Cholesterol in Childhood: Friend or Foe?

Commentary on the article by Merkens *et al.* on page 726

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In 1993, the Smith-Lemli-Opitz syndrome (SLOS), which previously had been a rare condition known best by dysmorphologists, became classified as an inborn error of metabolism (IEM) when it was discovered that affected patients lack the microsomal enzyme, 7-dehydrocholesterol reductase (3β -hydroxysterol- ρ 7-reductase; DHCR7) (1,2), which catalyzes the conversion of 7-dehydrocholesterol (7-DHC) to cholesterol in the final step of cholesterol biosynthesis. With this discovery, SLOS moved from its rather comfortable home with dysmorphologists to a newer, somewhat more uncertain home with biochemical geneticists.

The discovery that SLOS was caused by an inborn error of cholesterol biosynthesis led to investigations beyond SLOS itself. It raised new questions regarding the role of the cholesterol pathway in human development, and whether more effective treatments could be developed for conditions that cause the growth and mental retardation, which are secondary to underlying structural and 'metabolic' causes. But perhaps most importantly, it began a dialogue regarding the importance of cholesterol in fetal and early childhood development.

Prior to 1993, while the importance of cholesterol was known, the primary focus in adult, and to some extent pediatric, medicine centered on the prevention of hypercholesterolemia. For adults, much of the research focused on the development of medications, such as the statins, that interfere with cholesterol biosynthesis and decrease cholesterol levels, and on pharmacologic and dietary strategies in order to define and ultimately reduce absorption of dietary cholesterol. While hypercholesterolemia was not a significant contributor to pediatric disease, it was recognized that decreasing the cholesterol content in the diet was important. This point was evident in the composition of infant formulas, which were relatively cholesterol-poor when compared with human breast milk.

Recent identification of other inborn errors of cholesterol metabolism, which result in human phenotypes presenting with structural malformations, and growth and mental retardation, have contributed to an appreciation for the essential role that cholesterol plays in human development. Within 5 years of learning that patients with SLOS had low cholesterol levels and an accumulation of the cholesterol precursors 7-DHC and 8-DHC, both the biochemical and molecular basis of the

condition were confirmed. A deficiency in the microsomal enzyme DHCR7 was identified as the cause (3), and the gene was cloned and localized to chromosome 11q13 (4–6).

In addition, seven other conditions, hyperIgD syndrome, desmosterolosis, lathosterolosis, CHILD syndrome, CDPX2 (a form of chondrodysplasia punctata), HEM (hydrops-ectopic calcification-moth eaten) dysplasia, and some forms of Antley-Bixler syndrome, all of which are somewhat similar to SLOS in their presentation, have been linked to specific steps of the cholesterol metabolic pathway (7). These discoveries, along with advancement of our understanding of the role of cholesterol in glial synaptogenesis (8), in cell signaling and intracellular trafficking within lipid rafts (assemblies of cholesterol and sphingolipids) (9,10), and in fetal structural development via interaction with the various components of the hedgehog signaling pathway (11), underscore the importance of cholesterol and its precursors as important mediators of embryonic, fetal, and early childhood development.

Identification of SLOS as an IEM, along with the recognition of the importance of cholesterol in various physiological systems, led to efforts to develop more effective treatments for SLOS. However, SLOS did not fit the standard paradigm of an IEM, where one had either accumulation of a toxic metabolite or a deficiency of a product that could be replaced easily. Developing a treatment strategy for SLOS has been difficult because it is still unclear whether the clinical phenotype of SLOS is consequent to 1) the deficiency of cholesterol, 2) the accumulation of the cholesterol precursors, 7-DHC and/or 8-DHC, 3) an abnormality in lipid transfer from mother to fetus, 4) some other metabolite, which may be secondarily increased or decreased due to the primary deficiency of DHCR7, or 5) any combination of these factors. While cholesterol levels are low in SLOS, there are some unaffected individuals, who have low cholesterol levels, and other individuals with different IEMs that result in low cholesterol levels, who do not have the clinical phenotype seen in SLOS. Therefore, it appears that hypocholesterolemia alone is not sufficient to explain the clinical problems seen in this condition.

Not all features of the clinical phenotype are likely to be caused by a single biochemical abnormality. Structural anomalies may be explained by deficiency of cholesterol during embryonic development, while the neurodevelopmental problems and growth retardation may be due to a combined "toxic" effect, in which accumulating 7-DHC and 8-DHC is substituted for cholesterol in myelin or other cell membranes. Both 7-DHC and 8-DHC differ from cholesterol by one extra double bond,

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thus these precursors can easily substitute for cholesterol in cell membranes or in enzymatic reactions that accept cholesterol as a substrate (12). The treatment strategies that have been developed attempt to address all of these possibilities; however, because some of the neurodevelopmental problems seen in SLOS are due to fixed structural abnormalities of brain development, the ability to provide a complete neurodevelopmental "cure" has not been possible.

To date, treatment strategies have focused on supplying exogenous crystalline cholesterol by various vehicles (*ie*, oil-based or aqueous suspension) in an attempt to increase cholesterol levels and to secondarily decrease the levels of the precursors, 7-DHC and 8-DHC, through feedback inhibition of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. While investigators have reported that supplementation of exogenous cholesterol has led to improvement of the biochemical phenotype (cholesterol levels are elevated, and 7-DHC and 8-DHC levels are reduced), and to clinical improvement (improved growth and behavior), the response to therapy has varied between patients and protocols, such that it remains unclear as to whether one form of therapy is more beneficial than another (13–15). At least part of the confusion is due to the fact that patients with SLOS have variable degrees of biochemical effects, which range from mild to severe. This degree of variability interferes with other physiologic processes such as gastrointestinal function. In addition, whole-body cholesterol balance is a complex interaction of endogenous synthesis, intestinal absorption of exogenous cholesterol and biliary bile acid, and cholesterol secretion (16). Because, any or all of these factors may be affected by the hypocholesterolemia in SLOS, our current understanding of the absorption of exogenous cholesterol in non-affected individuals may not apply to SLOS patients, thereby increasing the challenge of developing an effective treatment strategy. However, because exogenous cholesterol is absorbed in the intestine and transported to the tissues via lipoproteins, it is important to determine the effect of dietary cholesterol supplementation on plasma lipoproteins in evaluating this form of therapy.

By measuring total sterols in lipoproteins and plasma on both cholesterol-free and high cholesterol diets, Merkens *et al.* (17), in this issue, not only attempted to answer this question, but also expanded our knowledge regarding the underlying sterol abnormalities in SLOS. In addition, they analyzed the distribution of cholesterol, 7-DHC, and 8-DHC in lipoproteins, and evaluated the effect of the apolipoprotein E genotype on the response to dietary cholesterol. The majority of patients received egg yolk supplementation, while others received crystalline cholesterol in either an oil-based or aqueous suspension in addition to egg yolks.

Their results are encouraging. They found that total sterols in plasma, LDL, and HDL increased with dietary cholesterol supplementation. LDL and HDL cholesterol increased significantly, while the concentrations of 7-DHC or 8-DHC in LDL did not change. This result indicates that the increase in sterols was due to cholesterol and not the precursor sterols. In addition, the distribution of sterols within each lipoprotein fraction was similar to that seen in plasma. While plasma cholesterol levels and the ratio of cholesterol to total sterols increased, a

significant decrease in 7-DHC was not seen. A significant negative correlation was noted between cholesterol and 7-DHC levels. Taken together these data indicate that those with the best response to dietary cholesterol in terms of cholesterol levels also had the lowest 7-DHC levels. However, the same group previously reported that significant decreases in 7-DHC levels were seen in their patient population after longer periods of treatment, which indicates that a longer time period than that involved in this study may be needed to affect precursor levels (15).

This study has clear implications for therapy of SLOS, and it answers several questions regarding response to dietary therapy. Since LDL and HDL are involved in transport of dietary cholesterol from the intestine to tissues, this study confirms the hypothesis that cholesterol feeding will result in an increase in cholesterol in both LDL and HDL, thereby providing a greater pool of cholesterol available for distribution. The response to exogenous cholesterol appears to be similar in the groups receiving egg yolk or other sources of cholesterol such as the crystalline cholesterol suspensions. Furthermore, while our previous understanding of the absorption of exogenous cholesterol indicated that absorption of dietary cholesterol was limited to approximately 40–60% of that ingested (16), the percentage increases noted in the present study were greater than the increases seen in adults given high cholesterol diets. This indicates that absorption of cholesterol may be enhanced in the presence of hypocholesterolemia. And finally, it appears that the apo E genotype did not appear to contribute to any variability in sterol levels.

The findings of Merkens *et al.*, would support the current practice of cholesterol supplementation as a treatment modality for SLOS in terms of improving the biochemical phenotype and providing a pool of cholesterol for transport to the tissues. Future challenges will include developing additional strategies to increase absorption of exogenous cholesterol and decrease the level of precursors, perhaps by means of the statins or other drugs. However, the ultimate goal will be in determining the effect of current treatment modalities on brain sterol levels, and developing a treatment strategy that can better affect sterol levels in the brain, especially in the early years of life. Current knowledge indicates that cholesterol in the central nervous system is synthesized locally and dietary cholesterol does not cross the blood-brain barrier; thus, methods need to be developed to deliver cholesterol to the brain. However, it is unclear whether this is true in SLOS since preliminary data utilizing MRS technology did reveal changes in brain lipid peaks in two patients prior to and on dietary therapy, indicating that exogenous cholesterol may in fact lead to change in brain lipids (18). Such advances will not only help us treat children with SLOS and perhaps other disorders of sterol metabolism, but will inevitably lead to a better understanding of the role of cholesterol and other sterols in human cognitive development.

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