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FETAL AND NEONATAL CHOLESTEROL METABOLISM

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ABSTRACT

Cholesterol is essential for mammalian development. It is a structural component in every cellular membrane, is involved with various signaling events, and is the precursor for key steroid hormones required for normal development. Fetuses have two sources of cholesterol, endogenous and exogenous, as do adults and children. An endogenous source of cholesterol comes from de novo synthesis. Cholesterol is synthesized in all tissues of all individuals, whether pre- or post-partum. In adults and children, the exogenous source comes from the diet. In contrast, the fetus is protected from direct contact with external factors in the maternal circulation. As such, fetal exogenous cholesterol is obtained from the maternal circulation after being taken up and transported across the placenta and possibly the secondary yolk sac. In this review we will discuss fetal cholesterol metabolism and the potential impact of maternal cholesterol on fetal cholesterol. We will also cover the impact of diet on neonatal cholesterol metabolism. Alterations in fetal and neonatal cholesterol metabolism are important to understand not only during infancy, but for the long term health of the individual as coronary heart disease been proposed to be linked to abnormal cholesterol metabolism in the fetus and newborn. For complete coverage of all related aeas of Endocrinology, please visit our on-line FREE web-text, WWW.ENDOTEXT.ORG.

FETAL CHOLESTEROL METABOLISM

Fetal lipoprotein metabolism

Plasma cholesterol concentrations in the newborn are markedly reduced compared to the adult. There are two lipoproteins that carry most of the circulating cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL), with lower amounts of cholesterol being carried as very low density lipoproteins (VLDL). According to the National Health and Nutrition Examination Survey (NHANES), in adults with an average age of 49±18 years and an average total cholesterol concentration of 193±42 mg/dl, a majority of plasma cholesterol is carried as LDL (115±35 mg/dl) with HDL carrying less cholesterol (53±15 mg/dl), making an average LDL-C/HDL-C ratio in adults of 2.17 (1). In contrast, total plasma cholesterol levels are much lower

in the fetus/newborn, with concentrations ranging from 51.4-96.8 mg/dl for term infants (2-13); for the sake of the review we will use the terms fetus and newborn interchangeably as blood samples for the newborn are often obtained from the umbilical vessels of the placenta at birth. In the fetus compared to the adult, a greater proportion of cholesterol is carried as HDL (22.1-44.9 mg/dl) versus LDL (22.0-44.9 mg/dl). Thus, the LDL-C/HDL-C ratio is much lower in a fetus/newborn compared to an adult. The ratio is 0.56-1.55 in the fetus or newborn, with an average ratio of 0.99 in term infants (2, 4-9, 12, 14).

Plasma cholesterol concentrations in fetuses are not constant throughout gestation, and concentrations often decrease as gestation progresses (8, 15-17). It appears that the biggest decreases occur in LDL-C such that LDL-C/HDL-C ratios are elevated in most studies earlier in gestation (up to 1.8 at 25 weeks of gestation) and decrease as gestation progresses (8, 15-17), possibly due to increased LDL receptor activity by the fetal liver late in gestation (18). This relationship has been found even in term infants (>37 weeks of gestation), depending on their gestational age (i.e. 37 vs 42 weeks of gestation) (16). While a negative correlation between gestational age and fetal cholesterol levels is found and preterm infants have higher plasma cholesterol levels than term infants in a number of studies, not all studies show this same relationship. Indeed, some studies have shown no effect of gestational age on fetal cholesterol levels or even an increase in plasma cholesterol level with gestational age (7, 19). The differences in results found in plasma collected from newborns born prematurely versus at term could relate to the design of the studies because some studies collect blood from the newborn infant while others collect cord blood from the placenta to analyze, which should be similar but may not be depending on the timing of sample collection. Also, gestational age may be defined differently depending on the method used to define gestational age (ultrasound or the last menstrual cycle of the female). Finally, differences could be related to the preterm population studied as some preterm infants are thought to have other metabolic issues that affect their sterol metabolism, leading to unexpected differences in plasma cholesterol levels. Indeed, preterm infants are at an increased risk to develop heart disease later in life (20), possibly due to altered sterol metabolism.

In addition to gestational age, other potential factors that could impact fetal plasma cholesterol levels are *in utero* growth rates. Too slow growth rates result in infants that are small-for-gestational age (SGA) or have intra-uterine growth retardation (IUGR). Too rapid of growth rates result in infants that are large-for-gestational age (LGA). Though several studies show fetal lipid concentrations are directly associated with fetal birthweight, the data are still quite variable with some studies showing reduced cholesterol in infants with LGA and some showing no effect (2, 4, 5, 7, 14). It has been proposed that it is actually the body type (body length, abdominal circumference, etc) and not the birthweight which is important in plasma cholesterol levels of the fetus (6), making it difficult to interpret results. It may also be the type of lipoprotein particles that are present and not just the cholesterol concentration that changes with size of the newborn. A recent study showed that the large HDL particles of smaller infants contained increased amounts of apolipoprotein C-I (apoC-I). These particles were shown to lead to apoptosis, thereby leading to a unique type of smaller infants with distinct metabolism (21). Interestingly, as with preterm infants, infants with abnormal intrauterine growth are at a greater risk for developing cardiovascular disease [see Developmental programming of adult cholesterol metabolism in the fetus and newborn]. It has been proposed that it may be metabolism in the mother that is responsible for the altered lipoprotein-C and not the fetal growth (2, 3, 22, 23).

The composition of the lipoprotein particles also differ between adults and fetuses. The most well-known apolipoproteins, including apoE, apoA-I and apoB, are all present in the fetal circulation. Most of the apolipoproteins are lower in the fetal versus adult circulation as well (10), which is expected when lipid levels are so much lower in the fetus.

One exception, however, is apoE, which is similar in adults and fetuses. The excess apoE is found on fetal HDL particles which are large in size (24-26). The presence of apoE on HDL increases the functions of HDL. The most commonly described function of HDL is to enhance cholesterol efflux from cholesterol-laden cells. The effluxed cholesterol is transported to the liver where the cholesterol can be removed from the body as biliary cholesterol or bile acids. ApoE can enhance the effux out of cells. More importantly, apoE is a ligand for a number of receptors of the LDL receptor family allowing for uptake of HDL-C by a greater number of tissues and potentially for increased transport of cholesterol between tissues. The apoE-containing HDL can also affect genes related to sterol metabolism and oxidation in fetal endothelial cells (cells separating the fetal circulation from the trophoblasts of the placenta) (27).

HDL is an interesting lipoprotein in that it carries over 90 proteins that mediate a myriad of functions (28). In adults, the proteins carried by HDL are involved in oxidation, inflammation, hemostasis, vitamin transport, immunity, and energy balance as well as lipid transport. Interestingly, fetal HDL is enriched in proteins involved in coagulation and transport, including apoE, and is lacking in proteins involved in anti-oxidative processes, such as paroxonase I (PON1) (26). The lack of PON1 on fetal HDL suggests that these particles do not have the same anti-oxidative capacity as that found in adults, but they have enhanced ability to transport cholesterol between tissues (26). Unlike HDL, changes in VLDL and LDL composition between the adult and fetal circulations are poorly defined. Finds of a single study, however, suggest that there are more small-dense LDL particles in the newborn compared to adults (29).

What is the significance of or newborn plasma cholesterol concentrations or composition to the clinician? Is it possible that plasma cholesterol may define individuals at risk to be hypercholesterolemic due to familial hypercholesterolemia? Plasma cholesterol levels at birth are not useful in this respect because concentrations are quite variable and they are dependent upon fetal growth rate and gestational age. To determine if an individual is at risk of high plasma cholesterol levels later in life, concentrations at one year of age are more representative of hypercholesterolemia than those at birth (30), taking into account if infants are fed cholesterol-containing breast milk or formula. Interestingly, infants that are at risk of high plasma cholesterol levels later in life and/or at an increased risk of heart disease are not apparent at birth because the hypercholesterolemia does not evolve until exposed to various factors in the environment or to aging. Regardless, infants that are premature or have abnormal fetal growth rates are at an increased risk to develop cardiovascular diseases, even if plasma cholesterol levels are not elevated at birth. The plasma cholesterol levels at birth also can be used to define various genetic disorders. One such rare disease, which can actually occur up to 1 in 10,000 to 40,000 live births, is the Smith-Lemli-Opitz syndrome (SLOS) [reviewed in (31-34)]. Individuals with this disorder have increased 7- and 8-dehydrocholesterol concentrations. Assays for these dehydrocholesterols must be done by gas chromatography, not the commonly used enzymatic assay which will measure the dehydrocholesterols along with cholesterol. Thus, if SLOS is suspected due to facial features or family history, plasma cholesterol should be measured using the appropriate assay.

Regulation of fetal lipoprotein metabolism

In the fetus as in the adult, plasma cholesterol levels are regulated by the amount of cholesterol entering versus that exiting the circulation. In adults that are in steady state, the amount of cholesterol entering the plasma is equal to that exiting the plasma. This does not apply to individuals that are not in steady state, as happens with rapid growth *in utero*. Cholesterol enters the circulation as lipoproteins and leaves the circulation after being taken up by lipoprotein receptors on a number of tissues. The liver synthesizes and secretes VLDL which is converted to LDL in the circulation. Since the liver is not functionally developed *in utero* (35, 36), lipoprotein production and secretion could be low, being at least part of the cause of the low fetal LDL-C levels. The reduced lipoprotein production is not due to a lack of cholesterol, however, because sterol synthesis rates, based upon markers in amniotic fluid, indicate that while fetal sterol synthesis rates are very low early in gestation, they increase markedly by mid gestation (37). The lower levels of fetal cholesterol in the circulation are also likely due to an increase in uptake of lipoprotein-C from the circulation. Using the *in vivo* catheterized pregnant sheep model, it was found that uptake of cholesterol by tissues is greater *in utero* than later in the neonatal lamb (38). This is not unexpected as tissues require significant amounts of cholesterol for membrane formation and for steroid hormone synthesis and lipoprotein receptors are expressed on fetal tissues (39-41). Why then are HDL-C levels relatively elevated in the fetus versus LDL-C levels? Unlike VLDL and subsequently LDL, HDL is produced in the circulation and as such is not dependent upon the fetal liver for lipoprotein production. To produce HDL, first cholesterol is effluxed from tissues onto lipid-poor apoA-I or apoE, followed by esterification of the cholesterol by lecithin cholesterol acyl transferase (LCAT), all of which are present in the fetal circulation (26).

Sources of fetal cholesterol

Because massive amounts of cholesterol are needed for growth, the question remains-where does the fetal cholesterol originate? Every membrane requires cholesterol with especially high amounts in neuronal cells. Thus, for a baby that weights 4.5 kg, almost 15 g of cholesterol is required by the body as the peripheral tissues and liver contain ≈2.2 mg cholesterol/g wet weight tissue and the brain contains ≈8 mg cholesterol/g tissue at birth [reviewed in (42, 43)]. As the fetus is not in steady state, more cholesterol is accrued by the fetal body as compared to that being removed. In fact, very little cholesterol is lost from the fetus as bile acid production is poorly developed in the fetal liver. The only net loss of cholesterol is in the form of steroid hormone synthesis, which does indeed occur in the adrenal glands during gestation (44).
The fetus has two sources of cholesterol. One source is that synthesized *de novo*. The rates of sterol synthesis are much greater in the fetus than in the adult in several species (38, 43, 45-48), including humans (43). In fact, a significant proportion of the fetal cholesterol can be accounted for by that which is synthesized *de novo* (45, 49-51). Cholesterol synthesis rates appear to be regulated less rigidly than that which occurs in adults (46), possibly reflecting the massive tissue requirements of the fetus. Whereas sterol synthesis rates are markedly suppressed in adult tissues with elevated cholesterol concentrations, sterol synthesis rates are suppressed only marginally in fetal tissues with similar elevations of cholesterol concentrations as that in adult tissues. One of the key regulators of cholesterol biosynthesis is sterol regulatory element-binding protein-2 (SREBP2) (52). Processing of SREBP-2 from the inactive form to the

mature active form enhances cholesterol synthesis. In adult tissues, increases in cellular cholesterol levels will reduce the processing of the SREBP-2 to the mature active form through a number of proteins present in the Golgi apparatus and endoplasmic reticulum. In the fetus, we found what appeared to be constitutive processing of the SREBPs, leading to a fully active sterol biosynthetic pathway, regardless of cholesterol levels within the tissues. This same lack of regulation in fetal tissues was found when fetal hepatocytes were treated with lipoprotein-cholesterol (53) and when fetuses were exposed to polyunsaturated fatty acids *in vivo* (54). Regulation still occurs however as estrogens, glucocorticoids, and progesterone all lead to increased fetal sterol synthesis rates (55).

The other potential source of cholesterol is that which originates in the maternal plasma. There has been much debate about the potential for maternal cholesterol to be transported to the fetus. There are two main reasons why it has not been well accepted that cholesterol is transported across the placenta even though transport of other lipids is well recognized. First, cholesterol levels are much greater in the maternal than the fetal circulation. Active transport of lipids across the placenta has been shown as some of the long chain fatty acids are actually at a greater concentration in fetal plasma compared to maternal plasma (56, 57). Second, there appears to be no correlation between maternal cholesterol and newborn cholesterol concentrations in term infants (11, 12). If maternal cholesterol did cross the placenta, it is assumed that there would be a direct association between the concentrations in both plasma pools. Conversely, several direct and indirect lines of evidence suggest that cholesterol can be transported from the maternal to fetal circulation. First, while there is no correlation between maternal and newborn cholesterol concentrations in term or late preterm infants, there is a direct relationship between maternal and fetal plasma cholesterol concentrations early in gestation (58). Thus, cholesterol might be transported early in gestation but not late in gestation. Interestingly, though there is not a direct correlation between maternal and fetal cholesterol, some studies have shown a direct correlation between maternal cholesterol levels and birthweight (59-61). Second, fetuses of mothers with higher plasma cholesterol levels have increased intimal plaque (58). Third, there are significant amounts of plant sterols in the newborn circulation, 40-50% of that found in the maternal circulation (30). As these sterols are only obtained from the diet of the mother, they must cross the placental barrier. Fourth, fetuses that are lacking the ability to synthesize cholesterol due to a defect in one of the enzymes of cholesterol biosynthetic pathway, such as those with the Smith-Lemli-Opitz syndrome, have measureable amounts of cholesterol in their body, even those with null-null mutations (62, 63). Fifth, indirect evidence that maternal cholesterol is present in the fetal circulation is that maternal plasma cholesterol levels increase during gestation, possibly to aid in development of the fetus. In a normal pregnancy, plasma cholesterol levels increase 25-50% by the third trimester (64-66). The increase could be driven by the increase in cholesterol synthesis that occurs in late gestation in rodent as well as human mothers (67, 68), possibly as a consequence of loss of cholesterol to the developing fetus or placenta or in anticipation of the future need of cholesterol by the fetal unit. Finally, the proteins required for uptake, transport and secretion, and efflux are present in tissues that separate the fetal and maternal circulations and in the fetus.

There are two different tissues that isolate the embryo or fetus from the maternal circulation [reviewed in (31, 69, 70)]. Early in gestation and prior to a functional placenta (first trimester), the secondary yolk sac would be responsible for any transport of cholesterol from the maternal

to fetal circulation. Briefly, maternal lipoproteins can be taken up by the yolk sac through receptor-independent processes and receptor processes as the yolk sac contains a number of lipoprotein receptors, including SR-BI, cubilin, and megalin. The yolk sac also synthesizes apolipoproteins and secretes newly formed lipoproteins which can be regulated by lipid availability. Because the yolk sac vasculature is integrated into that of the embryo, the maternally-derived lipids can enter the fetal circulation as newly secreted lipoproteins. At about 8 weeks of gestation, the spiral arteries of the placenta begin to flow, making the placenta functional. Once the placenta is functional and the secondary yolk sac regresses, the placenta takes over transport of maternal components to the fetus. The placenta is unique in that maternal blood enters the intervillous spaces of the placenta, directly bathing the syncytialized trophoblasts. As with the yolk sac, trophoblasts take up maternally-derived lipoproteins via receptor-independent and receptor-dependent processes; the placenta can take up lipoproteins through a number of receptors, including the LDL receptor, the VLDL receptor, the class A scavenger receptor, the LDL receptor-related protein (LRP), the apoE receptor 2, megalin, cubilin, and the scavenger receptor class B type I (SR-BI). Since the maternal blood within the intervillous space exchanges 3-4 times each minute, it is potentially an excellent source of maternal cholesterol for the fetus. Once taken up, sterol transport proteins would assist in channeling cholesterol across the cells to the fetal-facing basolateral membrane. The LDL or HDL could potentially be transcytosed across the cells after interaction with SR-BI as shown previously in other endothelial cells (71, 72). The route by which cholesterol exits the basolateral membranes and enters the fetal circulation remains a mystery as lipids likely need to pass through the fetal endothelial cells as well. Cholesterol exits the trophoblasts and endothelial cells after being effluxed to acceptors or after being secreted as newly formed lipoproteins. Interestingly, one group did show that the human placenta can secrete newly synthesized apoB-containing lipoproteins (73).

The proteins expressed on the fetal-facing membranes of endothelial cells and trophoblasts that can assist in efflux of cholesterol include SR-BI, ABCA1, and ABCG1 (31, 69, 70, 74, 75). When the expressions of these proteins are altered, either genetically or pharmacologically, efflux changes in parallel with the protein changes made. Acceptors of the effluxed cholesterol that are present in the fetal circulation include lipid-poor apoE or apoA-I and spherical HDL. A change in the amount or composition of acceptors can also affect efflux capacity. For example, we found that lipid-poor fetal HDL from an SLOS fetus is a better acceptor of trophoblast cholesterol than a typical fetal HDL particle (76). A newer player in this arena is the phospholipid transfer protein. This protein is located on the fetal side of fetal endothelial cells. When added to media with fetal HDL, efflux from endothelial cells increased (77, 78). Thus, while it is apparent that cholesterol can potentially be transported across cells of the yolk sac and the placenta and enter the fetal circulation, it is still not known how much cholesterol is transported and when during gestation this occurs.

Roles of fetal cholesterol

As stated earlier, cholesterol is essential for normal growth and development. It is an integral component of every membrane and is necessary to maintain structural integrity and for signaling. Though all membranes contain cholesterol for structural purposes, cholesterol is enriched in specific regions of the membranes, lipid rafts, where many phosphorylated proteins reside. Changing lipid raft composition can often lead to a change in various signaling events

with significant downstream metabolic consequences (79-81). Cholesterol is also required to activate hedgehog signaling through unique covalent bonds (82), including sonic hedgehog (SHH), a protein involved with patterning of various organs, mid-line brain structures, and others. As SHH is expressed as early as 3 weeks after fertilization, changes in activation could have very early and significant effects. Indeed, lower SHH signaling has been associated with altered signaling that occurs in individuals with SLOS (83). Cholesterol is also a precursor of steroid hormones, which are synthesized at elevated rates *in utero*, and oxysterols, regulators of metabolism through various pathways. Oxysterols can affect a number of pathways from activation of the liver X receptor (LXR) (84) to inhibition of SHH signaling (85).

ABNORMAL FETAL STEROL METABOLISM

Even though two sources of cholesterol exist for the fetus, a majority of fetal cholesterol is likely derived from synthesis, making fetal *de novo* cholesterol synthesis essential. An indication of the importance of fetal cholesterol is that individuals lacking the ability to synthesize cholesterol have mild to severe metabolic diseases and congenital defects. There are 7 known defects in the cholesterol biosynthetic pathway that result in altered fetal phenotypes [reviewed in (31-34)]. Most of the defects found in humans are post-squalene. Disruption of enzymes early in the sterol biosynthesis pathway leads to embryonic lethality in various murine models [reviewed in (31)].

The most well-known disorder due to altered sterol synthesis is the SLOS. This disorder is also the most common of this group of rare diseases. Individuals with SLOS have affected midline facial features, multiple organ and limb malformations, and intellectual disability. Cholesterol synthesis is halted at the last step when 7-dehydrocholesterol (7DHC) is converted to cholesterol by a defect in the 3β-hydroxysterol-Δ^7-reductase gene (DHCR7). Though it was thought that the syndrome was due to a lack of cholesterol, and some of the defects could be due to a lack of cholesterol, recent studies show that the buildup of 7DHC plays a role in the progression of the disease as well. Interestingly, the inability for normal neuronal cells to differentiate was due not to changes in SHH signaling, but to defects in Wnt/β-catenin signaling (86).

NEONATAL CHOLESTERL METABOLISM

The three major sources of nutrition in the United States during neonatal and early infancy are human milk, cow milk-based formulas, and soy milk-based formulas. The composition of these types of diet differs in several factors that may theoretically influence cholesterol homeostasis including cholesterol content, polyunsaturated/saturated fatty acid ratio (P/S ratio), protein composition, phytoestrogen content and the presence of hormones specific to breast milk. More recent components of milk include miRNAs and microbes, both of which can affect metabolism (87, 88).

As with the fetus, neonatal mammalian cells also require significant cholesterol for normal cellular function. Infants fed human milk receive much greater quantities of cholesterol than those fed commercial formulas. Human milk contains between 10-15 mg/dl of cholesterol, providing an average daily cholesterol intake of ≈75 mg per day for a breastfed 4 kg newborn. Cow milk-based formulas contain 1-4 mg/dl of cholesterol, giving an average daily cholesterol

intake of approximately 9 mg per day. Soy milk-based formulas contain no cholesterol. Not unexpectedly, breast-fed infants have higher serum cholesterol concentrations compared to formula-fed infants (89, 90). These differences have generally been attributed to the cholesterol content of human milk and commercial formula. Whether the low cholesterol content in commercial formulas poses any physiologic or pathophysiologic effects other than the difference in serum cholesterol concentration and synthesis rates remains to be understood.
The impact that dietary cholesterol has on sterol metabolism has also been studied. As discussed previously in this review, the fetus appears to be somewhat protected from down regulation of sterol biosynthesis. In contrast, neonates, like adults, can suppress sterol synthesis rates (91). In one study, infants were fed breast milk versus cow milk-based formula. After 4 months of diets with different cholesterol concentrations, total-C and LDL-C levels are higher in infants consuming more dietary cholesterol. Unlike fetal tissues, the fractional synthetic rate (FSR) of cholesterol was lower in infants consuming more cholesterol demonstrating the ability to regulate sterol biosynthesis in the neonate. The long term consequences of these changes are currently unknown (see below).

DEVELOPMENTAL PROGRAMMING OF ADULT CHOLESTEROL METABOLISM IN THE FETUS AND NEWBORN

In the early 1990s, Dr. David Barker unexpectedly discovered that persons growing up in less affluent areas of England and Wales were at an increased risk for ischemic heart disease and infant mortality compared to those growing up in more affluent areas (92). Dr. Barker and colleagues determined that the association was between heart disease and low birthweight. A similar relationship between SGA and age-related heart disease has been confirmed by other researchers in other populations [reviewed in (93, 94)]. An association with birthweight has expanded to include infants who are born LGA as well, forming a U-shaped curve. Thus, heart disease is now thought to be associated with abnormal *in utero* growth. Because of his early seminal work in this area that is ever expanding, the “programming” of metabolism by early life environment has been coined the “Barker hypothesis” or DOHaD (Developmental Origins of Health and Disease). Various mouse models have been used to study programming of adult diseases, though the number used to study programming of heart disease are not as prevalent as those for obesity and diabetes as mice do not routinely develop cardiovascular disease, though they will become hypertensive (95, 96). Some enlightening studies showing the importance of maternal lipids on cardiovascular disease were completed in mice using genetic alterations and additional stressors (97), however.

The long term changes in metabolism that persist into adulthood are the cause of programming are likely epigenetic changes in genes controlling metabolism [reviewed in (98)]. Several genes related to lipid metabolism have been found to be epigenetically altered *in utero*, including regulatory genes LXR and PPARα and the transporter GLUT4 (99). There are some recent treatments that are directed at changing the epigenome postnatally, including statins which are proposed to modify histones and various dietary regimes which can affect methylation status (100), and prenatally, including anti-oxidant compounds to reverse programming (101).
It is not only the *in utero* environment which has the potential to lead to programming of metabolic disease or heart disease. The type of diet fed to the newborn may also lead to profound and long-lasting effects on metabolism and heart disease (102). Since breast milk and formulas vary more than just in their cholesterol content, it is almost impossible to determine if

early life cholesterol affects age-related development of heart disease. The effect of the type of nutrition during infancy has additional confounders besides the composition of the diet, such as the amount of food consumed via the bottle versus breast (especially if milk production by the female is low), the way the infants are held, how much weight is gained, etc. However, if one were to focus solely on cholesterol, one hypothesis would be that Infants fed a cholesterol-containing diet are "programmed" to down-regulate their cholesterol synthetic rate to a greater extent than infants who had not been exposed to dietary cholesterol early in life. In this context, human milk with its higher cholesterol content compared to standard cow's milk- or soy-based formulas could be protective. Even though the effect of the type of nutrition during infancy on later cholesterol metabolism in adulthood is difficult to demonstrate because of many uncontrolled variables in a free living population, some studies do show a possible epigenetic effect. Current work in humans, which is largely inferential, is based upon plasma cholesterol concentrations. Adult men and women who were breast-fed in infancy had lower serum cholesterol concentrations compared to adults who were previously formula-fed (103) or higher HDL-C levels (104). Likewise, plasma total cholesterol was significantly higher in adult males that were breast fed for the shortest period of when compared to those who were breast fed for longer times (105). In contrast, plasma cholesterol concentrations in children and baboons fed either breast milk or formula had either no difference in plasma cholesterol levels or lower plasma cholesterol levels after being fed formula (106). A review of the literature suggested that the differences in studies were due to studies using exclusive breastmilk versus those using both breastmilk and formula (107). Additionally, the discrepancy between studies could also be due to the fact that some were completed in children so it is possible that age-related stressors have not been introduced to lead to an effect and some used different types of formula, i.e. cow- versus soy-based. It has also been suggested that some of the effects are mediated by the impact that breast milk has on BMI (108) or on food preferences in adulthood (109). Future studies are needed to better characterize the long term effects of early cholesterol exposure on cholesterol metabolism in later childhood and adulthood, and which genes may be affected by post-partum dietary cholesterol.

SUMMARY

To summarize this review, cholesterol is essential for normal growth and development. In the fetus, most cholesterol is derived from *de novo* synthesis, with a second source of cholesterol derived from the maternal circulation. The amount that is transported from the mother to the fetus is currently unknown. Due to its critical role in development, sterol synthesis rates are regulated less in the fetus and if synthesis is reduced due to genetic defects, abnormal development often occurs. The neonate also requires cholesterol for continued growth and development. The neonate obtains cholesterol from *de novo* synthesis as well as dietary cholesterol, with breast milk being the largest contributor of exogenous cholesterol. Unlike the fetus, sterol synthesis in neonates can be regulated.

In the future, a better understanding of how lipid metabolism in utero relates to lipid metabolism in adults is needed. This would be expanded to linking how lipid metabolism changes in the fetus result in cardiovascular disease later in life. One aspect would be to define how sterol metabolism is altered *in utero* when growth rates are abnormal and what epigenetic changes occur simultaneously. The same can be true for infants that are born prematurely. In fact, knowing which metabolic pathways are altered during times of abnormal growth could allow one

to devise potential interventions aimed at maternal and/or neonatal nutrition to reduce the occurrence of heart disease later in life. Possible targets would be anti-inflammatory factors in maternal diets or various factors in breast milk shown to be beneficial to long term health, i.e. certain microbes. In addition to targets developed for the young, interventions could be targeted for specific pathways known to be affected in the adult at a time when other risk factors arise. We hope that one day we can reach a point where modifications to the fetal environment or post-natal supplementation regimes can be used to reduce the long term incidence of cardiovascular and heart disease and other metabolic disorders.

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