Thyroid Hormone Status in Sitosterolemia Is Modified by Ezetimibe

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Objectives To assess the association between biomarkers of thyroid status and 5α-stanols in patients with sitosterolemia treated with ezetimibe (EZE).

Study design Eight patients with sitosterolemia (16-56 years of age) were studied during 14 weeks off EZE therapy and 14 weeks on EZE (10 mg/day). Serum thyroid biomarkers (free triiodothyronine [FT3], free thyroxine [FT4], FT3/FT4 ratio, thyroid-stimulating hormone), 5α-stanols (sitostanol and cholestanol), and cholestanol precursors (total cholesterol and its synthesis marker lathosterol, and 7α-hydroxy-4-cholesten-3-one cholestenol) were measured at baseline and during the 14 weeks off EZE and on EZE.

Results EZE increased FT3/FT4 (10% ± 4%; $P = .02$). EZE reduced plasma and red blood cells sitostanol (–38% ± 6% and –20% ± 4%; all $P < .05$) and cholestanol (–18% ± 6% and –13% ± 3%; all $P < .05$). The change in plasma cholestanol level on EZE inversely correlated with the change in FT3/FT4 ($r = –0.86$; $P = .01$). EZE lowered total cholesterol ($P < .0001$) and did not affect 7α-hydroxy-4-cholesten-3-one cholestanol. EZE increased ($P < .0001$) lathosterol initially, but the level was not sustained, resulting in similar levels at week 14 off EZE and on EZE.

Conclusion In patients with STSL, 5α-stanols levels might be associated with thyroid function. EZE reduces circulating 5α-stanols while increasing FT3/FT4, implying increased conversion of T4 to T3, thus possibly improving thyroid hormone status. *(J Pediatr 2017;188:198-204).*

Trial registration ClinicalTrials.gov NCT01584206.

Sitosterolemia (STSL) is a rare disease caused by mutations in either of the adenosine triphosphate-binding cassette transporter genes, *ABCG5* or *ABCG8*, that result in accumulation of plant sterols and their corresponding saturated 5α-stanols in the body.[1,2] Clinical features of STSL include xanthomas, premature atherosclerosis, and macrothrombocytopenia.[1] Endocrine disruption has also been reported.[3] Synthesis of thyroid hormones seem to be deranged in cerebrotendinous xanthomatosis (CTX), a disorder of bile acid synthesis,[4-6] and STSL.[7] Both disorders have elevated plasma and tissue cholestanol levels that might contribute to thyroid imbalance. Specifically, concurrent high levels of cholestanol, a 5α-stanol–saturated derivative of cholesterol,[8] and hypothyroidism have been observed in CTX[4-6] and STSL,[7] suggesting a link between underactive thyroid and 5α-stanols. However, the association between thyroid function and 5α-stanols has not, to our knowledge, been elucidated further.

For most individuals on a typical Western diet, 5α-stanols (cholestanol and sitostanol) are almost absent from the diet; thus, their presence in the body is mostly via endogenous production from cholesterol and sitosterol, respectively.[9-11] Cholestanol is biosynthesized from cholesterol[12,13] or its metabolite 7α-hydroxy-4-cholesten-3-one (7α-H-C4), which is also involved in bile acid synthesis.[14,15] Plasma cholestanol levels are normally low, but high in STSL[16] and CTX.[17] Biosynthesis of cholestanol precursors, including 7α-H-C4 and cholesterol (reflected by its synthesis marker lathosterol), are low in STSL,[18-20] so it is unclear if cholestanol accumulation arises from the diet or endogenously. Ezetimibe (EZE), the primary treatment for STSL that works by reducing plant sterol absorption, reduces intestinal sterol uptake,[21,22] but its effect on circulating 5α-stanols has not yet been examined. This study aimed to explore the nature of the relationship

7α-H-C4	7α-Hydroxy-4-cholesten-3-one
C4-d7	7-Hydroxy-4-cholesten-3-one-25,26,26,26,27,27,27-d7
CTX	Cerebrotendinous xanthomatosis
CV	Coefficient of variation
ddH_2O	Double distilled water
EZE	Ezetimibe
FT3	Free triiodothyronine
FT3/FT4	Ratio of FT3 to FT4
FT4	Free thyroxine
RBC	Red blood cells
STSL	Sitosterolemia
TC	Total cholesterol
TSH	Thyroid-stimulating hormone

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between 5α-stanols and thyroid hormones and to determine the effects of EZE on thyroid hormones as well as blood levels of sitostanol, and cholestanol and its precursors (total cholesterol [TC] and its synthesis marker lathosterol, and 7α-H-C4) in patients with STSL.

Methods

The study design from which these data were taken has been reported previously.[23] This report was part of a much larger pilot, interventional trial (ClinicalTrials.gov: NCT01584206) investigating the effect of EZE on sterol metabolism in patients with STSL. In summary, 8 patients (5 males and 3 females, between 16 and 56 years of age) with homozygous *ABCG8* S107X mutation (NM_022437.2:c.320C>G) were recruited from Hutterite communities. The study was approved by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development and the University of Manitoba Biomedical Ethics board, and written informed consent was obtained from all patients. After enrollment, patients were taken off their EZE treatment for 14 weeks (off EZE). For the full study design, blood was collected at weeks 2, 4, 6, 8, 10, 12, and 14. After 14 weeks off EZE, patients were instructed to take EZE (10 mg/day) for 14 weeks (on EZE). Blood was collected following the same schedule as in the off EZE phase. Only selected samples from the blood collection protocol were used for assessment of thyroid hormones (see below) owing to the limited availability of serum samples required. Serum, plasma, and red blood cells (RBC) fractions were separated by centrifugation at 3000 rpm for 20 minutes at 4°C, and stored at −80°C until analysis.

The concentrations of serum thyroid hormones were as follows: free triiodothyronine (FT3), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) were measured at baseline (beginning of the off EZE phase), and 8 and 14 weeks off EZE and on EZE by an outsourced laboratory (Gamma-Dynacare Medical Laboratories, Winnipeg, Manitoba, Canada) using automated immunoassays (Abbott Laboratories, Abbott Park, Illinois). The coefficient of variation (CV) for the assays were as follows: FT4, intra-CV <2.2% and inter-CV <4.9%; FT3, intra-CV <3.2% and inter-CV <5.9%; and TSH, intra-CV <1.2% and inter-CV <2.8%. The ratio of FT3 to FT4 (FT3/FT4) was calculated as an indirect index of deiodinase activity.[24]

Plasma and RBC 5α-stanols, and TC and lathosterol levels were measured using gas–liquid chromatography equipped with a flame ionization detector (Varian 430-GC; Agilent Technologies, Santa Clara, California) as published previously.[23] Measurement of RBC sterol levels indicates a longer term average of plasma levels and a better reflection of tissue stores. Plasma sterol and 5α-stanol levels were determined at baseline and biweekly up to 14 weeks off EZE and on EZE, whereas those in RBC were measured at baseline, 4, 8, 10, and 14 weeks off EZE and on EZE.

Serum 7α-H-C4 levels were measured at baseline, 4, 8, 10, and 14 weeks off EZE and on EZE using ultraperformance liquid chromatography tandem mass spectrometry.[25] Serum (50 μL) was diluted with 100 μL of double distilled water (ddH_2O), and 50 μL of the 40 ng/mL deuterated internal standard 7-hydroxy-4-cholesten-3-one-25,26,26,26,27,27,27-d7 (C4-d7) (Avanti Polar Lipids Inc, Alabaster, Alabama) and 0.2 mL of methanol were added. The mixture was applied to a Bond Elut C18 cartridge (Agilent Technologies Inc, Mississauga, Ontario, Canada) preconditioned with 2 mL methanol followed by 2 mL ddH_2O. The cartridge was washed twice with 2 mL ddH_2O and 2 mL of methanol. After evaporation, the residue was dissolved in 80 μL methanol and 3 μL was injected into the system. The separation was performed using a Kinetix XB-C18 column (2.1 × 100 mm, particle size 1.7 μm; Phenomenex, Torrance, California) at 35°C. The mobile phases were A (0.1% formic acid in ddH_2O) and B (0.1% formic acid in acetonitrile) and used at a flow rate of 0.20 mL/minute. The gradient program was started at 10% phase A and 90% phase B for 6 minutes, increased linearly to 100% phase B for 4 minutes, held at 100% phase B for 4 minutes, then returned to initial conditions and re-equilibrated for 4 minutes. The total run time for each sample analysis was 16 minutes. The quantitative data were acquired using multireaction monitoring mode. The multireaction monitoring transitions for 7α-H-C4 were 401.4 > 383.4 m/z and for C4-d7 were 408.4 > 390.4 m/z. The following settings were applied during each run: capillary voltage 3.50 kV; source temperature 100°C; desolvation temperature 400°C; nitrogen gas with flow rates of desolvation and cone gas of 400 and 50 L/hour, respectively; argon was used as the collision gas; cone voltage was 20V; and collision energy was 20 eV.

Statistical Analyses

Statistical analyses were performed using SPSS 21.0 (SPSS, Inc, Chicago, Illinois). All data are presented as mean ± SEM. Statistical significance was set at $P < .05$. Linear mixed-model analysis was used where treatment and time were specified as fixed factors, and age and body weight were specified as a covariate in the model. Significant treatment effects were examined with Bonferroni adjustment for multiple comparisons. Both treatment and time (with time representing the different time periods) were entered into the model. When a significant treatment effect, but no significant treatment-by-time interaction, was observed, the interpretation was that the treatment effect was consistent over the different time periods. Relationships between 2 variables were assessed with stepwise multiple linear regression analysis. Percentage change from baseline for each phase was analyzed using 2-tailed paired Student *t* test. Data that were not normally distributed, as determined by a Shapiro-Wilk test, were log or inverse transformed before statistical analysis.

Results

Baseline characteristics of the study patients are presented in **Table I**. All patients were euthyroid based on serum TSH. Mean FT3 concentration was at the lower limit of the normal range (0.3-0.7 ng/dL) with 1 patient at 0.4 ng/dL, 5 patients at 0.3 ng/dL, and 1 having a subnormal level of 0.2 ng/dL. Mean serum FT4 concentration was within the normal range (0.7-1.8 ng/