Plasma Plant Sterol Levels Do Not Reflect Cholesterol Absorption in Children with Smith-Lemli-Opitz Syndrome

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Objective To test the hypothesis that there is a correlation between the ratio of plant sterols to cholesterol in plasma and dietary cholesterol absorption in children with Smith-Lemli-Opitz syndrome (SLOS), a cholesterol synthesis disorder.

Study design We obtained measurements of cholesterol absorption with a direct radioisotope cholesterol absorption method during 9 visits of children with SLOS. We measured plasma sterols in 22 children with SLOS and 16 control children, and we measured dietary intake of cholesterol and sitosterol (n = 11 SLOS).

Results The correlations of 2 plasma plant sterol ratios (sitosterol/cholesterol and campesterol/cholesterol) with direct cholesterol absorption measurement were poor (R= −0.33 and R= −0.25, respectively), significantly lower than the published correlation in adults (R = 0.73; $P < .02$).

Conclusions Although the ratios of plant sterols to cholesterol in plasma has been used as a surrogate for cholesterol absorption in adults and children, these ratios may not accurately reflect cholesterol absorption in children with SLOS. These ratios should not be used as a surrogate for cholesterol absorption in children without further validation. *(J Pediatr 2009;154:557-61)*

The Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive condition with multiple malformations and mental retardation (OMIM# 270400) caused by mutations in the gene (*DHCR7*) encoding the final enzyme in the cholesterol biosynthetic pathway, 7-dehydrocholesterol Δ^7-reductase (DHCR7; E.C. 1.3.1.21).[1]

Monitoring the absorption of dietary cholesterol is important in SLOS, because dietary cholesterol is widely used as a potential treatment. The absorption of dietary cholesterol in children with SLOS has been demonstrated indirectly by the increase in plasma cholesterol level after cholesterol consumption.[2-6] In addition, we recently directly measured cholesterol absorption in children with SLOS.[7] A stable isotope method using ^{13}C-cholesterol administered orally and D_7-cholesterol administered intravenously has been reported in children to measure cholesterol absorption.[8] The stable isotopes, however, are expensive, and the instrumentation needed for isotope ratio mass spectrometric analysis is not widely available. Furthermore, because the measurements can only be done invasively with an intravenous catheter and multiple blood samples, this technique is limited to older, larger children. A simpler and lower risk method available for children of all ages is needed in the study of SLOS and other conditions affecting cholesterol metabolism.

A correlation between the ratios of plant sterols to cholesterol in plasma (μmol plant sterols/mmol cholesterol) and direct measure of cholesterol absorption has been described for adults.[9-11] There are no published data showing a similar correlation in children. If the correlation between the ratios of plant sterols to cholesterol in plasma and cholesterol absorption were validated in children with SLOS, then this method could allow for simple, relatively non-invasive monitoring of dietary cholesterol absorption.

GC	Gas chromatography	OHSU	Oregon Health & Science University
GCMS	Gas chromatography mass spectrometry	SLOS	Smith-Lemli-Opitz syndrome

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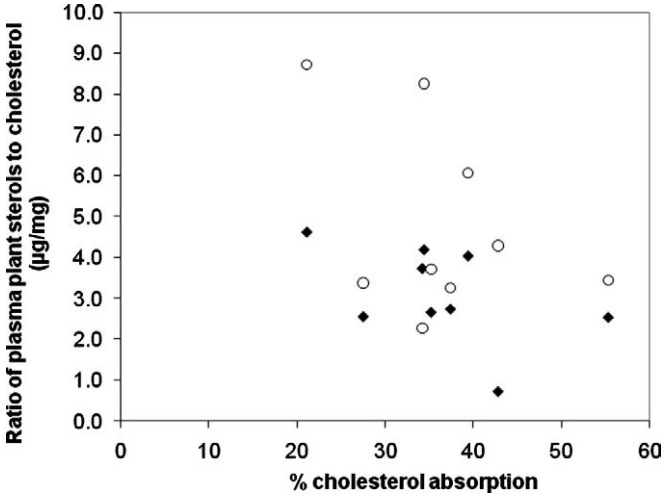

Figure 1. The correlation coefficients of ratios of sitosterol to cholesterol in plasma (*filled diamonds*; R = −0.33) and the ratios of campesterol to cholesterol in plasma (*open circles*; R = −0.25; µg plant sterol/mg cholesterol) with the fractional cholesterol absorption (percentage of the amount given) were poor. These were significantly lower than similar correlations in adults (R = 0.73).[9]

METHODS

Twenty-two children with SLOS were enrolled in this study, 2 of whom were siblings. Nineteen children were admitted to the Oregon Health & Science University (OHSU) General Clinical Research Center for 1-week visits as part of our ongoing research. In 3 of the 22 children, we measured the concentration of plasma sterols in blood samples that had been shipped to us between visits. Before visits of 5 of the children, the parents were instructed to feed their child an essentially cholesterol-free diet at home for 2 to 3 weeks before the admission; the dietary cholesterol intake ranged from 1.9 to 4.3 mg/kg/day. Thirteen children received a high cholesterol diet, usually with added egg yolk; the cholesterol content ranged from 14.2 to 50.0 mg/kg/day. Four children received a high cholesterol diet with simvastatin. Because of the common finding of feeding difficulties in SLOS, 8 of the children were fed via gastrostomy-tube. Sixteen control children were enrolled for blood samples; 4 were siblings of the SLOS children, and 12 were patients who required a blood draw for other reasons. The OHSU institutional review board approved these studies, and written informed consent was obtained. Plasma samples were saponified and extracted into hexane by means of the same procedures described previously.[5,12] Concentrations of the trimethylsilyl ether derivatives of individual plasma sterols were measured with capillary-column gas chromatography (GC) with a CP-Wax 57 column (25 M, 0.32 mm ID; Chrompack-Varian, Walnut Creek, California). Internal standard (5α-cholestane) and authentic standard of cholesterol were used for calibration. The most abundant plant sterols in plasma are campesterol and sitosterol. Because 7-dehydrocholesterol and campesterol co-elute on GC, campesterol was measured with gas chromatography-mass spectrometry (GCMS), as described earlier.[13] Because plasma sitosterol and campesterol are transported with cholesterol in lipoproteins, the absolute concentrations of sitosterol and campesterol (µg/dL) were adjusted for concentration of cholesterol (mg/dL; analyzed simultaneously from the same sample) and are expressed as a ratio: plasma plant sterol/cholesterol (µg plant sterol/mg cholesterol).

During each 1-week visit, all food given to the children was weighed before and after consumption, and exact amounts of food consumed were calculated by the General Clinical Research Center bionutrition staff. The study dietitian calculated the cholesterol content of the food consumed during each visit with the program Food Processor (ESHA Research, Salem, Oregon). Additionally, a composite, made up of 10% of the total gram weight of food consumed, was blended, and an aliquot was analyzed for cholesterol content with GC. Cholesterol measured in food aliquots was highly correlated with the cholesterol calculated from weighed samples (mg cholesterol/g food; R = 0.989). Because the database of plant sterol composition of foods is less comprehensive than that of cholesterol, the 3 predominant dietary plant sterols (sitosterol, campesterol, and stigmasterol) were not calculated by the study dietitian and were only measured in the food aliquot with GC. Sitosterol represented 70% ± 5% of the total plant sterols, and there was a close correlation of sitosterol with total plant sterols (R = 0.99), indicating that sitosterol, the most abundant plant sterol in the diet, is proportional to the total plant sterols consumed.

Direct measurement of dietary cholesterol absorption was obtained with fecal dual isotope ratio method during 9 visits by children with SLOS (1 child was examined twice during separate admissions). The concentrations of plasma plant sterols and cholesterol were available for each visit. This method was described previously.[7] The dosing plan was reviewed and approved by the OHSU Radiation Safety Committee. In brief, a radioactive test meal was provided with an age-based dose of the radioisotopes 4-^{14}C-cholesterol and 5,6-^{3}H sitostanol, and carrier cholesterol (0.4 mg) in canola oil mixed with cholesterol from egg yolk (14.5 ± 1.0 mg/kg). This cholesterol mix was added to appropriate food or formula and administered to the child. The same dietary protocols were used for children who were gastrostomy-tube fed or orally fed. Radioactivity of blood samples obtained at 24 and 48 hours after the meal verified the absorption of the ^{14}C-cholesterol. All stools collected during the 4 to 6 days after the meal were pooled, homogenized with equal amounts of water, and frozen until analyzed. The radioactivity of an aliquot of the stool (0.5-1.0 g) was determined according to the method described previously.[7,12] Although 13 cholesterol absorption tests were initially completed, 4 were excluded because of low recovery in the stools of the radioactive sitostanol, which is unabsorbed by the digestive tract. This suggests incomplete collection of the stools during the visit.

Data are expressed as means plus or minus SD. Correlations of the ratios of plant sterols to cholesterol with directly measured cholesterol absorption in children were determined