Post-prandial Remnant Lipoprotein Metabolism in Sitosterolemia

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Aim: We aimed to clarify post-prandial accumulation of remnant-like particles (RLP) in patients with sitosterolemia.

Methods: Oral fat tolerance test cream (Jomo Shokuhin, Takasaki, Japan) 50 g was given per body surface area (m²); blood sampling was performed at 2 h intervals up to 6 h. Plasma lipoprotein fractions and RLP fractions were determined in four sitosterolic subjects with double mutations in ATP-binding cassette (ABC) sub-family G member 5 or member 8 (ABCG5 or ABCG8) gene (mean age= 18 yr, median low-density lipoprotein cholesterol [LDL-C]= 154 mg/dL), six heterozygous carriers (mean age= 31 yr, median LDL-C= 105 mg/dL), and five subjects with heterozygous familial hypercholesterolemia (FH, mean age= 32 yr, median LDL-C= 221 mg/dL). The incremental area under curve (iAUC) of lipids, including LDL-C, apolipoprotein B-48 (apoB48), RLP cholesterol (RLP-C), and RLP triglyceride (RLP-TG) were evaluated.

Results: After oral fat load, there was no significant difference of the iAUC of LDL-C between sitosterolemia and heterozygous FH, whereas the iAUC of apoB48 was significantly larger in the sitosterolemic subjects compared with that of heterozygous FH (2.9 µg/mL×h vs. 1.3 µg/mL×h, p<0.05). Under these conditions, the iAUCs of RLP-C and RLP-TG levels were significantly larger in the sitosterolemic subject compared with those of heterozygous FH (9.5 mg/dL×h vs. 5.7 mg/dL×h, p<0.05; 149 mg/dL×h vs. 40 mg/dL×h, p<0.05, respectively), whereas those of heterozygous carriers were comparable with those with heterozygous FH.

Conclusions: Post-prandial lipoprotein metabolism in sitosterolemia appeared to be impaired, leading to their elevation in serum sterol levels. (UMIN Clinical Trials Registry number, UMIN000020330)

Key words: Sitosterolemia, Remnant, Familial hypercholesterolemia, Remnant-like-particles, OFTT

Introduction

Familial hypercholesterolemia (FH) is a common inherited disorder of plasma lipoprotein metabolism, characterized by an elevated level of low-density lipoprotein cholesterol (LDL-C), tendon xanthomas, and premature coronary artery disease¹. Monogenic causes of FH involve gene mutations such as LDL receptor, apolipoprotein B-100 (apoB100), and proprotein convertase subtilisin/kexin type 9 (PCSK9)². Post-prandial accumulation of lipoprotein remnants has been shown to be related with elevated cardiovascular risk³). Under these conditions, it has been shown that post-prandial lipoprotein metabolism is severely impaired in a dominant form of FH⁴), whereas we have shown that such lipoprotein metabolism is preserved in a recessive form of FH called autosomal recessive hypercholesterolemia caused by mutations in LDL receptor adaptor protein 1 (LDLRAP1) gene⁵). Investigating the extreme cases harboring mutations in a specific gene provides an opportunity to directly observe the role of certain molecules in lipoprotein metabolism.

See editorial vol. 25: 1183-1184
Sitosterolemia (OMIM #210250) is a rare, inherited, autosomal recessive disorder of lipid metabolism characterized by increased absorption and decreased biliary excretion of plant sterols and cholesterol, resulting in prominently elevated serum concentrations of plant sterols such as sitosterol and campesterol. This disease is caused by mutations in either of the two genes, named ATP-binding cassette (ABC) sub-family G member 5 and member 8 (ABCG5 and ABCG8). Subjects suffering from sitosterolemia present primarily with tendinous and tuberous xanthomas, premature coronary atherosclerosis such as FH. LDL-C levels are more variable in sitosterolemia than in other genetic hyperlipidemias, but can be extremely elevated in some patients, especially in breastfed infantile patients. This report indicates that patients with sitosterolemia might be vulnerable to post-prandial hyperlipidemia. Remnant-like particles (RLP) are known as a good marker to evaluate lipid metabolism after diet. Also, increased RLP levels are associated with high LDL-C, endothelium dysfunction, and coronary artery disease (CAD). Thus, this post-prandial condition may play an important role of plasma high LDL-C levels in sitosterolic patients. In this study, we examined post-prandial lipoprotein metabolism in sitosterolemia to determine their vulnerability to oral fat load for the first time.

### Materials and Methods

#### Study Design

This study is a single-arm, non-randomized, open-label, uncontrolled trial. Patients with homozygous sitosterolemia with double mutations, heterozygous carriers with single mutation, and heterozygous FH are enrolled in this study. They receive an oral fat tolerance test (OFTT) cream when they met the inclusion criteria. We then compare post-prandial plasma RLP cholesterol (RLP-C) levels between the patients with sitosterolemia with double mutations in ABCG5 or ABCG8 gene, heterozygous mutation carriers with single mutation, and heterozygous FH. This study has been registered at the University Hospital Medical Information Network (UMIN) (UMIN ID: 000020330).

#### Materials and Method

**Table 1. Characteristics of the study subjects**

|                    | Sitosterolemia (n=4) | Single mutation carrier (n=6) | Heterozygous FH (n=5) |
|--------------------|----------------------|-----------------------------|----------------------|
| Age                | 18 ± 13              | 31 ± 7                      | 32 ± 11              |
| Sex (male/female)  | 2/2                  | 3/3                         | 2/3                  |
| BMI (kg/m²)        | 23.1 ± 4.1           | 23.9 ± 6.5                  | 24.5 ± 4.0           |
| TC (mg/dl)         | 241 [226–275]        | 223 [189–233]               | 289 [284–305]        |
| TG (mg/dl)         | 201 [132–241]        | 99 [91–138]                 | 101 [94–146]         |
| HDL-C (mg/dl)      | 50 [47–60]           | 57 [50–58]                  | 49 [46–55]           |
| LDL-C (mg/dl)      | 154 [150–156]        | 105 [89–164]                | 221 [220–246]        |
| apoB48 (µg/ml)     | 5.7 [3.6–7.9]        | 2.8 [1.9–3.4]               | 1.4 [1.4–2.2]        |
| Lathosterol (µg/ml)| 3.0 [2.3–3.6]        | 1.5 [1.1–1.6]               | 3.8 [2.6–3.8]        |
| Campesterol (µg/ml)| 57 [49–70]           | 14.9 [13.9–15.5]            | 4.6 [3.3–4.9]        |
| Sitosterol (µg/ml)| 88.5 [73.6–113.2]    | 6.5 [5.1–8.4]               | 2.5 [1.9–2.9]        |
| RLP-C (mg/dl)      | 14.4 [11.8–16.5]     | 3.6 [3.7–8.4]               | 9.1 [7.2–10.8]       |
| RLP-TG (mg/dl)     | 41.5 [37.0–82.6]     | 14.2 [11.4–40.0]            | 12.7 [12.5–54.2]     |

FH: familial hypercholesterolemia, BMI: body mass index, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, apoB48: apolipoprotein B-48, RLP-C: remnant-like particle cholesterol, RLP-TG: remnant-like particle triglyceride.
**Statistical Analysis**

Values are expressed as medians (interquartile [IQR]) unless otherwise stated. Area under curve (AUC) for triglyceride (TG), LDL-C, apoB48, sitosterol, RLP-C, and RLP triglyceride (RLP-TG) at baseline and after fat load were calculated using the trapezoid rule. The differences in the incremental AUC (iAUC) of the plasma variables between the three groups were examined using the Kruskal–Wallis test. Post hoc analyses using Scheffé’s method were performed when the main effect was significant. All tests of statistical significance were assumed at a level of \( p < 0.05 \).

**Ethical Considerations**

This study was approved by the Ethics Committee of Kanazawa University and carried out in accordance with the Declaration of Helsinki (2008) of the
between sitosterolemia and heterozygous FH, whereas
the iAUC of TG was significantly larger in sitosterol-
emic subjects than that in heterozygous FH (154 mg/
dL × h vs. 50 mg/dL × h, p < 0.05, Fig. 3).

Interestingly, the iAUC of apoB48 was signifi-
cantly larger in the sitosterolemic subjects compared
with that of heterozygous FH (2.9 µg/mL × h vs. 1.3
µg/mL × h, p < 0.05, Fig. 3). In addition to those results,
the iAUC of sitosterol in the sitosterolemic subjects
was significantly larger than that of heterozygous FH
as expected (66 µg/mL × h vs. 0.5 µg/mL × h, p <
0.05). Under these conditions, the iAUCs of RLP-C
and RLP-TG levels were significantly larger in the
sitosterolemic subjects compared with those of hetero-
yzous FH (9.5 mg/dL × h vs. 5.7 mg/dL × h, p < 0.05;
149 mg/dL × h vs. 40 mg/dL × h, p < 0.05, respectively,
Fig. 4), whereas those of single mutation carrier in
ABCG5 or ABCG8 gene were comparable with those
with heterozygous FH.

Results

The baseline characteristics of the three study
groups are shown in Table 1. Genetic backgrounds of
the study subjects are shown in Supplemental Table 1.
These groups of patients were comparable in terms of
age, gender, and body mass index (BMI). Interestingly,
the baseline LDL-C level of sitosterolemic patients with
double mutations was significantly higher than that of
single mutation, whereas baseline LDL-C level of het-
erozygous FH was significantly higher than that in
sitosterolemic subjects. On the other hand, serum lev-
els of sitosterol and campesterol in sitosterolemic patients
with double mutations were significantly higher than
those with a single mutation as well as those in hetero-
yzous FH. In addition, apoB48, TG, RLP-C, and
RLP-TG levels were significantly higher in sitosterol-
emic subjects than those in heterozygous FH (Table 1).

After oral fat load, lipoproteins, other than LDL-
C, increased through 2 to 4 h (Figs. 1 and 2). There
was no significant difference in the iAUC of LDL-C
between sitosterolemia and heterozygous FH, whereas
the iAUC of TG was significantly larger in sitosterol-
emic subjects than that in heterozygous FH (154 mg/
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Fig. 4), whereas those of single mutation carrier in
ABCG5 or ABCG8 gene were comparable with those
with heterozygous FH.

Discussion

The main finding of the present study is that the
clearance of post-prandial RLP fraction was impaired
in sitosterolemia compared with heterozygous FH. This
is the first study to demonstrate the impaired post-
prandial RLP metabolism in sitosterolemia.

Impaired TG-rich lipoprotein metabolism under
the condition of disturbance of ABCG5/ABCG8 have
lipidemia. We confirmed that post-prandial RLP metabolism was disturbed in the patients with sitosterolemia for the first time. Moreover, apoB48 level, which has been shown to be associated with atherosclerotic cardiovascular diseases\textsuperscript{18}), was significantly elevated in sitosterolemic patients both in fasting state, as well as in post-prandial state. In addition, we observed that the peaks of the post-prandial lipoproteins were earlier in sitosterolemic patients than those in heterozygous FH. Moreover, sitosterol level in sitosterolemic patients increased after fat load, although such trends were not observed in heterozygous FH nor in single mutation carriers. Thus, dietary counseling for the patients with sitosterolemia should be one of the reasonable approaches been implicated by the observational and interventional studies of sitosterolemic subjects\textsuperscript{14, 15}) as well as by the experimental study using ABG5/ABG8 knockout mice \textit{(sitosterolemic mice)}\textsuperscript{16}). Previously, we demonstrated that breastfed infantile cases with sitosterolemia harboring double mutations in ABG5 gene exhibit transient extreme hyper-LDL cholesterolemia\textsuperscript{9}). In addition, there have been great diversities in the LDL-C levels among the subjects with sitosterolemia described so far\textsuperscript{17}). Such observations as well as the facts that ABG5/ABG8 are playing an important role in excretion of sterols in the intestine could lead us to investigate if the patients with sitosterolemia harboring ABG5/ABG8 mutations are vulnerable to diet-induced hyper-

\textbf{Fig. 3.} The iAUC of lipoproteins

\begin{description}
\item[Boxplots illustrating the iAUC of (A) TG, (B) LDL-C, (C) apoB48, and (D) sitosterol in three groups.]
\item[Red: Sitosterolemia]
\item[Pink: Single mutation carrier]
\item[Light blue: Heterozygous FH]
\end{description}
by actual “post-prandial” lipemia itself. Despite those limitations, we believe that this study provides new insights into the roles of ABCG5/ABCG8 in the post-prandial lipoprotein metabolism.

Acknowledgements and Notice of Grant Support

We express our special thanks to Kazuko Honda and Sachio Yamamoto (staff of Kanazawa University) for their outstanding technical assistance. We also express our special thanks to Drs. Takuya Nakahashi, Yoshihiro Tanaka, Taro Ichise, Takashi Kobayashi, Azusa Ohbatake, and Akari Wada for their assistance in data collection. We have received the research grants from Sakakibara Memorial Research Grant from the Japan Research Promotion Society for Cardiovascular Diseases and Astellas Foundation for Research on Metabolic Disorders.

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Hayato Tada has received a research grant from Sanofi K.K. Atsushi Nohara and Hiroshi Mabuchi have received research grants from MSD K.K., Sanofi K.K., Shionogi & Co., Ltd., Kowa Co., Ltd., Astellas Pharma Inc., AstraZeneca K.K., Keiai-Kai Medical Corp., and
Biopharm of Japan Co. Masakazu Yamagishi has received research grants from MSD K.K., Astellas Pharma Inc., Daiichi-Sankyo Co., Ltd., and Otsuka Pharmaceutical Co., Ltd., and he has received payments for lectures from Astellas Pharma Inc., Daiichi-Sankyo Co., Ltd., Shionogi & Co., Ltd., and Kowa Co., Ltd. Masa-aki Kawashiri has received payments for lectures from Amgen Astellas Biopharma K.K. and Astellas Pharma Inc.

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### Supplemental Table 1. Genetic backgrounds of the subjects

| Sitosterolemia  $(n = 4)$ | Heterozygous carrier  $(n = 6)$ | Heterozygous FH  $(n = 5)$ |
|--------------------------|---------------------------------|----------------------------|
| c.1256G $>$ A/c.1763-1G $>$ A (ABCG5) | c.1256G $>$ A (ABCG5) | c.1702C $>$ G (LDLR) |
| c.454C $>$ T/c.1403_1404delTC (ABCG8) | c.1763-1G $>$ A (ABCG5) | c.1845 + 2T $>$ C (LDLR) |
| c.1306G $>$ A/c.1813_1817delCTTTT (ABCG5) | c.1306G $>$ A (ABCG5) | c.2054C $>$ T (LDLR) |
| c.130T $>$ G/c.1306G $>$ A (ABCG5) | c.1813_1817delCTTTT (ABCG5) | c.2431A $>$ T (LDLR) |
|  | c.1306G $>$ A (ABCG5) | c.94G $>$ A (PCSK9) |
|  | c.130T $>$ G (ABCG5) |  |

FH: familial hypercholesterolemia, ABCG5: ATP-binding cassette (ABC) sub-family G member 5, ABCG8: ATP-binding cassette (ABC) sub-family G member 8, LDLR: low-density lipoprotein receptor, PCSK9: proprotein convertase subtilisin/kexin type 9