⁾. Serum levels of campesterol and lathosterol were measured by SRL, Inc. (Tokyo, Japan).

Table 1. Characteristics of the patients with or without CAD and statin treatment

	Statin (–)			Statin (+)		
	Non-CAD (<i>n</i> = 42)	CAD (<i>n</i> = 42)	<i>p</i> value	Non-CAD (<i>n</i> = 40)	CAD (<i>n</i> = 103)	<i>p</i> value
Male, <i>n</i> (%)	27 (64.3)	40 (95.2)	<0.001	15 (37.5)	75 (72.8)	<0.001
Age (years)	59.0 ± 12.4	69.1 ± 10.3	<0.001	67.0 ± 7.3	67.6 ± 10.5	0.658
BMI (kg/m ²)	22.7 ± 2.7	24.1 ± 4.3	0.069	24.5 ± 3.6	24.5 ± 3.5	0.875
Metabolic syndrome, <i>n</i> (%)	1 (2.4)	17 (40.5)	<0.001	19 (47.5)	58 (56.3)	0.319
Hypertension, <i>n</i> (%)	19 (45.2)	37 (88.1)	<0.001	23 (47.5)	92 (89.3)	<0.001
Diabetes Mellitus, <i>n</i> (%)	0	19 (45.2)	<0.001	11 (27.5)	50 (48.5)	0.011
Dyslipidemia, <i>n</i> (%)	5 (11.9)	21 (50.0)	<0.001	40 (100)	103 (100)	–
Current smoking, <i>n</i> (%)	8 (19.1)	9 (21.4)	0.786	4 (10.0)	23 (22.3)	0.114
TC (mg/dL)	195.6 ± 29.1	175.4 ± 34.8	0.005	179.2 ± 28.0	160.7 ± 33.0	0.001
HDL-C (mg/dL)	60.6 ± 20.5	46.9 ± 10.3	<0.001	57.4 ± 14.8	48.6 ± 14.5	0.002
LDL-C (mg/dL)	115.2 ± 24.3	106.5 ± 30.1	0.146	99.7 ± 22.6	90.5 ± 24.0	0.045
Triglycerides (mg/dL) [§]	108.1 ± 50.9	128.1 ± 65.2	0.123	122.1 ± 52.4	129.3 ± 59.6	0.425
Campesterol/TC (μg/mg)	2.83 ± 0.97	2.89 ± 0.94	0.754	2.44 ± 1.42	3.48 ± 1.25	<0.001
Lathosterol/TC (μg/mg)	1.45 ± 0.53	1.28 ± 0.87	0.626	0.44 ± 0.16	0.61 ± 0.33	0.013
Campesterol/lathosterol	2.24 ± 1.18	2.74 ± 2.63	0.032	6.49 ± 4.87	7.64 ± 5.31	0.044
RLP-C (mg/dL) [§]	6.5 ± 4.1	7.5 ± 4.8	0.335	5.9 ± 3.6	6.5 ± 4.0	0.290
Apolipoprotein B (mg/dL) [§]	81.8 ± 15.4	78.9 ± 19.3	0.449	76.2 ± 13.4	71.4 ± 15.6	0.096
Apolipoprotein B48 (μg/mL) [§]	4.5 ± 2.2	5.4 ± 3.6	0.028	4.1 ± 2.4	4.6 ± 2.6	0.042
FPG (mg/dL) [§]	94.4 ± 13.8	102.9 ± 31.0	0.111	97.1 ± 29.6	105.0 ± 26.0	0.190
eGFR (mL/min/1.73 m ²)	72.2 ± 18.1	66.1 ± 15.7	0.098	70.0 ± 16.5	61.9 ± 17.9	0.011
Lipid lowering drug, <i>n</i> (%)	0	3 (7.1)	0.078	40 (100)	103 (100)	–
Statin, <i>n</i> (%)	0	0	–	40 (100)	103 (100)	–
Fibrate, <i>n</i> (%)	0	0	–	1 (2.5)	0	<0.001
EPA, <i>n</i> (%)	0	3 (7.1)	0.078	1 (2.5)	6 (5.8)	0.298
Ezetimibe, <i>n</i> (%)	0	0	–	1 (2.5)	1 (1.0)	0.435

Values (mean ± SD) or numbers (% in parentheses) are shown. BMI, body mass index; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol; TC, total cholesterol. *P*-values were calculated using chi-square test for categorical values and *t*-test unpaired for continuous variables.

[§]Based on analysis normalized by logarithmic transformation.

Statistical Analysis

Values are expressed as mean ± SD or frequencies (%). Variables (triglycerides, RLP-C, apoB, apoB48 and fasting plasma glucose) with skewed distribution were normalized by natural logarithmic transformation and were analyzed as follows: *t*-test unpaired, Spearman test, and one-way ANOVA were used to compare continuous variables. Chi-square test was used to compare categorical values. Stepwise multivariate logistic regression analysis was used to determine the best independent predictor of coronary risk in patients with de novo lesion progression compared with those with ISR or no lesions, with the *p* value-to-enter set at 0.10. All statistical analyses were performed using Stata 13.1 software (Stata, Texas, USA). *p* < 0.05 was considered statistically significant. We adjusted significance levels by Bonferroni correction in multiple com-

parison tests.

Results

Controlled Serum Lipid Profile but Enhanced Cholesterol Absorption in CAD/Statin+ Patients

Baseline characteristics and lipid profiles in terms of CAD and statin use are shown in **Table 1**. The CAD groups, with or without statin use, showed a high prevalence of HT and DM compared with the non-CAD groups. The CAD patients were taking intensive LDL-lowering therapy for secondary prevention as recommended¹⁴. As a result, the CAD/statin+ patients had significantly lower total cholesterol (TC) and LDL-C than the non-CAD/statin+ patients. However, the HDL-C level was lower and apoB48 concentration was higher in CAD patients than in

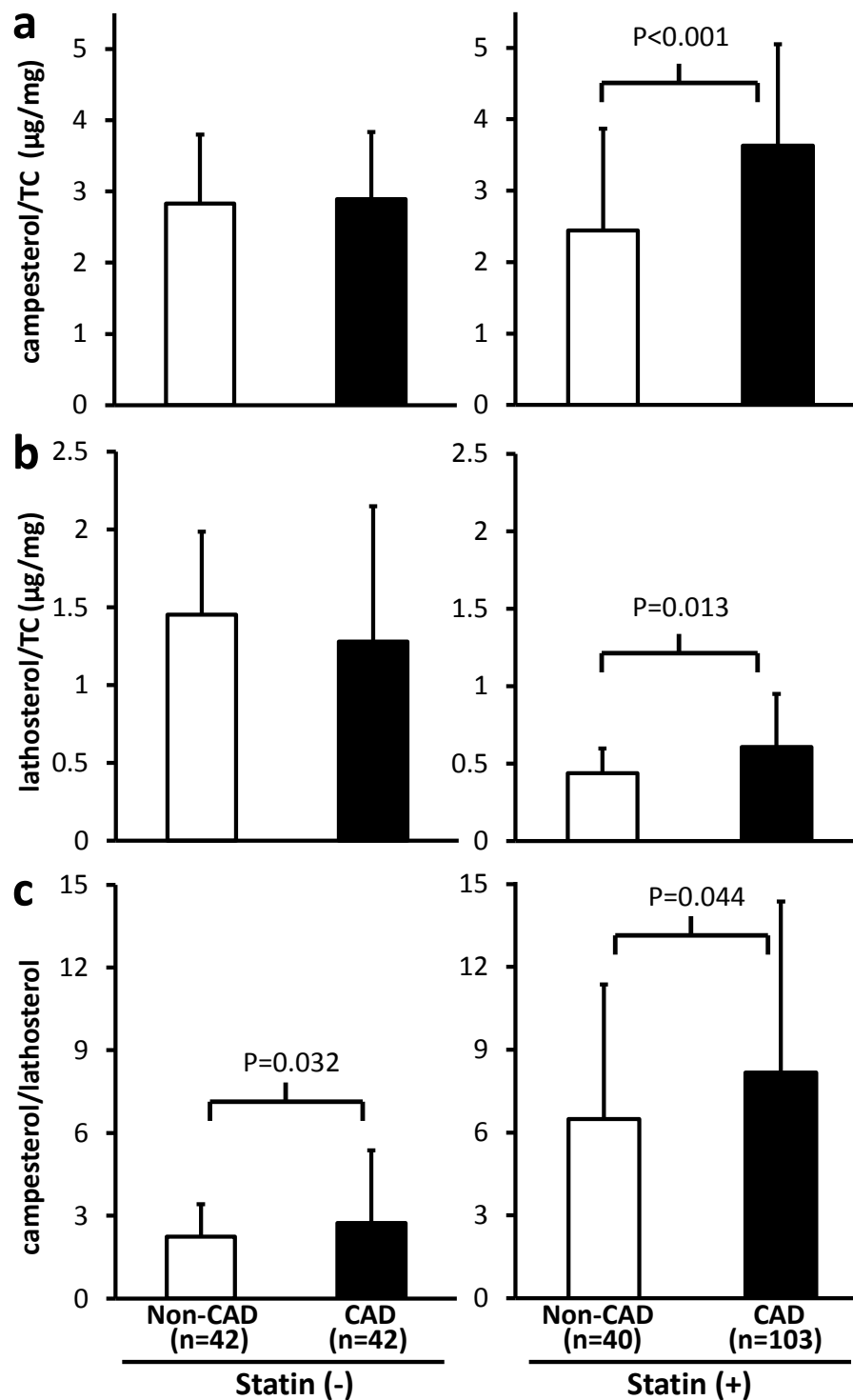


Fig. 1. Cholesterol absorption and synthesis markers in CAD patients with or without statin

During statin therapy, compared with the non-CAD patients, cholesterol absorption marker (campesterol/TC, a) was significantly increased in CAD patients. Cholesterol synthesis marker (lathosterol/TC, b) was decreased in the patients with statin therapy compared with the patients without statin, whereas in the statin (+) group, it was still higher in patients with CAD than in those without CAD. The campesterol/lathosterol ratio (c) was higher in patients with CAD than in corresponding patients without CAD, with and without statin therapy. CAD, coronary artery disease and TC, total cholesterol. *p* values were calculated using *t*-test unpaired.

Table 2. Patient characteristics of statin-treated CAD patients after PCI by lesion prognosis

	No lesion (<i>n</i> = 70)	ISR (<i>n</i> = 15)	De novo (<i>n</i> = 18)	<i>p</i> value
Male, <i>n</i> (%)	50 (71.4)	12 (80.0)	13 (72.2)	0.794
Age (years)	65.3 ± 10.7	65.3 ± 11.0	65.4 ± 9.4	0.771
BMI (kg/m ²)	24.1 ± 3.6	25.3 ± 2.9	25.1 ± 3.5	0.610
Metabolic syndrome, <i>n</i> (%)	38 (54.3)	9 (60.0)	11 (61.1)	0.832
Hypertension, <i>n</i> (%)	64 (91.4)	14 (93.3)	14 (77.8)	0.213
Diabetes Mellitus, <i>n</i> (%)	33 (47.1)	7 (46.7)	10 (55.6)	0.806
Dyslipidemia, <i>n</i> (%)	70 (100)	15 (100)	18 (100)	–
Current smoking, <i>n</i> (%)	19 (27.1)	2 (13.3)	2 (11.1)	0.230
TC (mg/dL)	156.4 ± 32.0	163.3 ± 27.5	175.3 ± 38.2	0.987
HDL-C (mg/dL)	47.5 ± 12.9	50.7 ± 19.9	51.2 ± 15.7	0.564
LDL-C (mg/dL)	87.0 ± 23.1	91.2 ± 15.2	103.6 ± 29.4	0.831
Triglycerides (mg/dL) [§]	128.1 ± 62.7	139.5 ± 67.2	125.8 ± 39.2	0.186
Campesterol/TC (μg/mg)	3.11 ± 0.92	3.08 ± 0.81	5.21 ± 1.24*, **	< 0.001
Lathosterol/TC (μg/mg)	0.60 ± 0.34	0.68 ± 0.23	0.58 ± 0.35	0.192
Campesterol/lathosterol	6.85 ± 3.88	5.25 ± 2.81	12.73 ± 8.14*, **	< 0.001
RLP-C (mg/dL) [§]	6.2 ± 3.8	6.8 ± 2.8	7.2 ± 3.8	0.120
Apolipoprotein B (mg/dL) [§]	70.0 ± 15.6	71.3 ± 10.0	77.9 ± 18.3	0.077
Apolipoprotein B48 (μg/mL) [§]	4.2 ± 2.3	4.4 ± 1.6	5.0 ± 2.6*, **	0.034
FPG (mg/dL) [§]	104.1 ± 26.4	106.1 ± 17.1	107.6 ± 29.2	0.121
eGFR (mL/min/1.73 m ²)	59.0 ± 15.9	71.3 ± 19.4	65.8 ± 21.4	0.222
Lipid-lowering drug, <i>n</i> (%)	70 (100)	15 (100)	18 (100)	–
Fibrate, <i>n</i> (%)	0	0	0	–
EPA, <i>n</i> (%)	4 (5.7)	2 (13.3)	0	0.265
Ezetimibe, <i>n</i> (%)	1 (1.4)	0	0	0.788

Values (mean ± SD) or numbers (% in parentheses) are shown. ISR, in-stent restenosis; de novo, de novo lesion progression at the non-percutaneous coronary intervention site; no lesion, patients with neither ISR nor de novo lesion on the follow-up coronary angiography. Other abbreviations as in Table 1. *P*-values were calculated using chi-square test for categorical values and one-way ANOVA for continuous variables. We adjusted significance levels by Bonferroni correction in multiple comparison tests.

[§] Based on analysis normalized by logarithmic transformation.

p* < 0.05 as compared to the no lesion group. *p* < 0.05 as compared to the ISR group.

non-CAD patients with or without statin treatment. There were no differences in triglycerides and RLP-C between these groups, with or without statin.

As shown in **Fig. 1** and **Table 1**, the cholesterol absorption marker (campesterol/TC) in the CAD/statin+ patients was significantly higher than in the non-CAD/statin+ (**Fig. 1a**, right panel), while the level was similar in patients without statin (**Fig. 1a**, left panel). Cholesterol synthesis marker (lathosterol/TC) in patients receiving statin therapy was markedly low compared with that in patients not receiving statin (statin+ group: 0.56 ± 0.31 μg/mg vs. statin- group: 1.41 ± 0.72 μg/mg, *p* < 0.001) (**Fig. 1b**). However, lathosterol/TC in CAD/statin+ was still higher than that in non-CAD/statin+, despite inhibition of synthesis by statin (**Fig. 1b**, right panel). Thus, the increase of campesterol/TC was not inversely proportional to the decrease in cholesterol absorption. Campesterol/

lathosterol ratio in CAD patients was higher than that in non-CAD patients both with and without statin therapy (**Fig. 1c**). Increases in campesterol/TC, campesterol/lathosterol ratio, and apoB48 concentration in CAD/statin+ patients suggested that the cholesterol absorption in these patients was enhanced during LDL-C lowering therapy with statin.

Cholesterol Absorption Marker and ApoB48 Concentration were Associated with Coronary Lesion Prognosis

We investigated the association between cholesterol absorption or synthesis markers and coronary lesion progression during statin treatment. In the statin-treated CAD patients (*n* = 103), 15 patients showed ISR, 18 patients had de novo lesions, and 70 patients did not show either ISR or de novo lesions at the time of follow-up CAG. As shown in **Table 2**,

there was no difference in the prevalence of HT, DM, DL and MetS. Serum levels of HDL-C, LDL-C, triglycerides, and RLP-C were similar among the three groups. Thus, these conventional risk markers or serum lipid profiles did not predict coronary lesion prognosis in the secondary prevention. By contrast, there were significant differences in serum concentration of apoB48 and cholesterol absorption marker; in de novo group, campesterol/TC was higher than those in no lesion- and ISR groups (**Fig. 2a** and **Table 2**). The cholesterol synthesis marker was not different among the three groups (**Fig. 2b** and **Table 2**). Accordingly, the campesterol/lathosterol ratio was significantly higher in the de novo group than in no lesion- and ISR groups (**Fig. 2c**, **Table 2**) because of the enhanced cholesterol absorption. These results suggest that the high levels of campesterol/TC, campesterol/lathosterol ratio together with apoB48 concentration may represent the increase in cholesterol absorption and intestinal chylomicron production in CAD patients with de novo coronary stenosis, not with ISR. However, there was no significant correlation between cholesterol absorption marker and apoB48 concentration, both in all subjects ($R=0.174$, $p=0.49$) and in the CAD/statin+ group ($R=0.275$, $p=0.29$).

Campesterol/TC and ApoB48 Concentration are Predictors for de novo Lesion Progression

To confirm the impact of cholesterol absorption or synthesis markers on the prognosis of CAD, we performed univariate and stepwise multivariate logistic regression analysis, adjusted classical risk factors (age, gender, HT, DM, current smoking, TC, HDL-C and LDL-C)¹⁵⁾, in addition to campesterol/TC, campesterol/lathosterol ratio and apoB48 concentrations. These analyses were performed between patients with de novo lesion progression and no lesion or ISR (**Table 3**). Univariate analysis showed that campesterol/TC, campesterol/lathosterol ratio, and apoB48 concentration were significantly associated with de novo lesion progression compared with no lesion progression or ISR. Furthermore, stepwise multivariate analysis revealed that both campesterol/TC and apoB48 concentrations were significant risk factors for de novo lesion progression (**Table 3**). These findings suggest that intestinal lipid absorption and intestinal chylomicron production are linked, and serve as predictive risk markers for de novo lesion progression in CAD patients during statin treatment.

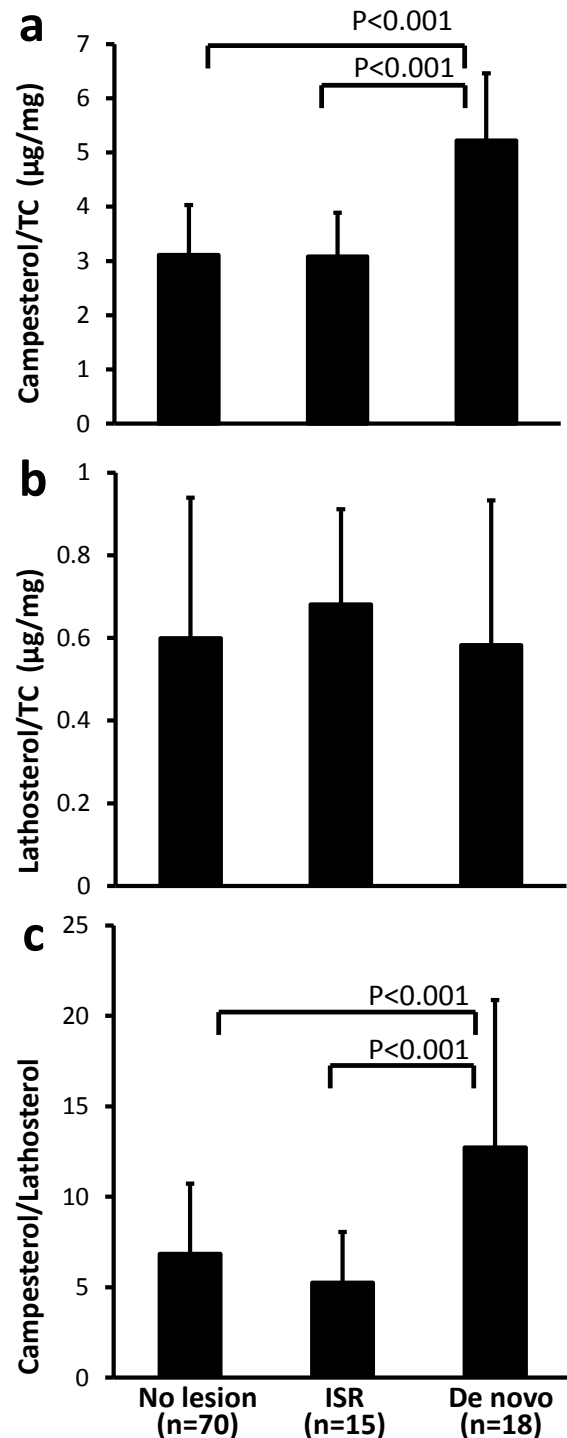


Fig. 2. Impact of cholesterol absorption and synthesis markers on CAD prognosis

Cholesterol absorption marker (campesterol/TC, a) and campesterol/lathosterol ratio (c) was higher in CAD patients with de novo lesion progression after coronary intervention than in patients with in-stent restenosis (ISR) or no lesion. Cholesterol synthesis marker (lathosterol/TC, b) was similar in the three groups. Abbreviations similar to those in Fig. 1. p values were calculated using one-way ANOVA in the three groups. We adjusted significance levels by Bonferroni correction in multiple comparison tests.

Table 3. Univariate and stepwise multivariate logistic regression analyses for de novo lesion progression

Factors	No lesion vs. de novo				ISR vs. de novo			
	OR	[95% CI]		<i>P</i> value	OR	[95% CI]		<i>P</i> value
Univariate analysis								
Campesterol/TC	9.76	3.28	28.99	<0.001	23.53	2.72	203.16	0.004
Lathosterol/TC	0.87	0.18	4.14	0.857	0.31	0.29	3.42	0.342
Campesterol/lathosterol	1.20	1.08	1.34	0.001	1.32	1.05	1.68	0.019
Age	0.97	0.92	1.02	0.250	1.00	0.93	1.07	0.987
Gender (male)	1.04	0.33	3.30	0.070	0.65	0.13	3.33	0.605
Hypertension	0.32	0.08	1.31	0.116	0.25	0.02	2.53	0.240
Diabetes mellitus	1.40	0.49	3.97	0.525	1.43	0.36	5.66	0.661
Current smoking	0.34	0.07	1.60	0.170	0.81	0.10	6.58	0.846
TC (mg/dL)	1.02	1.00	1.03	0.040	1.01	0.99	1.03	0.310
HDL-C (mg/dL)	1.02	0.98	1.05	0.296	1.00	0.96	1.04	0.935
LDL-C (mg/dL)	1.03	1.00	1.05	0.017	1.02	0.99	1.06	0.154
ApoB48 (µg/mL) §	2.09	1.29	3.40	0.003	2.88	1.32	6.29	0.008
Stepwise multivariate analysis								
Campesterol/TC	8.73	3.07	24.85	<0.001	17.51	2.09	146.42	0.008
ApoB48 (µg/mL) §	1.73	0.95	3.74	0.076	1.89	1.16	4.10	0.014
LDL-C (mg/dL)	1.03	0.99	1.07	0.063	none remained			

Univariate and stepwise multivariate analyses were performed in the patients with de novo lesion progression ($n=18$) as compared to ISR ($n=15$) or no lesion ($n=70$), in CAD patients with statin treatment. Stepwise multivariate analysis adjusted for age, gender, hypertension, diabetes mellitus, current smoking, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, campesterol/TC, campesterol/lathosterol ratio and apolipoprotein B48. ApoB48, apolipoprotein B48; CI, confidence interval; OR, odds ratio. Other abbreviations as in Table 1 and 2.

[§]Based on analysis normalized by logarithmic transformation.

Discussion

Enhanced Cholesterol Absorption in CAD Patients Receiving Statin

The present study has demonstrated that the level of cholesterol absorption marker was significantly higher in CAD/statin+ patients with de novo lesion progression than in those with no lesion or with ISR, despite comparable control of serum LDL-C levels in these groups. The relative increase of absorption was likely attributable to the compensation of the inhibition of hepatic cholesterol synthesis by statin⁴. However, statin treatment did not increase campesterol/TC in non-CAD patients (Table 1 and Fig. 1a), and the lathosterol/TC in CAD/statin+ group was still higher than that in the non-CAD/statin+ group (Fig. 1b). The findings indicate that the increase of campesterol/TC in the CAD/statin+ cannot be explained simply by the compensatory effect of statin. We speculate that CAD patients may have some predisposing factor(s) to increase cholesterol absorption. Previous studies have reported that cholesterol absorption is increased in patients with CAD⁹. Also, cholesterol absorption is increased in patients with type 2 DM¹⁶ and renal insufficiency¹⁷. Lally *et al.* have reported that the

expression of intestinal cholesterol transporter, Niemann-Pick C1-like 1 protein (NPC1L1) is elevated in patients with DM¹⁸. The enhanced cholesterol absorption may contribute to the prevalence of CAD in these pathological states.

Possible Mechanisms Underlying the Impact of Cholesterol Absorption on Atherosclerosis

Recent clinical studies have shown that the enhanced absorption and reduced synthesis of cholesterol may be related to coronary plaque volume or vulnerability^{19, 20}. In combination with the present study, we speculated that an increase in diet-derived exogenous cholesterol might be, at least in part, responsible for the residual risks during statin treatment. As for a plausible mechanism, enhanced cholesterol absorption may be associated with enhanced absorption of proatherosclerotic oxidized cholesterol or oxysterols, such as alpha epoxysterol²¹⁻²³.

Variation of Cholesterol Absorption/Synthesis in Plasma Lipid Profile and Atherosclerosis

Increased dietary cholesterol intake may increase serum cholesterol in some individuals, while there may be no response in other subjects^{24, 25}. However,

increased cholesterol absorption can cause hypercholesterolemia⁶⁾; in such a population, this would represent a CAD risk. There is a known variation in the effect of ezetimibe²⁶⁾ and the effect is a counterpart of that of statin²⁷⁾. As a result, patients with low cholesterol absorption are expected to respond better to statins and worse to ezetimibe, and vice versa²⁸⁾. Inactivating mutations of NPC1L1 have been reported to reduce plasma LDL-C levels and the prevalence of CAD²⁹⁾. On the other hand, a partial ileal bypass is known to improve plasma lipid profile and CAD prognosis³⁰⁾. Taken together, the dietary and/or pharmaceutical inhibition of cholesterol absorption may reduce CAD events during statin treatment, particularly in subjects with enhanced cholesterol absorption.

Significance of Other Diet-derived Lipids in Atherosclerosis

Findings from a number of studies suggest that other markers for exogenous lipids may play a role in the development of CAD. Increased level of chylomicron remnants in fasting and postprandial states is correlated to atherogenicity, and high apoB48 concentrations significantly impact accumulation of the MetS or CAD^{12, 31, 32)}. While most of the lipids in remnant lipoprotein are triglycerides, intestinal cholesterol absorption is another regulator of remnant lipoproteins, as is apoB production^{33, 34)}. Furthermore, PCSK9, which down-regulates LDL-receptor, reportedly produces and stabilizes intestinal apoB48 by microsomal triglyceride transfer protein³⁵⁾. Statin treatment activates SREBP2 and thereby upregulates PCSK9, which modulates expression of various transporter molecules controlling cholesterol absorption and excretion³⁶⁾. These effects may contribute to the increase of intestinal triglyceride-rich apoB48 containing particles. On the other hand, elaidic acid (*trans* 9-C18:1) can be used as a tracer for exogenous fatty acids because humans cannot synthesize it. The serum elaidic acid level is elevated in young patients with CAD¹³⁾. Taken together, the lipid- and protein component in chylomicron remnants appear closely linked, and may affect the prognosis of CAD, irrespective of plasma LDL-C concentration.

Limitations

First, this study was cross-sectional, conducted at a single-center with a limited number of patients. The major result was confined to statin-treated CAD patients after PCI, but not the whole population, including statin-untreated CAD after PCI and non-CAD patients. Second, we did not include emergency cases of acute coronary syndrome or severe congestive heart failure. The impact of cholesterol absorption in

patients with acute coronary syndrome has recently been reported²⁰⁾, and is consistent with our present study. Third, the serum LDL-C level was incompletely controlled in some subjects of CAD/statin- group, which may have some potential influence on the CAD prognosis. Fourth, we could not include data for the type/doses of statin, other anti-hyperlipidemic drugs including ezetimibe, and anti-diabetic drugs, because of the limited sample number. The correlation between cholesterol absorption/synthesis and potency of statin, or the LDL-C change should be clarified. Further studies, including a large-scale prospective study, are required to establish the impact of cholesterol absorption in risk management for CAD.

Conclusions

During intensive lipid-lowering therapy with statins, increased cholesterol absorption and intestinal chylomicron synthesis were associated with the progression of de novo coronary lesions. The finding suggests that inhibition of exogenous cholesterol absorption and chylomicron synthesis could be a possible therapeutic target for the secondary prevention of CAD during statin treatment.

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Role of Co-authors in This Manuscript

K.M. performed biochemical and statistical analysis, and wrote the manuscript.

T.I. supervised the project and revised the manuscript.

S.T. and T.O. collected serum samples and interpreted the data.

M.S., T.H. and Y.I. took part in the biochemical assays, and clinical data analysis.

R.T. and K.H. participated in the data analysis and interpretation.

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