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A Pilot Study of the Association of Markers of Cholesterol Synthesis with Disturbed Sleep in Smith Lemli-Opitz Syndrome

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Abstract

OBJECTIVE—Smith-Lemli-Opitz syndrome (SLOS) is a rare genetic disorder characterized by cholesterol synthesis impairment. A host of physical, developmental, and behavioral presentations are associated with SLOS, many of which have been related with disorder severity. Sleep disturbance is commonly reported in SLOS. The current study is the first to examine the association between sleep disturbance and biomarkers of cholesterol synthesis defect.

METHODS—Twenty youth with SLOS participated. Biomarkers of cholesterol synthesis were obtained, including plasma sterols (i.e., 7-dehydrocholesterol, 8-dehydrocholesterol, cholesterol), mevalonic acid, and 24-S hydroxycholsterol. A ratio of plasma cholesterol precursors to cholesterol levels was used as a measure of biochemical severity. Parents reported on their children's sleep problems using the Children's Sleep Habits Questionnaire.

RESULTS—Most markers of cholesterol synthesis disruption were associated with overall sleep disturbance. Biochemical severity of SLOS was also associated with specific sleep problems (e.g., decreased sleep duration and increased sleep onset delay) and was identified as a significant predictor of these factors.

CONCLUSION—The current study is the first to demonstrate associative relationships between cholesterol levels and sleep disturbance in youth with SLOS. These results add to the current understanding of how cholesterol levels may contribute to the behavioral phenotype of SLOS. These findings may inform future studies related to the role cholesterol synthesis defects play in the behavioral phenotype of SLOS and, subsequently, modalities of intervention for behavioral symptoms.

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Keywords

Sleep; Smith Lemli-Optiz Syndrome; phenotype

Smith-Lemli-Opitz syndrome (SLOS) is a rare autosomal recessive disorder characterized by a disruption in the biosynthesis of cholesterol ([1]), occurring in an estimated 1 in 10,000 to 1 in 80,000 live births ([2]). Individuals with SLOS have biallelic mutations in the 7-dehydrocholesterol reductase gene. This results in low plasma cholesterol concentrations and elevated concentrations of 7-dehydrocholesterol (7-DHC) and 8-dehydrocholesterol (8-DHC), the immediate precursor of cholesterol and its isomer, respectively. Clinical manifestations of SLOS are varied and often include intellectual disability, language impairments, hyperactivity, inattention, aggression, self-injury, and symptoms of autism spectrum disorder ([3–5]). Severity of the cholesterol biosynthesis defect, which occurs along a continuum, is associated with physical, developmental, and behavioral features of the syndrome ([6–8]).

Sleep problems appear common for youth with SLOS, with early studies indicating that a majority of these youth may experience general sleep disturbance ([9, 10]). Most recently, Zarowski and colleagues ([11]) found a high rate of sleep problems in 18 individuals with SLOS (all but two children) using a standardized parent-report measure. Results indicated that a majority of the sample experienced sleep-disordered breathing, sleep onset difficulty, frequent waking, early waking, and difficulty falling back to sleep after waking. Overall, converging evidence demonstrates sleep problems as a prevalent clinical issue for youth affected by SLOS. Yet, little is known about sleep disturbance and its relation to the changes of metabolites in the cholesterol pathway as a result of the deficiency of the 7-dehydrocholesterol reductase in SLOS. One case study demonstrated that cholesterol supplementation may improve behavioral functioning in youth with SLOS, including better sleep health ([9]), suggesting that low cholesterol may contribute to sleep disturbance.

Given the positive association between sleep quality and daytime behavioral functioning in children both with and without developmental disabilities ([12, 13]), and reported sleep disturbance in SLOS, continued examination of sleep problems in youth with SLOS is warranted. The current study aims to provide new insights into sleep disturbance and SLOS through novel examination of the association between changes in metabolites and sleep behavior. It is the first to examine metabolic biomarkers as correlates or predictors of sleep disturbance in SLOS. While numerous studies have described various aspects of the behavioral phenotype of SLOS, studies that relate metabolic biomarkers (e.g., cholesterol, 7-DHC, 8-DHC) to behavior are few. Increased understanding of the association between sleep and the metabolic phenotype of SLOS may, ultimately, help elucidate the complex behavioral phenotype of SLOS and the potential causal pathways therein.

This exploratory study aims to provide an initial examination of the association between cholesterol and sleep problems in youth with SLOS. The diminished conversion of 7-DHC to cholesterol, the final step in the cholesterol biosynthesis pathway, in SLOS potentially influences metabolites proximal and distal to this final step. As a result, a large number of biochemical markers may be affected in SLOS and thus are available for examination in

phenotyping research. Three will be examined here. First, the (7-DHC+8-DHC)/Cholesterol ratio is accepted as a measure of biochemical severity in SLOS ([14]) and has been used in previous research examining the association between developmental and behavioral outcomes ([6, 7]). Further, mevalonic acid (MVA) is an early biomarker of the biosynthetic cholesterol pathway that is associated with a host of physiological processes ([15]). The implications of MVA levels are pleiotropic and inclusive of human circadian rhythm ([16]), which may render this biomarker as uniquely appropriate in the investigation of sleep in SLOS. Lastly, brain-based cholesterol is distinct from cholesterol across the blood-brain barrier and may be especially relevant in the study of developmental or behavioral presentation ([17]). Here, 24(S)-hydroxycholesterol (24S), the predominant metabolite of brain-based cholesterol synthesis and a minimally invasive proxy of brain-based cholesterol turnover ([17]), will also be examined.

In addition to serving as the first investigation of the association between biochemical severity and sleep, the current study improves upon existing research by utilizing a more comprehensive measure of sleep disturbance. Thus far, studies of sleep in SLOS have relied on screeners or measures with a dichotomous “yes/no” response system ([5, 9, 11]). Use of a well-validated and comprehensive measure of various sleep problems and overall sleep disturbance may provide a more thorough account of sleep in SLOS.

Given previous research regarding the association between the severity of the biosynthesis defect and developmental and behavioral outcomes ([6–8]), and the case study suggesting improved sleep with cholesterol supplementation ([9]), we hypothesized that greater biochemical severity would be associated with and predict worse sleep disturbance.

METHODS

Participants

Twenty youth (9 males, 11 females) with confirmed diagnosis of SLOS ranging in age from 1 to 18 years of age (M = 8.6 years) participated (Table 1). All were enrolled in a longitudinal study designed to determine the effect of cholesterol supplementation on developmental outcomes, including sleep ([18–20]). Using a rating of the anatomy of 10 organ systems (0 = normal, 2 = most severity affected; normalized total scores range from 0 to 100 with higher scores indicative of greater severity; 21, 22), the study population was mildly to moderately affected by SLOS (clinical severity score ranged from 5 to 40; mean severity score was 19.7). Mean plasma concentration 7-DHC was 8.3 mg/dl (SD = 1.3) and mean concentration of cholesterol was 89.88 mg/dl (SD = 8.11). Taking into account that no (or trace amounts of) 7-DHC is typically detected in the blood of healthy children and plasma cholesterol concentrations in 8–12 year old children is approximately 175–180 mg/dl ([23]), these sterol data confirm the biochemical hallmark of SLOS and 7-dehydrocholesterol reductase deficiency in our study population.

Measures

Children’s Sleep Habits Questionnaire (CSHQ; 24)—The CSHQ is a 45-item parent-report form used to assess children’s sleep behavior occurring during the most recent

typical week. Originally established for use with youth ages 4–10 years, subsequent examination suggests its utility in characterizing sleep patterns in youth with disabilities ranging from infancy through adolescence ([12], [25–28]). Items are rated on a 3-point scale ("usually" = if the sleep behavior occurs 5 to 7 times per week, "sometimes" for 2 to 4 times per week, and "rarely" for 0 to 1 time per week). Individual item scoring is adjusted so that a higher score is indicative of more disturbed sleep. Item scores are tallied to a Total Sleep Disturbance score and nine subscale scores (i.e., Bedtime Resistance, Sleep Onset Delay, Sleep Duration, Sleep Anxiety, Night Wakings, Parasomnias, Sleep Disordered Breathing, and Daytime Sleepiness). Raw score, mean, and t-score can be obtained for the total scale and subscales. Mean scores were used in the current analysis, as some participants fell outside of the age of the normative group to establish t-scores. For three cases, items on the CSHQ were incomplete. In these instances, participant's subscale mean was calculated and imputed as the raw score for that missing item; that imputed score was then used when calculating final Total and subscale mean scores. If more than one item was missing on an individual subscale on the CSHQ, that participant's subscale score was neither calculated nor included in analysis. If more than one item was missing on the entire CSHQ, a Total Sleep Disturbance mean score was not calculated.

Biochemical severity—The primary biochemical indicator of disease severity was the (7-DHC+8-DHC)/Cholesterol ratio ([14]). However, because this represents the first investigation of the association between biochemical variables and sleep in SLOS, we also independently examined associations between 7-DHC, 8-DHC, and total plasma cholesterol. Plasma sterol concentrations were measured by capillary-column gas chromatography on a Perkin Elmer gas chromatograph (model AutoSystemXL) with a CP-Wax57 column (25M, 0.32 mm ID; 0.25 μm film; Chrompack Co., Rariton, NJ) or Agilent gas chromatograph (model 6890N) with a ZB1701 column (30M; 0.25 mm ID; 0.25 μm film; Phenomenex). Internal standards (5α-cholestane or epicoprostanol) and authentic cholesterol standards were used for calibration ([18]).

Mevalonic acid concentration was determined in 24-hr urine samples using a radioenzymatic isotope dilution method reported previously and expressed in μmol/day ([29]). Plasma concentrations of 24(*S*)-hydroxycholesterol (24S) were determined by liquid chromatograph-mass spectrometry (LC-MS) as previously described ([30]). The within- and between-run precision (coefficients of variation) for 24S measurements was less than 8.5% across the range measured with a lower limit of detection of 38 ng/ml.

Procedure

The study was approved by the Institutional Review Board at Oregon Health & Science University, and parents of all participants provided informed consent. As part of a longitudinal study of SLOS, each participate completes repeated, annual inpatient stays that last up to one week and during which a variety of medical, developmental, and behavioral evaluations are completed. All participants with paired sleep (CSHQ) and biochemical (7-DHC, 8-DHC, and cholesterol) data were included in the present data analysis. If multiple sets of paired data were available, those from the most recent visit were utilized. Data on

other biochemical markers (mevalonic acid via urine [U-MVA] and 24S) were included in the analysis as well when available.

Statistical Analysis

The participants' individual data are presented (see Table 1) and Kendall rank correlation coefficients were computed to examine associations between sleep disturbance, age, and biochemical indicators. The strength of statistically significant correlations was interpreted using Cohen's ([31]) guidelines. Hierarchical linear regression models were used to examine bivariate associations of scores on the CSHQ and biochemical markers. Significance was set at $p < 0.05$.

RESULTS

Association between Sleep Disturbance and Biomarkers

Summary scores for CSHQ Total Sleep Disturbance and subscale scores are presented in Table 2. In the current sample, 70.6% of all children, and 100% of children within the age range with which the measure was originally normed, with completed CSHQs obtained Total Sleep Disturbance scores that fell above the clinical cut-off of 41 ([24]), demonstrating significant sleep concerns. The association between CSHQ Total Sleep Disturbance and subscale scores, as well as the variable of age and various biochemical markers, was examined (Table 3). There were significant correlations between biochemical markers and various parent-reported sleep problems. Specifically, the Sterol Ratio was significantly and positively correlated with CSHQ Sleep Onset Delay, Sleep Duration, and Total Sleep Disturbance. Levels 7-DHC and 8-DHC were positively correlated with Total Sleep Disturbance and negatively correlated with Sleep Anxiety, while level 8-DHC was positively correlated with Sleep Duration. In contrast, cholesterol level was negatively correlated with Sleep Onset Delay and Total Sleep Disturbance. Age was significantly negatively correlated with Bedtime Resistance, Parasomnias, and Total Sleep Disturbance. Neither U-MVA nor 24S correlated with any CSHQ score.

Hierarchical Multiple Regressions

A series of hierarchical multiple regressions were completed to assess the value of the Sterol Ratio and age as predictors of sleep problems (Table 4). The Sterol Ratio was chosen because it reflects the combined variation of all three sterol markers (7-DHC, 8-DHC, and cholesterol), while age was examined due to the evidenced significant correlation with Total Sleep Disturbance. Kendall rank correlation coefficients were identified. Three CSHQ scales (Sleep Onset Delay, Sleep Duration, and Total Sleep Disturbance) were significantly associated with Sterol Ratio; thus, three models were evaluated with these variables as criterion. In all models, age was entered at Step 1, while the Sterol Ratio was entered at Step 2 (see Table 4).

In assessing the ability of the Sterol Ratio to predict Sleep Onset Delay, age alone was found to explain a small, non-significant amount of the variance in Sleep Onset Delay (3.9%). Entry of the Sterol Ratio in Step 2 was found to explain an additional 41.9% of the variance in Sleep Onset Delay, and the total variance explained by the model was 45.8% and was

statistically significant. The Sterol Ratio was a significant predictor of Sleep Onset Delay. When examining the ability of the Sterol Ratio to predict Sleep Duration problems, age was again found to explain a small, non-significant amount of variance (4.0%). While the entry of the Sterol Ratio into the model was found to explain an additional, statistically significant 21.8% of the variance in Sleep Duration, the model as a whole did not reach statistical significance. Lastly, the ability of the Sterol Ratio to predict Total Sleep Problems was evaluated. When age alone was entered into the model, it was found to explain a non-significant 17.4% of the variance of Total Sleep Problems. The Sterol Ratio contributed an additional 25.3% of the variance, and the model as a whole explained 42.8% of the variance and was statistically significant. The Sterol Ratio was the only statistically significant predictor for Total Sleep Problems.

DISUSSION

Current findings represent the first test of an association between biochemical phenotype and sleep problems in SLOS and most are consistent with our hypothesis that sleep abnormalities are correlated with biochemical indices of disease severity. Importantly, the biochemical markers of impaired cholesterol synthesis were either positively (i.e., Sterol Ratio, 7-DHC, 8-DHC) or negatively (i.e., cholesterol) associated with parental ratings of general sleep disturbance in SLOS in expected directions. Further, the Sterol Ratio was positively associated with two of the eight specific CSHQ subscales (Sleep Onset Delay and Sleep Duration). This suggests that cholesterol deficiency and/or cholesterol precursor accumulation specifically may impact certain sleep behaviors. Last, we demonstrated that the Sterol Ratio predicted parent ratings of sleep problems above and beyond the variance accounted for by age. The results provide a novel understanding of sleep abnormalities in SLOS, and thus contribute to a better understanding of the underpinnings of the behavioral phenotype of SLOS.

Interestingly, Sleep Anxiety was negatively correlated with 7-DHC and 8-DHC, which is incongruous with both our hypotheses and other findings. Reasons for this are unclear and warrant further study.

The mechanism(s) by which sleep and cholesterol are associated in SLOS were not addressed in the current study. Sleep disturbance and pervasive developmental differences have been posited as having bidirectional effects on one another ([32]). Whether disturbed sleep seen in SLOS ([9–11]) contributes negatively to variations in plasma cholesterol above and beyond the direct result of the genetic defect in biosynthesis, or whether the error in cholesterol metabolism causes sleep problems remains unclear. That sleep and plasma cholesterol levels are linked to one another has been consistently shown. However, research primarily focuses on the impact of sleep on cholesterol levels ([33–35]). Cholesterol synthesis exhibits a diurnal rhythm, in that both precursors to cholesterol synthesis ([16]) and cholesterol synthesis itself increase at nighttime and decrease during the daytime ([36]). Further, transcripts that code for cholesterol synthesis and transport exhibit a higher expression during sleep ([37]). As cholesterol plays a vital role in myelination of the central nervous system ([38]), it may be that individuals with SLOS have reduced capability to synthesize and transport cholesterol, resulting in reduced ability to produce and repair the

myelin in the central nervous system. This may prevent those with SLOS from fully recuperating from restful sleep, resulting in more sleep disturbance as time passes. Another possibility is that impaired sterol synthesis may negatively impact ligand binding and signaling in receptors that play a role in sleep. For example, oxytocin has been shown to influence sleep onset and wakefulness in animal models ([39]), and oxytocin receptors appear to depend on cholesterol for both efficient expression and stabilization ([40]). Therefore, disruption of cholesterol synthesis may limit the effectiveness of oxytocin receptors, resulting in disrupted oxytocin transmission and subsequent disrupted sleep. As cholesterol plays a vital role in a variety of biological processes, and has links to sleep, it is plausible that deficits in cholesterol synthesis impact a system that has an influence on sleep. As of yet, though, the specifics of those pathways have not been discovered.

Study participants had all been treated with cholesterol supplementation (target intake: 30 mg of cholesterol per kg body weight and per day; 18–20) for several months at the time sleep behavior measurements were performed. Since cholesterol supplementation impacts serum cholesterol levels in SLOS patients ([19]), the treatment may have influenced the sterol-sleep relationships reported in Table 3. It is, however, reasonable to assume that at the time the sleep behavior assessments were made, serum cholesterol levels had reached a steady-state level that reflected homeostatic adaptation to cholesterol supplementation. Thus it is possible that the sleep-cholesterol relationship we observed was influenced not only by the original defect in cholesterol synthesis but also by the patients' homeostatic response to cholesterol supplementation.

Limitations

Current findings must be considered within the context of study limitations. The sample size is small, given the rarity of SLOS. While the total number of participants is comparable to other studies of behavioral phenotypes and SLOS ([6], [11]), it is important to interpret results with caution given minimized statistical power. Due to this limitation, we were unable to examine potential mediating or moderating variables of the cholesterol-sleep associations found here. Further, whether the sleep disturbance is better accounted for by other aspects of the SLOS behavioral phenotype (e.g., irritability) ([3–5]), developmental differences (e.g., intelligence quotient) ([6–7]), or other biomarkers affected by the cholesterol synthesis defect cannot be ruled out based on our study.

Additionally, while the measure of sleep disturbance used in the current study is well-validated, it is reliant upon subjective and retrospective information, rather than observed sleep patterns, which may decrease accuracy ([41]). Finally, the current results are limited by single time points, rather than examination of cholesterol levels, sleep patterns, and the associations thereof over time.

Future Directions

The examination of sleep disturbance in individuals with SLOS provides a unique avenue towards better understanding behavioral phenotypes associated with this disorder, as well as the potential biological etiologies thereof. Continued research in this area will help to build a larger body of knowledge on this topic, which is of great importance given the rare nature of

SLOS. Study of plasma samples is feasible given that obtaining them is relatively non-invasive and provides convincing measures of the biochemical severity of SLOS. Adding more objective assessments of sleep disturbances and sleep physiology (e.g., actigraphy, polysomnography, melatonin levels) in future studies will be instrumental in furthering the understanding of cholesterol-sleep associations. Further, studies utilizing other designs (e.g., longitudinal) may help elucidate dynamic associations between deficient cholesterol synthesis, cholesterol supplementation, and sleep.

The results presented here and future research may also have clinical application. Sleep hygiene is considered an important health initiative for youth and may be associated with behavioral disturbance in youth with developmental disabilities ([32]). To date, studies of intervention for youth with SLOS have focused on cholesterol supplementation ([7, 9, 15]) as opposed to behavioral intervention. Sleep disturbance and related biochemical severity may provide a useful opportunity for comparing different modes of intervention for youth with SLOS (e.g., behavioral versus dietary). Such research may further the ability to differentiate between learned versus syndrome-bound behavior symptoms in youth with SLOS ([6]) and, possibly, other rare genetic disorders.

Conclusion

In conclusion, our study represents an important contribution to the understanding of sleep in SLOS. This is the initial demonstration of an association between reported sleep problems and markers of impaired cholesterol biosynthesis in this population. As such, the findings add to our growing understanding of how the defect in cholesterol biosynthesis relates to physical, developmental, and behavioral outcomes. Our findings suggest that continued inquiry into sleep in SLOS may be useful. Studying SLOS, whose precise biochemical defect in cholesterol metabolism is known, may have important implications for our understanding of how cholesterol can affect behavior more generally.

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Table 1

Participant Characteristics

Participant	Age (years)	Gender	Sterol Ratio[†]	U-MVA[‡]	24S+	Severity Score[*]
1	14.33	Male	0.039	0.863	47.6	11.0
2	10.33	Male	0.39	4.928	41.13	35.0
3	5.83	Male	0.34	0.763	51.7	38.0
4	8.25	Female	0.47	0.850	47.2	25.0
5	5.17	Female	0.08	1.503	78.4	–
6	17.33	Female	0.09	1.573	43.3	10.0
7	16.50	Female	0.09	1.530	32.5	10.0
8	1.00	Female	0.65	0.385	–	–
9	5.58	Male	0.19	1.420	64.1	20.0
10	10.00	Male	0.10	1.286	65.6	30.0
11	4.92	Male	0.37	0.628	46.88	–
12	5.93	Female	0.09	0.444	–	5.0
13	3.92	Male	0.14	0.308	–	5.0
14	6.58	Male	0.00	0.594	109	20.0
15	3.08	Female	0.04	–	–	–
16	18.17	Female	0.70	1.201	24.6	33.0
17	15.42	Female	0.23	–	–	10.0
18	5.92	Male	0.06	0.610	132	6.0
19	1.08	Female	0.75	0.300	40.0	40.0
20	11.92	Female	0.042	0.184	–	17.0

Note.

[†]= (7-DHC+8-DCH)/Cholesterol;

[‡]= Mevalonic Acid via Urine (μmol/day);

[+]24(S)-hydroxycholesterol;

[*]= SLOS Clinical Severity Score ([21], [22]).

Table 2

Participant Scores on the Children's Sleep Habits Questionnaire

Variable	Raw Score *M* (SD)	Mean Score *M* (SD)
Bedtime Resistance	7.40 (1.64)	1.23 (0.27)
Sleep Onset Delay	1.45 (0.69)	1.45 (0.69)
Sleep Duration	4.00 (1.41)	1.33 (0.47)
Sleep Anxiety	5.58 (1.26)	1.39 (0.32)
Night Wakings	4.74 (1.19)	1.58 (0.40)
Parasomnias	8.83 (1.50)	1.26 (.21)
Sleep Disordered Breathing	3.58 (1.30)	1.19 (0.43)
Daytime Sleepiness	10.05 (3.05)	1.26 (0.38)
Total Sleep Disturbance	43.12 (4.27)	1.30 (0.13)

Table 3

Kendall's Tau Correlations Between Age, Sleep Disturbance, and Cholesterol Biochemical Markers

	Age	Sterol Ratio†	7-DHC	8-DHC	Chol	U-MVA‡	24S+
Total Sleep Disturbance	−.42*	.41*	.37*	.38*	−.35*	−.17	−.05
Bedtime Resistance	−.46**	.08	.11	.07	−.06	−.31	.12
Sleep Onset Delay	−.15	.41*	.31	.26	−.54**	−.11	−.39
Sleep Duration	−.01	.42*	.32	.36*	−.31	.10	−.26
Sleep Anxiety	.11	−.35	−.42*	−.45*	.06	.37	−.08
Night Wakings	−.03	.14	.21	.17	−.03	−.06	.27
Parasomnias	−.42*	.20	.18	.21	−.25	−.16	.20
Sleep Dis. Breathing	−.02	−.11	−.10	−.09	−.04	.06	.26
Daytime Sleepiness	−.16	.10	.15	.15	.01	−.14	−.11

Note.

† = (7-DHC+8-DCH)/Cholesterol;

‡ = Mevalonic Acid via Urine (µmol/day); Chol = cholesterol; ;

+ = 24(S)-hydroxycholesterol; Two-tailed.

* $p < 0.05$;

** $p < 0.01$

Table 4

Linear Regression Models Predicting Endorsement of Sleep Concerns

Model	R^2	$R^2\Delta$	β	*p*
Predicted: Sleep Onset Delay				
Model 1 Predictor: Age	.039	.039	−.20	.406
Model 2 Predictors: Age & Sterol Ratio	.458**	.419		
Age			−.07	.725
Sterol Ratio			.66	.002
Predicted: Sleep Duration				
Model 1 Predictor: Age	.040	.040	.20	.395
Model 2 Predictors: Age & Sterol Ratio	.258	.218		
Age			.30	.183
Sterol Ratio			.48	.039
Predicted: Total Sleep Disturbance				
Model 1 Predictor: Age	.174	.174	−.42	.075
Model 2 Predictors: Age & Sterol Ratio	.428*	.253		
Age			−.40	.051
Sterol Ratio			.50	.017

Note. Overall model fit is significant at

* $p < 0.05$;

** $p < 0.01$