Relationship between cholesterol synthesis/absorption marker and vascular function in healthy subjects

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Abstract:

Recently, inhibition of absorbed cholesterol has been reported to reduce the recurrence of vascular events in patients with acute coronary syndrome. However, the effect of absorbed cholesterol on atherosclerosis in healthy subjects remains unclear. In the present study, we investigated the relationship between cholesterol synthesis and absorption biomarkers, and vascular elasticity or morphological changes in healthy subjects. **Methods:** Among 580 subjects who underwent a complete medical checkup between 2012 and 2015, 256 healthy subjects (male/female: 161/95; mean age, 55 ± 10 years) who did not receive any medication were included in the present study. We measured blood pressure, heart rate, blood glucose level, lipid parameters, lathosterol level (cholesterol synthesis marker), and campesterol level (cholesterol absorption marker). We then analyzed the relationship between these measured values and vascular elasticity (cardio-ankle vascular index [CAVI]) or carotid arterial plaque score (CAPS). **Results:** The CAVI and CAPS significantly correlated with the campesterol-to-lathosterol ratio (CAVI; $r=-0.20$, $p=0.015$; CAPS; $r=-0.16$, $p=0.023$). Multivariate analysis of conventional atherosclerotic factors and campesterol-to-lathosterol ratio for CAVI and CAPS as the objective variables showed that age, systolic blood pressure, low-density lipoprotein-to-high-density lipoprotein ratio, and campesterol-to-lathosterol ratio were the significant determining factors for CAVI, and age and campesterol-to-lathosterol ratio were the determining factors for CAPS. **Discussion:** We found that absorbed cholesterol is closely related to vascular elasticity and morphological changes, and that a higher balance of absorption could induce favorable effects on atherosclerosis in healthy subjects. These findings might propose new nutritional guidance for healthy subjects.

Key words:

Campesterol, Lathosterol, Arterial stiffness, Arterial plaque

Introduction

Cholesterol as an atherosclerotic factor is supplied by synthesis in the liver and absorption from the small intestine. Clinical studies have shown that decreased serum cholesterol level due to synthesis and absorption inhibitors could reduce vascular events¹⁻³⁾. Furthermore, the balance of synthesized and absorbed cholesterol could play a role for progression of atherosclerosis⁴⁾. However, study results regarding the relationship between the balance of synthesized and absorbed cholesterol and atherosclerosis are conflicting. Thus, whether absorbed cholesterol could affect atherosclerosis re-

mains controversial. Although the confliction could be due to the difference in study participants with or without vascular events, only few studies have been conducted in subjects without vascular events. In the present study, we investigated the relationship among synthesized or absorbed cholesterol markers, vascular elasticity, and vascular morphological changes in healthy subjects.

Methods

Among 580 subjects who underwent a complete medical checkup between 2012 and 2015, 256 healthy subjects

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Table 1. Demographics of the subjects

	Total (256)	Male (161)	Female (95)	<i>p</i>
Age	56±10	54±10	56±9	ns
BMI (kg/m ²)	22.8±3.2	23.3±3.0	21.9±3.2	ns
Abd.circum (cm)	82±9	84±9	78±9	ns
Smoking (n)	24	23	1	ns
SBP (mmHg)	125±14	128±13	121±15	ns
DBP (mmHg)	76±10	78±9	71±10	ns
TCHO (mg/dl)	227±32	222±31	235±33	ns
TG (mg/dl)	120±81	138±93	91±44	ns
HDL (mg/dl)	65±17	61±15	73±18	ns
LDL (mg/dl)	137±30	135±29	139±31	ns
FBS (mg/dl)	99±14	102±15	95±8	ns
HbA1c (%)	5.5±0.5	5.5±0.6	5.5±0.4	ns
Lathosterol (µg/ml)	3.05±1.52	3.42±1.50	2.45±1.34	ns
Campesterol (µg/ml)	6.24±2.80	6.04±2.63	6.58±3.02	ns

Abd. circum: abdominal circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, TCHO: total cholesterol, TG: triglyceride, HDL: HDL cholesterol, LDL: LDL cholesterol, FBS: fasting blood sugar

(male/female, 161/95; mean age, 55 ± 10 years) were selected. The levels of blood glucose, lipids (total cholesterol: TCHO, HDL cholesterol: HDL, triglyceride: TG, and LDL cholesterol: LDL), and lathosterol (Latho-S) and campesterol (Camp-S), which are markers of synthesized and absorbed cholesterol, respectively, were measured. The relationship between these measurements and vascular elasticity (cardio-ankle vascular index: CAVI) or carotid artery plaque score (CAPS) were analyzed. The right and left CAVIs were measured using a CAVIplus VS-1000 system (Fukuda Den-shi Ltd, Tokyo, Japan), and the higher CVAI value was used for analysis. Carotid artery ultrasonography was performed using a Vivid S5 system (GE Health Care Japan, Tokyo, Japan) or an Avius ultrasonography imaging system (Hitachi, Tokyo, Japan). The intima-medial thicknesses (IMTs) were measured at 1.5 cm on the central side of the right and left external-internal carotid artery bifurcations and additional 3 segments toward the peripheral side of the carotid artery at a 1.5-cm interval. The maximal IMT in each segment, with a thickness of ≥1.1 mm, was obtained, and summation of the maximal IMTs from 4 segments was defined as CAPS. The lathosterol and campesterol levels were measured using gas chromatography. All the subjects were asked to fast, except water, after 21pm before the examination. Smoking was also prohibited from a night before.

Statistical analysis of Camp-S/Latho-S ratio and the absolute value of each marker were performed using a JMP ver. 12. Statistical significance was defined as a *p* value of <0.05. The study was approved by the institutional ethics committee of Shimane University, and written informed consent was obtained from each subject.

Results

Table 1 shows the demographic characteristics of the subjects. The subjects showed no sex-related differences in

age; BMI; systolic and diastolic blood pressures; and levels of TCHO, TG, HDL, LDL, blood glucose, HbA_{1c}, lathosterol, or campesterol. **Figure 1** shows the distributions of each sterol (panel A) and CAVI (panel B). The lathosterol and campesterol levels varied, with the lathosterol levels showing larger variations. By contrast, the right and left CAVIs showed slight differences with small variations, and the higher values were used for the analysis. **Figure 2** shows the relationship between LDL/HDL ratios and absorbed/synthesized cholesterol marker ratio (Camp-S/Latho-S ratio) in all the subjects. **Figure 3** shows the relationship between CAVI (panel A) or CAPS (panel B) and Camp-S/Latho-S ratio. A significant negative correlation was found between LDL/HDL and Camp-S/Latho-S ratio ($r=-0.77$, $p=0.0003$). CAVI and CAPS also negatively correlated to Camp-S/Latho-S ratio with statistical significance ($r=-0.20$, $p=0.015$ and $r=-0.16$, $p=0.023$, respectively).

The univariate analysis of each factor, using the CAVI and CAPS as objective variables, revealed significant correlations of both CAVI and CAPS to age, systolic blood pressure, diastolic blood pressure, campesterol and Camp-S/Latho-S ratio (**Table 2**). Multivariate analysis using the factors with *p* values of <0.1 as variables revealed that systolic blood pressure, LDL/HDL ratio, and Camp-S/Latho-S ratio were significant determinant factors for CAVI, and that age and Camp-S/Latho-S ratio were significant determinant factors for CAPS (**Table 3**).

Furthermore, we performed multivariate analysis for CAVI as objective variable using age, Camp-S/Latho-S ratio and CAPS, because we found a significant positive relationship between CAVI and CAPS ($r=0.16$, $p=0.046$). In this analysis, age and Camp-S/Latho-S ratio were significant ($p<0.0001$ and $p=0.005$, respectively).

Discussion

In the current cross-sectional study, the absorbed/synthesized cholesterol marker ratio was a significant determinant factor for CAVI and CAPS, indicating that the ratio was equivalent to conventional atherosclerotic risk factors such as age and blood pressure. Our results revealed that balance of absorbed and synthesized cholesterol is closely linked to atherosclerosis progression.

High cholesterol level, especially LDL cholesterol, has been well known to be closely linked to atherosclerosis progression. In humans, cholesterol is supplied by synthesis in liver and absorption from the intestinal tract. Cholesterol synthesis and metabolism pathways in the liver have been well documented, and HMG-CoA reductase inhibitors (statins) are widely used as modulators of the pathway. Statin effects on anti-cerebrovascular and anti-cardiovascular events have already been proven by many large-scale clinical studies^{1,5}. Meanwhile, recently, the effects of inhibition of cholesterol absorption on cardiovascular events was reported. Thus, the inhibitor of Niemann-Pick C1-like protein (NPC1L1) (ezetimibe), which selectively transports choles-

Figure 1. Relationship between absorbed cholesterol (campesterol, vertical axis) and synthesized cholesterol (lathosterol, horizontal axis) in all the subjects (left panel). Relationship between the right (horizontal axis) and left (vertical axis) CAVI measurements (right panel). Both plots indicate variations of the indexes.

Figure 2. Relationship between LDL/HDL ratio (vertical axis) and absorbed/synthesized cholesterol balance (Camp-S/Latho-S ratio, horizontal axis) in all the subjects. A significant negative correlation was found between the parameters ($r=-0.77$, $p=0.0003$).

terol into small intestinal cells^{6,7}, significantly reduced cardiovascular death in 18,144 ACS patients by combined treatment with statin². Recently, Japaridze et al reported that atorvastatin plus ezetimibe therapy significantly reduced vascular events by 11.1% compared with atorvastatin monotherapy in 323 patients with recently diagnosed acute coronary syndrome³. However, the results of the present study showed that the absorbed cholesterol is not a unique factor of atherosclerosis progression. Thus, the negative correlation between absorbed/synthesized cholesterol ratio and CAVI (vascular elasticity) or CAPS (morphological changes in artery) suggests that higher balance of absorbed blood cholesterol might reduce the atherosclerosis score in healthy subjects.

Fassbennder et al.⁸ reported in 2008 that the coronary artery disease risk in 1242 healthy subjects with higher ab-

sorbed/synthesized cholesterol ratio was reduced at ages ≥ 65 years. Furthermore, Gylling et al.⁹ measured CAVI in 92 healthy subjects, and Ras et al.¹⁰ measured pulse-wave velocity in 240 patients with dyslipidemia and reported that the increased absorbed cholesterol improved vascular elasticity. By contrast, Buchwald et al.¹¹ reported in 1990 that partial resection of the small intestine in 838 patients who survived a first myocardial infarction reduced cardiovascular events (coronary disease death and nonfatal myocardial infarction) by 35% (ROSC study). Furthermore, Strandberg et al.¹² reported an observational study of 376 patients with coronary artery disease in 2006. They found that patients with high ratio of absorbed cholesterol had higher cerebrovascular and cardiovascular events. In addition, the Myocardial Infarction Genetics Consortium Investigators in which Kathiresan was the principal investigator¹³ identified the NPC1L1 gene exon sequence and detected NPC1L1 gene mutation carriers (nonsense mutation, splice mutation, and frameshift mutation) in 7364 coronary artery disease patients and 14,728 control subjects without coronary artery disease who were of European, African, and South Asian origin in 2014. They also performed genotyping in 22,590 coronary artery disease patients and 68,412 control subjects for specific gene mutations (p.Arg406X), and investigated the relationship between mutant gene expression, LDL level, and coronary artery disease risk in 2 groups. Fifteen NPC1L1 inactivated gene mutations were detected by sequencing, and the frequency of the heterozygous carrier for each mutation was approximately 1/650. They also found that the mean LDL value in the gene mutation carriers was 12 mg/dL lower than that in non-carriers ($p=0.04$), and the mutation carriers showed 53% lower coronary artery disease risk, with statistical significance ($p=0.008$). However, the authors stated that further investigations were necessary to confirm their results to support the usefulness of cholesterol absorption inhibitors.

The previous reports suggest mixed results regarding the

Figure 3. Relationship between CAVI (left panel) or CAPS (right panel) and absorbed/synthesized cholesterol balance (Camp-s/Lathol-s ratio). Both CAVI ($r=-0.20$, $p=0.015$) and CAPS ($r=-0.16$, $p=0.023$) showed weak but significant negative correlations to the absorbed/synthesized cholesterol balance.

Table 2. Univariate analysis of each risk factor for CAVI and CAPS

	CAVI		CAPS	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Age	0.58	<0.0001	0.33	<0.0001
BMI	0.13	0.107	0.09	0.197
SBP	0.26	0.001	0.18	0.011
DBP	0.14	0.094	0.16	0.019
TG	0.14	0.081	0.11	0.121
LDL/HDLratio	0.15	0.068	0.11	0.123
FBS	0.07	0.390	0.11	0.057
Lathosterol	0.07	0.424	0.12	0.096
Campesterol	0.24	0.002	0.16	0.019
Camp-S/Latho-S ratio	0.20	0.015	0.16	0.023

Camp-S/Latho-S ratio: campesterol and lathosterol ratio, CAPS: carotid artery plaque score, CAVI: cardio-ankle vascular index

Table 3. Multivariate analysis of risk factors for CAVI and CAPS

	CAVI		CAPS	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Age	75.57	<0.0001	25.70	<0.0001
SBP	6.14	0.014	2.17	
DBP	0.86		3.24	
TG	2.24			
LDL/HDLratio	9.17	0.003		
FBS			0.35	
Lathosterol			1.21	
Campesterol	0.11		0.48	
Camp-S/Latho-S ratio	8.42	0.004	6.12	0.014

Camp-S/Latho-S ratio: campesterol and lathosterol ratio, CAPS: carotid artery plaque score

effect of absorbed cholesterol on atherosclerosis. Thus, studies that showed a favorable effect of absorbed cholesterol were conducted in healthy subjects, whereas studies that showed a negative effect were conducted in patients with atherosclerotic vascular diseases. Therefore, as shown in previous studies, the inhibition of both synthesis and absorption of cholesterol is effective in patients with atherosclerosis progression, and absorbed cholesterol may be effective to prevent atherosclerosis in subjects who has not yet experienced cardiovascular events. Our results support the hypothesis that absorbed cholesterol can prevent atherosclerosis in subjects without experience in cardiovascular events.

This study has several limitations. First, this was a cross-sectional study, which did not assess the cardiovascular events of the participants. This limitation can be solved by a prospective observation of participating subjects, which may also provide us new insights. Second, in the present study, some study participants may have had various background risk factors of atherosclerosis, especially diabetes mellitus or impaired glucose tolerance. Increased cholesterol absorption

activity has been reported in diabetic patients¹⁴. A previous large-scale clinical study of cholesterol absorption inhibitor showed that the cholesterol absorption inhibitor did not reduce the cerebrovascular and cardiovascular events in non-diabetic patients, whereas the events in diabetic patients were significantly reduced, suggesting that cholesterol absorption activity is influenced by patients' background factors. Thus, in diabetic patients, absorbed cholesterol may induce progression of atherosclerosis, resulting in different conclusions. Further detailed studies with larger numbers of subjects are necessary. Third, the quality of absorbed cholesterol, including oxidative (degraded) cholesterols, should be investigated. Oxidative cholesterols are produced by excess heat or storage in the air of meat and egg with enriched cholesterol. Retort foods, fried foods (potato chips, etc.), and other fast foods also contain oxidative cholesterols. One study reported a positive correlation between the number of fast-food stores and cerebral infarction events¹⁵, which does not support our results that higher balance ratio of absorbed cholesterol can prevent atherosclerosis progression. To confirm our results, not only absorbed cholesterol but also oxi-

dative cholesterol (oxysterol) should be measured. Fourth, the study participants did not take any medication. Recent studies have shown that the number of patients who received statin therapy has increased and that statin therapy upregulates cholesterol absorption. It is also important to investigate whether other non-statin lipid-modifying therapies have similar effects on cholesterol absorption. Lastly, we could not provide the mechanism of absorbed cholesterol balance effect on atherosclerosis prevention. In this study, balance of absorbed and synthesized cholesterol was significant predictive value for CAVI and CAPS, and CAVI significantly correlated with CAPS. These findings suggest the presence of mediators among these parameters. Further studies of atherosclerosis progression factors such as oxidative cholesterol intake (described above), inflammation markers, small dense LDL or oxidative LDL are necessary. We will continue to investigate to resolve these study limitations in the future.

We found in the present study that absorbed cholesterol is closely related to vascular elasticity and morphological changes in lipid metabolism, causing atherosclerosis. While in the current standard guideline of lifestyle, restriction of lipid intake is considered as the primary step to improve abnormal lipid metabolism, our results may provide new insights into nutritional guidelines for healthy subjects with pre-atherosclerotic condition.

Conflicts of Interest

There are no conflicts of interest

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