Plant sterol hyperabsorption caused by uncontrolled diabetes in a patient with a heterozygous ABCG5 variant

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ABSTRACT
Plant sterol intake is widely recommended for patients with cardiovascular risk factors based on the inhibitory effect on intestinal cholesterol absorption. Although plant sterols, once absorbed, can promote atherosclerosis, their intake is believed to be safe because of poor absorption, except in rare hyperabsorbers with homozygous ABCG5/8 mutations. We report a case of new-onset type 1 diabetes accompanied by hypercholesterolemia. At the initial presentation with diabetic ketoacidosis, the patient showed marked hypercholesterolemia. Whole-exome sequencing revealed a heterozygous pathogenic variant in ABCG5 (p.R419H). The initial serum plant sterol levels were markedly high (sitosterol 32.5 μg/mL, campesterol 66.0 μg/mL), close to the range observed in patients with homozygous ABCG5/8 mutations, which were largely reduced by insulin treatment without ezetimibe. The addition of ezetimibe normalized plant sterol levels. These findings provide the first evidence that uncontrolled diabetes plays a causal role in the pathogenesis of phytosterolemia.

INTRODUCTION
Plant sterols (or phytosterols), such as sitosterol and campesterol, inhibit intestinal cholesterol absorption because of their structural similarity to cholesterol. Dietary plant sterols taken up by the intestinal mucosa are largely excreted into the feces by adenosine triphosphate-binding cassette subfamily G members 5 (ABCG5) and 8 (ABCG8). Based on their cholesterol-lowering effect, plant sterol consumption has been widely recommended for patients at risk of cardiovascular disease, including those with diabetes. However, once absorbed, plant sterols can promote atherosclerosis, which is observed in patients with sitosterolemia caused by biallelic loss-of-function mutations in ABCG5/8 genes. In patients with diabetes, although some studies suggested increased plant sterol absorption, there is no direct evidence of its changes in response to the treatment of hyperglycemia. Therefore, it is unclear whether the recommendation to increase plant sterol consumption is safe for every patient with diabetes. We herein report a patient with a heterozygous loss of function ABCG5 mutation, who showed marked phytosterolemia and hypercholesterolemia at the onset of type 1 diabetes. Insulin treatment substantially decreased his serum plant sterol levels, and the addition of ezetimibe further reduced his serum plant sterol levels to the normal range. In the present study, we provide the first direct evidence that uncontrolled diabetes has a causal role in phytosterolemia.

CASE REPORT
A 21-year-old Japanese man (height 174.3 cm, weight 54.8 kg and body mass index 18.0 kg/m²) without remarkable medical history was admitted to Kitano Hospital, Osaka, Japan, complaining of polydipsia, polyuria and unintentional bodyweight loss for 2 months. At the presentation, physical examination revealed tachycardia, dry oral mucous membranes and deep respiration, but no physical findings characteristic of familial hypercholesterolemia, such as xanthomas and corneal rings. Based on ketonuria, metabolic acidosis and hyperglycemia, he was diagnosed with diabetic ketoacidosis (Table 1). Given the presence of anti-glutamic acid decarboxylase antibody and...
severely blunted serum C-peptide response during the glucagon stimulation test, we diagnosed the patient as acute-onset type 1 diabetes. Intensive insulin therapy was initiated immediately after admission, and his diabetic ketoacidosis resolved on hospital day 2. The patient was discharged on hospital day 19 with his diabetes. Intensive insulin therapy was initiated immediately after admission, and his diabetic ketoacidosis resolved on hospital day 2. The patient was discharged on hospital day 19 with insulin degludec and insulin aspart for routine glycemic control, and insulin treatment drastically reduced his glycated hemoglobin (15.8% at the baseline to 6.2% at 2 months).

Table 1 | Laboratory data of the patient at the initial presentation

| Variable                        | Result     | Reference |
|---------------------------------|------------|-----------|
| Peripheral blood                |            |           |
| White blood cells (/μL)         | 14,100     | 3,300–8,600 |
| Neutrophil (%)                  | 75.8       | 41.7–33.7 |
| Eosinophil (%)                  | 0.0        | 0.7–8.1   |
| Red blood cells (x10^6/μL)      | 606        | 435–555   |
| Hemoglobin (g/dL)               | 18.6       | 13.7–16.8 |
| Hematocrit (%)                  | 58.1       | 40.7–50.1 |
| Platelets (x10^3/μL)            | 18.8       | 15.8      |
| Blood biochemistry              |            |           |
| Total protein (g/dL)            | 8.4        | 6.6–8.1   |
| Albumin (g/dL)                  | 5.5        | 4.1–5.1   |
| AST (U/L)                       | 18         | 13–30     |
| ALT (U/L)                       | 20         | 10–42     |
| LDH (U/L)                       | 189        | 124–222   |
| ALP (U/L)                       | 341        | 106–322   |
| γ-GTP (U/L)                     | 44         | 13–64     |
| BUN (mg/dL)                     | 15.6       | 8.0–20.0  |
| Creatinine (mg/dL)              | 1.04       | 0.65–1.07 |
| Urine acid (mg/dL)              | 6.6        | 3.7–7.8   |
| Creatinine kinase (U/L)         | 348        | 59–248    |
| Sodium (mmol/L)                 | 134        | 138–145   |
| Potassium (mmol/L)              | 46         | 36–48     |
| Chloride (mmol/L)               | 102        | 101–108   |
| Total cholesterol (mg/dL)       | 670        | 142–248   |
| C-reactive protein (mg/dL)      | 0.05       | 0–0.14    |
| Total ketone body (μmol/L)      | 14,796     | <130      |
| Acetoacetate (μmol/L)           | 2,235      | <55       |
| β-Hydroxybutyrate (μmol/L)      | 12,561     | <85       |

ALP, alkaline phosphatase; ALT, alanine aminotransferase; Anti-GADAb, anti-glutamic acid decarboxylase antibody; Anti-IA-2 antibody, anti-insulinoma-associated protein-2 antibody; Anti-InsulinAb, anti-insulin antibody; Anti-TgAb, anti-thyroglobulin antibody; Anti-TPOAb, anti-thyroid peroxidase antibody; Anti-ZnT8Ab, anti-zinc transporter protein 8 antibody; AST, aspartate aminotransferase; BUN, blood urea nitrogen; FT3, free triiodothyronine; FT4, free thyroxine; HbA1c, glycated hemoglobin; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyltranspeptidase.

CYP27A1 and ABCG8 in the patient’s genome. Based on these results, we measured serum plant sterol levels of the patient at the initial presentation, which showed marked elevation of both sitosterol and campesterol (32.5 μg/mL and 66.0 μg/mL, respectively) close to the range of patients with homozygous ABCG5/8 mutations.6 His mother and brother did not have the ABCG5 variant, and showed normal serum plant sterols levels (Figure 1a). We were unable to obtain his father’s lipid profile and genomic DNA, because his father had already died of amyotrophic lateral sclerosis. Therefore, we could not determine whether the ABCG5 variant is paternally derived or de novo. All genetic analyses of the patient and his family members were approved by the local ethics committee of Kitano Hospital (No. 18040601, approved on 17 April 2018). Intriguingly, after improved glycemic control with insulin, plant sterol concentrations were decreased to approximately 25% of initial levels without ezetimibe (Figure 1b). At that point, ezetimibe was initiated considering the possibility of increased plant sterol absorption. In addition, although a detailed dietary interview...
showed that the patient had not consumed excess plant sterol-enriched foods before the onset of type 1 diabetes, we instructed the patient to avoid dietary intake of plant sterols. Thereafter, serum sitosterol and campesterol levels were normalized. To further confirm the effects of ezetimibe or atorvastatin, we serially measured serum plant sterols after the sequential discontinuation of each medication. The results showed elevation of plant sterol levels only after discontinuation of ezetimibe, even under optimal glycemic control. The clinical course of glycemic and lipid profiles of the patient are summarized in Table 2 and Figure 1b.

DISCUSSION

In the present study, we characterized marked phytosterolemia in a patient with uncontrolled diabetes who has a heterozygous ABCG5 mutation. The current findings have two important implications: (i) pathogenesis of phytosterolemia in uncontrolled diabetes; and (ii) the potential risk of plant sterol intake in patients with uncontrolled diabetes and/or heterozygous ABCG5/8 mutations.

Mechanistically, dynamic changes in serum plant sterol levels might reflect both insulin deficiency (or hyperglycemia) and the heterozygous ABCG5 mutation. Initial marked phytosterolemia and its substantial reversal by glycemic control without ezetimibe indicate a major causal role of uncontrolled diabetes in phytosterolemia. This notion is supported by previous studies showing elevated serum plant sterol levels in patients with type 1 diabetes. The present data provide the first direct evidence of increased plant sterol levels in uncontrolled diabetes and a dramatic decrease in response to glycemic control. Although the precise mechanisms of plant sterol hyperabsorption in diabetes remain uncertain, a study using a streptozotocin-induced rat diabetes murine model suggested that reduced ABCG5/8 mRNA expression in both liver and intestine leads to increased cholesterol absorption. Another study also showed that ABCG5/8 mRNA expression levels in duodenal biopsies were lower in patients with type 2 diabetes compared with healthy individuals. Taken together with the association between hyperinsulinemia and decreased cholesterol absorption, insulin deficiency might contribute to increased plant sterol absorption through reduced ABCG5/8 expression.

On the other hand, normalization of serum plant sterols was achieved only by adding ezetimibe, suggesting that the heterozygous ABCG5 mutation predisposed the patient to elevated basal plant sterol levels. The extent of plant sterol absorption in heterozygous ABCG5/8 mutation carriers remains controversial. Recent studies showed that heterozygous carriers have significantly higher serum plant sterol and cholesterol.
levels compared to healthy individuals. The present case also suggests that ABCG5/8 heterozygous carriers are potential ‘hyperabsorbers’ for plant sterols.

Altogether, it seems plausible that the initial marked phytosterolemia in the current case (serum sitosterol and campesterol, 32.5 and 66.0 μg/mL, respectively) resulted from the coincidence of two factors that potentially deteriorate ABCG5 function and increase plant sterol accumulation: the development of type 1 diabetes and the loss of function heterozygous mutation in ABCG5. Insulin treatment without ezetimibe largely decreased the levels of serum plant sterols (serum sitosterol and campesterol, 9.5 and 16.9 μg/mL, respectively), which conceivably reflected the restoration of ABCG5/8 expression. In addition, the normalization of serum plant sterol levels was achieved only with ezetimibe under optimal glycemic control, as the heterozygous ABCG5 mutation contributed to slightly increased absorption of plant sterols.6

Due to their cholesterol-lowering effect, plant sterol intake has been widely recommended to reduce cardiovascular risk among patients both with and without diabetes. Meanwhile, if absorbed from the intestine, plant sterols can promote atherosclerosis, which is suggested by the studies of the patients with sitosterolemia and the general population. Based on these observations and the present findings, the current recommendation of plant sterol intake should be carefully applied to patients with uncontrolled diabetes. It is noteworthy that the estimated incidence of deleterious ABCG5/8 mutations is one in 220 individuals, indicating that a substantial number of patients with diabetes would be potential plant sterol ‘hyperabsorbers’. As most heterozygous carriers present with hypercholesterolemia, clinicians should be cautious in dietary guidance and drug choice for patients with diabetes and hypercholesterolemia. Plant sterol measurement can be helpful in order not to overlook ‘hyperabsorbers’.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: All clinical and genetic analyses were approved by the local ethics committee of Kitano Hospital (No. 180400601, approved on 17 April 2018). Informed consent: Written informed consent was obtained from the patient and family members for the genetic testing and for the publication of this article.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1** | Serum lipoprotein fractions measured by electrophoresis on polyacrylamide gel on hospital day 4.