FETAL LIPID METABOLISM

Laura A. Woollett

Department of Pathology and Laboratory Medicine, University of Cincinnati, 231 Albert B. Sabin Way, Cincinnati, OH 45267-0529

TABLE OF CONTENTS

1. ABSTRACT

The fetus grows at a rate unparalleled by that during any other stage of life. To maintain its rapid growth rate, the fetus requires a significant amount of cholesterol and fatty acids. For structural purposes alone, the fetus requires 1.5 mg of cholesterol per gram of tissue, not including the brain. Cholesterol is also required as a precursor for various steroidogenic hormones that are critical to normal development, such as estrogen, and for metabolic regulators, such as oxysterols. More recently, it was found that cholesterol is necessary for the activation of Sonic hedgehog (Shh) (1), an organizer involved in early spatial patterning of the forebrain (2). Fatty acids are needed as structural components of tissues, as a source of energy, and if metabolic regulation in the fetus is similar to that in the adult, as activators of transcription factors. The fetus, as in any tissue, acquires its cholesterol and fatty acids from two different sources, endogenous and exogenous.

2. FETAL CHOLESTEROL

2.1. Endogenous source of cholesterol

The endogenous source of cholesterol in the fetus, as in any tissue, is that synthesized de novo. Activities for HMG-CoA reductase, the rate limiting step for cholesterol synthesis, and *in vivo* sterol synthesis rates are much greater in the fetus than the adult when presented on a per gram tissue basis (3-10). In fact, a large proportion of the cholesterol accrued, if not all, in some species such as the rat (3, 5), can be accounted for by de novo sterol synthesis (3-5). Sterol synthesis rates, measured *in vivo* and by HMG-CoA reductase activities, are not static throughout gestation and decrease as gestation progresses in the whole fetus (3, 4, 8), the fetal liver, and the fetal kidney (11). In the brain, sterol synthesis rates

actually increase during gestation (11), possibly to account for its exponential increase in cholesterol content which appears to be obtained primarily from de novo synthesis (5, 12, 13).

The regulation of sterol synthesis rates in the fetus is uncharacterized, as is the mechanism responsible for the gestation-related decrease in sterol synthesis rate. In adult tissues, sterol synthesis rates are regulated by the sterol regulatory element binding proteins (SREBPs) (reviewed in (14-16)). There are three active SREBPs in adult tissues, SREBP-1a, -1c and –2; SREBP-1c is much more prevalent in tissues than is SREBP-1a (17). The SREBPs are membrane-bound transcription factors that are synthesized as precursors and bound to the endoplasmic retituclum (ER) and nuclear envelope (14). The carboxy terminus of the membrane-bound SREBPs bind to the SREBP cleavage-activating protein (SCAP) and are transported to a post-ER compartment, most likely the Golgi, where the SREBPs are cleaved by Site-1 and Site-2 proteases (18, 19). The mature forms of the SREBPs are then transported to the nucleus where they increase transcription of numerous genes, including HMG-CoA reductase and synthase, farnesyl diphosphate synthase and squalene synthase (20-22). Several factors regulate the levels of cellular SREBP, including cholesterol and fatty acids. In the presence of a high cellular cholesterol concentration, the SREBPs are not processed to the mature form due to the rapid degradation of SCAP within the ER; the mature SREBP concentration is relatively low. When sterol concentration is low, SCAP attaches to the SREBPs to stabilize the protein and aid in transport to the Golgi where the Site-1 protease is located, thereby increasing the amount of mature SREBP (23, 24). Polyunsaturated fatty

acids reduce the mature form of SREBP-1 independent of a change in the amount of the membrane bound form (25). Even though both SREBPs are involved in the overall maintenance of sterol homeostasis, each regulatory element has a specific role in maintaining balance: SREBP-1c is responsible for maintaining basal rates of sterol synthesis whereas SREBP-2 is responsible for the induction of synthesis rates during times of sterol depletion (26).

Both SREBP-1 and SREBP-2 are necessary for normal fetal development (27). When SREBP-2 expression is halted by disruption of the gene, all embryos die in utero at 7-8 days of gestation. When SREBP-1c is not expressed, only 50-85% of the embryos die in utero at a later stage of development. As one might then expect, both SREBP-1 and -2 are expressed in the human fetal liver (28). Interestingly, the membrane-bound precursor form of SREBP-1 is absent in some of the fetal livers (28).

It is not evident from these results alone that cholesterol deficiency is the primary cause of embryonic lethality in the SREBP-/- embryos since a lack of SREBPs will also lead to decreases in fatty acid synthesis rates and in various cholesterol synthesis intermediates, such as farnesyl, that are needed for other cellular processes. A significant collection of data exists, however, that demonstrates the importance of cholesterol synthesis in fetal development. Currently, there are 4 known disruptions in the sterol synthesis pathway that lead to congenital defects in the human fetus. It is possible that the frequency of defects is underestimated since embryos may be spontaneously aborted very early in gestation as a result of severe abnormalities, as in mice with a disrupted squalene synthase gene (29). The most common alteration in sterol biosynthesis which leads to a congenital defect is a deficiency of 3beta-hydroxysteroid-delta[7]-reductase. Embryos lacking this enzyme develop the Smith-Lemli-Opitz syndrome (SLOS) (30) which has an estimated prevalence of 1:7,000 to 1:20,000 live births, depending on the population studied (31-33). Persons with this biochemical defect have very low cholesterol concentrations and elevated 7- and 8-dehydrocholesterol concentrations in tissues and plasma (30). The characteristics of these individuals include craniofacial and neurological abnormalities, at times including forms of holoprosencephaly (HPE) which is the lack of a forebrain, and affected organs and limbs (34, 35). The defects do not appear to be related to an excess of 7-dehydrocholesterol, but rather to a lack of cholesterol (36). Other congenital defects associated with a reduction in the amount of cholesterol synthesized include mevalonic acid kinase deficiency in which mevalonic acid is not converted to mevalonate pyrophosphate (37), desmosterolosis in which delta[24]-reductase is absent and desmosterol builds up (38, 39), and chondrodysplasia punctata 2 in which 3beta-hydroxysteroid-delta[8],delta[7]-isomerase is absent and delta[8]-cholestenal and 8-dehydrocholesterol accumulates (40, 41). These three less common syndromes are all associated with severe neurological and/or craniofacial abnormalities.

Congenital defects can also be generated in various animal models given drugs that inhibit cholesterol synthesis. The earliest evidence of this was inadvertently discovered when it was observed that consumption of *Veratrum californicum* by pregnant ewes led to cyclopia in their offspring (42, 43). The consequences of the ingestion of this plant were traced to two alkaloids, jurvine and cyclopamine, which are structurally similar to cholesterol and which inhibit cholesterol synthesis at a distal step in the pathway (44). The effects on offspring were found to be consistent in all ruminants and rodents studied (45). Similarly, consumption of AY9944 and BMI15.766, two other compounds which inhibit cholesterol synthesis, will also lead to neurological defects, including HPE (46-48). It should be noted that the alkaloids, AY9944, and BMI15.766 may also affect cholesterol esterification within cells (44, 49). Inhibition of cholesterol synthesis early in the sterol synthesis pathway is also detrimental and can lead to deformities as demonstrated by consumption of the early generations of HMG-CoA reductase inhibitors (50).

2.2. Exogenous sources of cholesterol

It is apparent from the previous discussion that de novo cholesterol synthesis is critical for fetal development. The fetus, as in any tissue, has a second potential source of cholesterol which is derived exogenously. There has been much debate over the entry of exogenous cholesterol, specifically maternal-derived cholesterol, into the fetus because of 1) extremely high rates of fetal sterol synthesis (3-10), 2) *in vivo* sterol synthesis rates that can account for essentially all of fetal cholesterol accrued in the fetal rat (3, 5), 3) the inconsistent rate of transfer of radiolabeled cholesterol from the maternal circulation to the fetus (51-56), and 4) the fact that the fetus does not come in direct contact with the maternal circulation. Recent data does support a role for exogenous cholesterol in fetal development, however. First, a drug-induced SLOS-like syndrome in rodents can be reversed with an increase in maternal plasma cholesterol (36, 47, 57). Second, apoAI-null fetuses within dams of a similar genotype are smaller and have a lower cholesterol content than do fetuses expressing apoAI within like dams (58). This effect is not directly due to the apoAI deficiency within the fetus itself, but rather to the low plasma HDL-cholesterol concentration within the maternal circulation. Third, fetuses of hypercholesterolemic mothers have a greater accumulation of lipid in the aorta, implying that maternal cholesterol is crossing into the fetus and changing metabolism within the fetal tissues (59).

The fetus is unique from other tissues because, if it does obtain exogenous cholesterol, it does so from the two extra-embryonic fetal tissues that form a barrier between the fetus and the maternal circulation, the yolk sac and the placenta. A variety of essential nutrients, such as oxygen and glucose, must reach the fetus through various transport processes. Since some nutrients pass through the yolk sac and placenta and some do not, these tissues are essentially the gatekeepers for the fetus. To fully understand how the yolk sac and placenta might transport cholesterol to fetal vessels, a basic understanding of the physiology and anatomy of these tissues is necessary.

The yolk sac is formed first during gestation and is derived from the inner cell mass of the blastocyst. The

endoderm cells of the yolk sac of most species, including the rodent, human and avian, are polarized with an apical and a basolateral side. Rodents have an inverted yolk sac whereas humans and birds do not. The yolk sac can take up numerous maternal constituents from the yolk sac cavity via a variety of transport processes. Some of these constituents can cross the endoderm cells and can be secreted into the vitelline vessels leading to the fetus (reviewed in (60, 61)). Shortly after the formation of the yolk sac, the trophoblasts coalesce to form the placenta. The human syncytiotrophoblasts and the outer layer of trophoblasts of rodents are bathed in maternal blood and tissue secretions and also can take up a variety of maternal constituents. Some of these maternal-derived components can also cross the trophoblasts and be secreted into the umbilical vein leading to the fetus (reviewed in (62, 63)).

Assuming exogenous cholesterol does enter the fetus, it will originate from cholesterol synthesized within the yolk sac and/or placenta or from cholesterol within the maternal circulation. Thus, for exogenous cholesterol to have an impact upon fetal development, a mechanism must be present by which the yolk sac and/or placenta can take up cholesterol from the maternal circulation, transport lipids across the cells and/or secrete the maternal-derived or newly synthesized sterol to the vessels leading to fetus. Numerous data does exist that demonstrate the yolk sac and placenta take up circulating maternal cholesterol in the form of LDL and HDL (3, 60, 64-67). The lipoproteins are taken up by receptor-mediated and receptor-independent transport processes (64). The lipoprotein receptors expressed by these tissues are numerous and include, the LDL receptor (64, 68), scavenger receptor, class B, type I (SR-BI) (64, 69), the VLDL receptor (70), the apoE receptor 2 (71) the acetylated LDL receptor (67), megalin (72, 73) and cubulin (gp280) (74-76).

In the yolk sac, the maternal-derived lipoprotein-cholesterol or newly synthesized cholesterol is potentially transported to the basolateral side of the cells by being incorporated into lipoproteins formed within the endoderm cells (77-79). Various factors can affect the lipoprotein formation in the endoderm cells, including exogenous fatty acid concentration (80) and age (80, 81). Triglyceride and cholesterol concentration may also affect lipoprotein formation, assuming the regulation of lipoprotein formation in these cells is similar to that in hepatocytes (82, 83) (Xie, Woollett and Dietschy, unpublished data). Thus, an excess of maternal-derived cholesterol in the yolk sac, as seen in dams with high plasma cholesterol concentrations (McConihay and Woollett, unpublished data), could either be incorporated into the lipoproteins themselves and/or stimulate lipoprotein secretion as a result of a change in tissue sterol concentration.

The transport of cholesterol across trophoblasts is not presently defined. However, recent observations have allowed for speculation of the processes involved in this transport. In one study, lipid droplets, possibly including cholesterol, were found to be secreted into the umbilical vein (84). Another recent observation which may help delineate the role of the placenta in the accretion of fetal cholesterol was that mRNA levels for the ATP binding cassette transporter 1 (ABC-A1), a protein involved in cholesterol transport (85-87),

were very high (88). When this transporter was deleted from tissues by a targeted mutation, a portion of the fetuses lacking functional ABC-AI died in utero (89). These data lead to the conclusion that this transporter may aide in the efflux of cholesterol from trophoblasts to various acceptors in the fetal circulation. Thus, mechanisms may be present in both the endoderm cells and trophoblasts by which maternal plasma cholesterol and/or cholesterol synthesized de novo within the extra-embryonic fetal tissues can cross the tissues and be secreted into vessels leading to the fetus.

Similar to what is found in animals and humans with abnormal sterol synthesis, a significant disruption in the uptake or transport of maternal lipid across the extra-embryonic fetal tissues can result in severe fetal abnormalities in the rodent. At the present time, it is not possible to distinguish if the defects are due to a lack of cholesterol or other lipids, such as Vitamin E. There appear to be three distinct types of exogenous sterol or lipid deficiency. First, when lipoprotein formation by the yolk sac is halted due to a truncated form of apoB, absent apoB or absent microsomal transfer protein, abnormal brain development occurs early in gestation (90-92). Cholesterol is implicated as a contributing factor for this defect since the cholesterol concentrations of fetuses of apoB knockout dams are less than those of fetuses of control dams (78). In addition, these fetal rodents are phenotypically similar to those found in animals with low sterol synthesis rates. It should be noted that the human fetus successfully develops without apoB formation in the yolk sac as demonstrated by abetalipoproteinemia (93). The yolk sac does make other apolipoproteins which may account for this discrepancy (79). Second, when various lipoprotein receptors on the endoderm cells of the yolk sac are depleted, embryonic lethality and/or HPE develops. The receptors that are fundamentally important for normal fetal development include megalin, the lipoprotein receptor-related protein (LRP), SR-BI, and cubulin (gp280) (74, 94-96). A decrease in cholesterol uptake by the endoderm cells could have a two-fold effect. 1) If less cholesterol is taken up by the endoderm cells, the concentration of cholesterol within the cell will be lower (58). A lower cholesterol concentration could lead to fewer lipoproteins being synthesized and less lipid being presented to the fetus. 2) If less cholesterol is taken up by the endoderm cells, then less cholesterol will be secreted into the vitelline vessels leading to the fetus. The lack of sterol uptake would have to be significant since a marginal reduction can be compensated for, at least partially, by an increase in sterol synthesis rates within the endoderm cells themselves (58). Another possibility is that the fetuses themselves require expression of these lipoprotein receptors for normal development. The third type of exogenous lipid deficiency is due to a disruption in normal placentation. The placenta secretes lipids, possibly including cholesterol, into the umbilical vein. Inhibition of lipid droplet formation results in fetal lethality (84).

2.3. Modification of Sonic hedgehog by cholesterol in the fetus

A renewed interest in the role of cholesterol in fetal development has occurred during recent years primarily as a result of two interrelated findings. First, Sonic hedgehog (Shh), one of the signaling molecules

involved in spatial patterning of the forebrain, requires covalent attachment of cholesterol to induce autoproteolysis and activation (1, 97). Second, the drug-induced development of severe craniofacial defects can be halted by increasing maternal cholesterol early in gestation (36, 47, 57).

Shh is a member of a family of secreted signaling molecules involved in cellular differentiation during development (reviewed in (98, 99)). It is found in numerous embryonic structures, including the notochord, the floor plate of the neural tube and the zone of polarizing activity (reviewed in (100, 101)). Shh is synthesized as an inactive protein which is activated after autoprocessing, including attachment of cholesterol and cleavage into a catalytic activity-containing C-terminus and a receptor-binding N-terminus (1). Once cleavage occurs, the cholesterol remains bound to the N-terminus or the signaling domain (1); palmitate is also found bound to this domain (102). It has been theorized that the lipids linked to Shh are involved in the tissue and spatial distribution of the protein (reviewed in (100, 101)). Many studies examining the role of the hedgehog family of genes, including Shh, have been completed with the Drosophila hedgehog protein (Hh). The receptor for Hh and Shh is Patched (Ptc) (103, 104), a transmembrane protein with a sterol sensing region similar to that found in other proteins involved in cellular sterol homeostasis (105). Patched appears to repress signaling by way of Smoothened (Smo). Once Hh is bound to Ptc, the inhibitory effect on Smo is released and the major transcriptional factors of Hh are activated, such as the Gli/Ci family of DNA-binding proteins (reviewed in (100, 106)). Hh and Shh are pivotal for normal development in Drosophila and vertebrates, respectively, and mutations in Shh lead to severe malformations in the forebrain in both rodents and humans (97, 107).

As might be expected, rats given drugs which inhibit sterol synthesis rates have lower cholesterol concentrations and lower levels of Shh (108). In addition, the alkaloids that lead to cyclops formation in pregnant ewes also inhibit the Shh signaling (44, 109, 110). It appears that the alkaloids may also be affecting how the target tissue which contains the sterol-sensitive patched responds to the binding of Shh (44). A lack of Shh activity has been implied in SLOS fetuses since this population of individuals has a lower tissue cholesterol concentration (30) and a higher occurrence of HPE than the general population (111). Thus, the ability to increase fetal cholesterol concentration at a time when the forebrain is beginning to develop and Shh is most active could have a significant impact on the outcome of some pregnancies.

3. FETAL FATTY ACIDS

3.1 Endogenous sources of fatty acids

The fetus has significant fatty acid synthesis rates even though it obtains a significant amount of its fatty acids from the maternal circulation (112, 113) (Schmid and Woollett, unpublished data). As with cholesterol, the regulability of these elevated synthesis rates fetus is unknown. In the adult, fatty acid synthesis rates are regulated by various factors and hormones (16, 114, 115), including SREBP-1c and the peroxisomal proliferating activator receptor (PPAR). The factors affecting SREBP-1c expression were discussed in a previous section. There are three types of PPARs that belong to the nuclear receptor superfamily, each with a different tissue expression profile (alpha, delta, and gamma) (116). Upon activation by fatty acids, fibrates, eicosanoids and thiazolidinediones, the PPARs form a heterodimer with the retinoic acid receptor (RXR) and affect transcription of various genes involved in the maintenance of lipid metabolism (reviewed in (115, 117)). PPAR alpha and gamma affect fatty acid synthesis differently in various tissues, whereas the role of PPAR delta in fatty acid homeostasis is unclear at the present time (reviewed in (115, 117)). Interestingly, PPAR delta mRNA is expressed first in the fetal mouse by day 9.5 of gestation and is the only PPAR expressed until late into gestation (118). Thus, the regulation of fatty acid synthesis may be different in the fetus and the adult due to the sole presence of PPAR delta and the as yet undefined regulation of SREBPs in the fetus (112).

3.2. Exogenous sources of fatty acids

Exogenous fatty acids readily cross into the fetal circulation via both the placenta and yolk sac. The yolk sac takes up fatty acids in the form of lipoprotein-triglyceride from the maternal circulation (64). The triglycerides are hydrolyzed within the lysosomes (reviewed in (60)) and, if the endoderm cells synthesize lipoproteins as hepatocytes do, the maternal or newly synthesized fatty acids are packaged and secreted into the vitelline vessels as lipoproteins (83). In the placenta, fatty acids either are taken up as circulating maternal fatty acids via one of several fatty acid binding proteins expressed on the maternal surface of the trophoblasts, or are taken up after triglyceride hydrolysis on the cell surface by lipoprotein lipase or endothelial lipase (119). The maternal and newly synthesized fatty acids are then transported across the cell by fatty acid binding proteins and secreted into the umbilical vein leading to the fetus (120). The maternal exogenous fatty acids are necessary since about 50% of the fatty acids within the developing brain consist of the long chain, maternal-derived PUFA (121). Interestingly, it appears that these fatty acids which are critical to neurological development are preferentially taken up by the trophoblasts, transported across the cells and secreted into the umbilical vein (120, 122).

3.3. Fatty acid oxidation

In the adult, a major source of energy is derived from oxidation of fatty acids. However, in the fetal tissues, glucose oxidation seems to be a primary source of energy (123). Some fatty acid oxidation does occur in the fetus, however, possibly as a source of acetyl CoA units to be used for cholesterol synthesis within the brain (124). The non-oxidized fatty acids are stored in the fetus to be oxidized for energy perinatally (125).

4. SUMMARY

Lipids are essential to the fetus and can originate from exogenous and endogenous sources. A change in

concentration or composition of the lipids obtained by the fetus from either source could have a dramatic impact on the outcome of certain high-risk pregnancies. For example, the ability to increase cholesterol synthesis rates or cholesterol uptake from the maternal circulation very early in gestation when the neural crest is just beginning to form could enhance Shh activity and improve forebrain development. In addition, dietary PUFA could dramatically change lipid metabolism within the fetus via SREBP and PPAR, depending on how the fetus responds to known stimulants of these metabolic regulators. The use of this information could be critical in the prevention of congenital defects or spontaneous abortions in women with a known predisposition for these defects, or in treatment of small-for-age fetuses. Even though there are numerous factors that lead to some of the congenital defects, including HPE, the number of fetuses that could be affected are significant since 1:250 concepti have HPE (126). However, more data needs to be collected before recommendations can be made.

5. REFERENCES

1. Porter, JA, KE Young and PA Beachy: Cholesterol modification of hedgehog signaling proteins in animal development. *Science* 274, 255-259 (1996)

2. Ingham, PW: Signalling by hedgehog family proteins in *Drosophila* and vertebrate development. *Curr Opin Genet Dev* 5, 492-498 (1995)

3. Belknap, WM and JM Dietschy: Sterol synthesis and low density lipoprotein clearance in vivo in the pregnant rat, placenta, and fetus. Sources for tissue cholesterol during fetal development. *J Clin Invest* 82, 2077-2085 (1988)

4. Woollett, LA: Origin of cholesterol in the fetal Golden Syrian hamster: contribution of de novo sterol synthesis and maternal-derived lipoprotein cholesterol. *J Lipid Res* 37, 1246-1257 (1996)

5. Jurevics, HA, FZ Kidwai and P Morell: Sources of cholesterol during development of the rat fetus and fetal organs. *J Lipid Res* 38, 723-733 (1997)

6. Carr, BR and ER Simpson: Cholesterol synthesis in human fetal tissues. *J Clin Endocrinol Metab* 55, 447-452 (1982)

7. Dietschy, JM, T Kita, KE Suckling, JL Goldstein and MS Brown: Cholesterol synthesis in vivo and in vitro in the WHHL rabbit, an animal with defective low density lipoprotein receptors. *J Lipid Res* 24, 469-480 (1983)

8. Haave, NC and SM Innis: Perinatal development of hepatic cholesterol synthesis in the rat. *Biochim Biophys Acta* 1985, 35-44 (1991)

9. Smith, JL, SR Lear and SK Erickson: Developmental expression of elements of hepatic cholesterol metabolism in the rat. *J Lipid Res* 36, 641-652 (1995)

10. Yount, NY and DJ McNamara: Dietary regulation of maternal and fetal cholesterol metabolism in the guinea pig. *Biochim Biophys Acta* 1085, 82-90 (1991)

11. Cavender, CP, SD Turley and JM Dietschy: Sterol metabolism in fetal, newborn, and suckled lambs and their response to cholesterol after weaning. *Am J Physiol* 269, E331-E340 (1995)

12. Turley, SD, DK Burns, CR Rosenfeld and JM Dietschy: Brain does not utilize low density lipoprotein-cholesterol during fetal and neonatal development in the sheep. *J Lipid Res* 37, 1953-1961 (1996)

13. Turley, SD, DK Burns and JM Dietschy: Preferential utilization of newly synthesized cholesterol for brain growth in neonatal lambs. *Am J Physiol* 274, E1099-E1105 (1998)

14. Brown, MS and JS Goldstein: The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 89, 331-340 (1997)

15. Brown, MS and JL Goldstein: A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc Natl Acad Sci USA* 96, 11041-11048 (1999)

16. Horton, JD and I Shimomura: Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis. *Curr Opin Lipidol* 10, 143-150 (1999)

17. Shimomura, I, H Shimano, JD Horton, JL Goldstein and MS Brown: Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J Clin Invest* 99, 838-345 (1997)

18. Sakai, J, A Nohturfft, D Cheng, YK Ho, MS Brown and JL Goldstein: Identification of complexes between the COOH-terminal domains of sterol regulatory element binding proteins (SREBPs) and SREBP cleavage-activating protein (SCAP) *J Biol Chem* 272, 20213-20221 (1997)

19. Sakai, J, A Nohturfft, JL Goldstein and MS Brown: Cleavage of sterol regulatory element binding proteins (SREBPs) at site-1 requires interaction with SREBP cleavage-activating protein. Evidence from *in vivo* competition studies. *J Biol Chem* 273, 5785-5793 (1998)

20. Horton, JD, I Shimomura, MS Brown, RE Hammer, JL Goldstein and H Shimano: Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. *J Clin Invest* 101, 2331-2339 (1998)

21. Shimano, H, JD Horton, RE Hammer, I Shimomura, MS Brown and JL Goldstein: Overproduction of cholesterol and fatty acids causes massive liver

enlargement in transgenic mice expressing truncated SREBP-1a. *J Clin Invest* 98, 1575-1584 (1996)

22. Horton, JD, Y Bashmakov, I Shimomura and H Shimano: Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci USA* 95, 5987-5992 (1998)

23. Nohturfft, A, D Yabe, JL Goldstein, MS Brown and PJ Espenshade: Regulated step in cholesterol feedback localized to budding of SCAP from ER membranes. *Cell* 102, 315-323 (2000)

24. Nohturfft, A, RA DeBose-Boyd, S Scheek, JL Goldstein and MS Brown: Sterols regulate cycling of SREBP cleavage-activating protein (SCAP) between endoplasmic reticulum and Golgi. *Proc Natl Acad Sci USA* 96, 11235-11240 (1999)

25. Yahagi, N, H Shimano, AH Hasty, M Amemiya-Kudo, H Okazaki, Y Tamura, Y Iizuka, F Shionoiri, K Ohashi, J Lsuga, K Harada, T Gotoda, R Nagai, S Ishibashi and N Yamada: A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. *J Biol Chem* 274, 35840-35844 (1999)

26. Sheng, Z, H Otani, M Brown and JL Goldstein: Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. *Proc Natl Acad Sci USA* 92, 935-938 (1995)

27. Shimano, H, I Shimomura, RE Hammer, J Herz, JL Goldstein, MS Brown and JD Horton: Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. *J Clin Invest* 100, 2115-2124 (1997)

28. Diomede, L, M Salmona, D Albani, M Bianchi, A Bruno, S Salmona and U Nicolini: Alteration of SREBP activation in liver of trisomy 21 fetuses. *Biochem Biophys Res Comm* 499-503 (1999)

29. Tozawa, R, S Ishibashi, J Osuga, H Yagyu, T Oka, Z Chen, D Ohashi, S Perrey, F Shionoiri, N Yahagi, K Harada, T Gotoda, Y Yazaki and N Yamada: Embryonic lethality and defective neural tube closure in mice lacking squalene synthase. *J Biol Chem* 274, 30843-30848 (1999)

30. Tint, GS, M Irons, ER Elias, AK Batta, R Frieden, TS Chen and G Salen: Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. *N Eng J Med* 330, 107-113 (1994)

31. Lowry, RB and S-L Young: Borderline intelligence in the Smith-Lemli-Opitz syndrome. *Am J Med Genet* 5, 137-143 (1980)

32. Cuniff, C, LE Kratz, A Moser, MR Natowicz and RI Kelley: Clinical and biochemical spectrum of patients with RSH/Smith-Lemli-Opitz syndrome and abnormal cholesterol metabolism. *Am J Med Genet* 68, 263-269 (1997)

33. Opitz, JM. The RSH syndrome: paradigmatic metabolic malformation syndrome? In: Diagnosis and treatment of the unborn child. Ed: New, MI., Idelson-Gnocchi, Reddick, FL, 45-55 (1998)

34. Smith, DW, L Lemli and JM Opitz: A newly recognized syndrome of multiple congenital anomalies. *J Pediatr* 64, 210-217 (1964)

35. Salen, G, S Shefer, AK Batta, GS Tint, G Xu, A Honda, M Irons and ER Elias: Abnormal cholesterol biosynthesis in the S.mith-Lemli-Opitz syndrome. *J Lipid Res* 37, 1169-1180 (1996)

36. Gaoua, W, C Wolf, F Chevy, F Ilien and C Roux: Cholesterol deficit but not accumulation of aberrant sterols is the major cause of the teratogenic activity in the Smith-Lemli-Opitz syndrome animal model. *J Lipid Res* 41, 637-646 (2000)

37. Houten, SM, GJ Romeijn, J Koster, RGF Gray, P Darbyshire and GPA Smit: Identification and characterization of three novel missense mutations in mevalonate kinase cDNA causing mevalonic aciduria, a disorder of isoprene biosynthesis. *Hum Mol Gen* 8, 1523-1528 (1999)

38. Clayton, P, K Mills, J Keeling and F K.: Desmosterolosis: a new inborn error of cholesterol biosynthesis. *Lancet* 348, 404 (1996)

39. FitzPatrick, DR, JW Keeling, MJ Evans, AE Kan, JE Bell, ME Porteous, K Mills, RM Winter and PT Clayton: Clinical phenotype of desmosterolosis. *Am J Med Genet* 75, 145-152 (1998)

40. Happle, R: X-linked dominant chondrodysplasia punctata. Review of literature and report of a case. *Hum Gen* 53, 66-73 (1979)

41. Happle, R: X-linked dominant chondrodysplasia punctata/ichtyosis/cataract syndrome in males. *Am J Med Genet* 57, 493 (1995)

42. Keeler, RF and W Binns: Teratogenic compounds of Veratrum californicum (Durand) II. Production of ovine fetal cyclopia by fractions and alkaloid preparations. *Can J Biochem* 44, 829-838 (1966)

43. Keeler, RF and W Binns: Teratogenic compounds of Veratrum californicum (Durand) V. Comparison of cyclopian effects of steroidal alkaloids from the plant and structurally related compounds from other sources. *Teratology* 1, 5-10 (1968)

44. Cooper, MK, JA Porter, KE Young and PA Beachy: Teratogen-mediated inhibition of target tissue response to *Shh* signaling. *Science* 280, 1603-1607 (1998)

45. Keeler, RF: Cyclopamine and related steroidal alkaloid teratogens: Their occurrence, structural relationship, and biologic effects. *Lipids* 13, 708-715 (1978)

46. Barbu, V, C Roux, R Dupuis, J Gardette and JC Maziere: Teratogenic effect of AY 9944 in rats: Importance of the day of administration and maternal plasma cholesterol level. *Proc Soc Exper Biol Med* 176, 54-59 (1984)

47. Roux, C, C Horvath and R Dupuis: Teratogenic action and embryo lethality of AY9944R: Prevention by a hypercholesterolemia-provoking diet. *Teratology* 19, 35-38 (1979)

48. Kolf-Clauw, M, F Chevy, B Siliart, C Wolf, N Mulliez and C Roux: Cholesterol biosynthesis inhibited by BM15.766 induces holoprosencephaly in the rat. *Teratology* 56, 188-200 (1997)

49. Yoshikawa, H: Effects of drugs on cholesterol esterification in normal and Niemann-Pick type C fibroblasts: AY-9944, other cationic amphiphilic drugs and DMSO. *Brain Dev* 13, 115-120 (1991)

50. Minsker, DH, JS MacDonald, RT Robertson and DL Bokelman: Mevalonate supplementation in pregnant rats suppresses the teratogenicity of mevinolinic acid, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme a reductase. *Teratology* 28, 449-456 (1983)

51. Lin, DS, RM Pitkin and WE Connor: Placental transfer of cholesterol into the human fetus. *Am J Obstet Gynecol* 128, 735-739 (1977)

52. Connor, WE and DS Lin: Placental transfer of cholesterol-4-^{14}C into the rabbit and guinea pig fetus. *J Lipid Res* 8, 558-564 (1967)

53. Pitkin, RM, WE Connor and DS Lin: Cholesterol metabolism and placental transfer in the pregnant rhesus monkey. *J Clin Invest* 51, 2584-2592 (1972)

54. Plotz, EJ, JJ Kabara, ME Davis, GV LeRoy and RG Gould: Studies on the synthesis of cholesterol in the brain of the human fetus. *Am J Obstet Gynecol* 101., 534-538 (1968)

55. Goldwater, WH and D Stetten, Jr.: Studies in fetal metabolism. *J Biol Chem* 169, 723-738 (1947)

56. Chevallier, F: Transferts et synthese du cholesterol chez le rat au cours de sa croissance. *Biochim Biophys Acta* 84, 316-339 (1964)

57. Lanoue, L, DB Dehart, ME Hinsdale, N Maeda, GS Tint and KK Sulik: Limb, genital, CNS, and facial malformations result from gene/environment-induced cholesterol deficiency: Further evidence for a link to sonic hedgehog. *Am J Med Genet* 73, 24-31 (1997)

58. McConihay, JA, AM Honkomp, NA Granholm and LA Woollett: Maternal high density lipoproteins affect fetal mass and extra-embryonic fetal tissue sterol metabolism in the mouse. *J Lipid Res* 41, 424-432 (2000)

59. Napoli, C, FP D'Armiento, FP Mancini, A Postiglione, JL Wiztum, G Palumbo and W Palinski: Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of LDL and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest* 100, 2680-2690 (1997)

60. Jollie, WP: Development, morphology, and function of the yolk-sac placenta of laboratory rodents. *Teratology* 41, 361-381 (1990)

61. Mossman, HW. Vertebrate fetal membranes: comparative ontogeny and morphology, evolution, phylogenetic significance, basic functions, research opportunities, Rutgers University Press, New Brunswick (1987)

62. Ahokas, RA and GD Anderson. The placenta as an organ of nutrition. In: The human placenta. Clinical perspectives. Ed: Lavery, J, Aspen Publishers, Inc., Rockville, MD, 207-220 (1987)

63. Tropper, PJ and RH Petrie. Placental exchange. In: The human placenta. Clinical Perspectives. Ed: Lavery, J, Aspen Publishers, Inc, Rockville, MD, 199-206 (1987)

64. Wyne, KL and LA Woollett: Transport of maternal LDL and HDL to the fetal membranes and placenta of the Golden Syrian hamster is mediated by receptor-dependent and receptor-independent processes. *J Lipid Res* 39, 518-530 (1998)

65. Winkel, CA, JM Snyder, PC MacDonald and ER Simpson: Regulation of cholesterol and progesterone synthesis in human placental cells in culture by serum lipoproteins. *Endocrinology* 106, 1054-1060 (1980)

66. Winkel, CA, PC MacDonald and ER Simpson: The role of receptor-mediated low-density lipoprotein uptake and degradation in the regulation of progesterone biosynthesis and cholesterol metabolism by human trophoblasts. *Placenta* 3 (suppl), 133-143 (1981)

67. Alsat, E, F Mondon, R Rebourcet, M Berthelier, D Erlich, L Cedard and S Goldstein: Identification of specific binding sites for acetylated low density lipoprotein in microvillous membranes from human placenta. *Mol Cell Endocrinol* 41, 229-235 (1985)

68. Shi, W, KF Swan, SR Lear, JS O'Neil, SK Erickson and MC Henson: Regulation of pathways determining cholesterol availability in the baboon placenta with advancing gestation. *Biol Reprod* 61, 1499-1505 (1999)

69. Hatzopoulos, AK, A Rigotti, RD Rosenberg and M Krieger: Temporal and spatial pattern of expression of the HDL receptor SR-BI during murine embryogenesis. *J Lipid Res* 39, 495-508 (1998)

70. Gafvels, ME, LG Paavola, CO Boyd, PM Nolan, F Wittmaack, A Chawla, MA Lazar, M Bucan, B Angelin and JF Strauss, III.: Cloning of a complementary deoxyribonucleic acid encoding the murine homolog of the very low density lipoprotein/apolipoprotein-E receptor: expression pattern and assignment of the gene to mouse chromosome 19. *Endocrinology* 135, 387-394 (1994)

71. Kim, DH, H Iijima, K Goto, J Lakai, H Ishii, HJ Kim, H Suzuki, H Kondo, S Saeki and T Yamamoto: Human apolipoprotein E receptor 2. A novel lipoprotein receptor of the low density lipoprotein receptor family predominantly expressed in brain. *J Biol Chem* 271, 8373-8380 (1996)

72. Zheng, G, DR Bachinsky, I Stamenkovic, DK Strickland, D Brown, G Andres and RT McCluskey: Organ distribution in rats of two members of the low-density lipoprotein receptor gene family, gp330 and LRP/2MR, and the receptor-associated protein (RAP) *J Histochem Cytochem* 42, 531-542 (1994)

73. Stefansson, S, DA Chappell, KM Argraves, DK Strickland and WS Argraves: Glycoprotein 330/low density lipoprotein receptor-related protein-2 mediates endocytosis of low density lipoproteins via interaction with apolipoprotein B-100. *J Biol Chem* 270, 19417-19421 (1995)

74. Sahali, D, N Mulliez, F Chatelet, R Dupuis, P Ronco and P Verroust: Characterization of a 280-kD protein restricted to the coated pits of the renal brush border and the epithelial cells of the yolk sac. Teratogenic effect of the specific monoclonal antibodies. *J Exp Med* 167, 213-218 (1988)

75. Hammad, SM, S Stefansson, WO Twal, CJ Drake, P Fleming, A Remaley, HB Brewer Jr. and WS Argraves: Cubulin, the endocytic receptor for intrinsic factor-vitamin B12 complex, mediates high-density lipoprotein holoparticle endocytosis. *Proc Natl Acad Sci USA* 96, 10158-10163 (1999)

76. Kozyraki, R, J Fyfe, M Kristiansen, C Gerdes, C Jacobsen, S Cui, EI Christensen, M Aminoff, A de la Chapelle, R Krahe, PJ Verroust and SK Moestrup: The intrinsic factor-vitamin B_{12} receptor, cubulin, is a high-affinity apolipoprotein A-I receptor facilitating endocytosis of high-density lipoprotein. *Nature Med* 5, 656-661 (1999)

77. Plonne, D, L Winkler, H Franke and R Dargel: The visceral yolk sac - an important site of synthesis and secretion of apolipoprotein B containing lipoproteins in the feto-placental unit of the rat. *Biochim Biophys Acta* 1127, 174-185 (1992)

78. Farese, RV, Jr., S Cases, SL Ruland, HJ Kayden, JS Wong, SG Young and RL Hamilton: A novel function for apolipoprotein B: lipoprotein synthesis in the yolk sac is critical for maternal-fetal lipid transport in mice. *J Lipid Res* 37, 347-360 (1996)

79. Shi, W-K, B Hopkins, S Thompson, JK Heath, BM Luke and CF Graham: Synthesis of apolipoproteins, alphafoetoprotein, albumin, and transferrin by the human foetal yolk sack and other foetal organs. *J Embryol Exp Morphol* 85, 191-206 (1985)

80. Plonne, D, A Stacke, KU Weber, U Endisch and R Dargel: The pattern of apolipoprotein B100 containing lipoprotein subclasses produced by the isolated visceral rat yolk sac depends on developmental stage and fatty acid availability. *Biochim Biophys Acta* 1299, 54-66 (1996)

81. Terasawa, Y, SJ Cases, JS Wong, H Jamil, S Jothi, MG Traber, L Packer, DA Gordon, RL Hamilton and RV Farese, Jr.: Apolipoprotein B-related gene expression and ultrastructural characteristics of lipoprotein secretion in mouse yolk sac during embryonic development. *J Lipid Res* 40, 1967-1977 (1999)

82. Mason, TM: The role of factors that regulate the synthesis and secretion of very-low-density lipoprotein by hepatocytes. *Crit Rev Clin Lab Sci* 35, 461-487 (1998)

83. Dixon, JL and HN Ginsberg: Regulation of hepatic secretion of apolipoprotein B-containing lipoproteins: information obtained from cultured liver cells. *J Lipid Res* 34, 167-179 (1992)

84. Barak, Y, MC Nelson, ES Ong, YZ Jones, P Ruiz-Lozano, KR Chien, A Koder and RM Evans: PPAR gamma is required for placental, cardiac and adipose tissue development. *Mol Cell* 4, 585-595 (1999)

85. Bodzioch, M, E Orso, J Klucken, T Langmann, A Bottcher, W Diederich, W Drobnik, S Barlage, C Buchler, M Porsch-Ozcurumez, WE Kaminski, HW Hahmann, K Lette, G Rothe, C Aslanidis, KJ Lackner and G Schmitz: The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nature Genet* 22, 347-351 (1999)

86. Brooks-Wilson, A, M Marcil, SM Clee, LH Zhang, K Roomp, M van Dam, L Yu, C Brewer, JA Collins, HOF Molhuizen, O Loubser, BFF Ouelette, K Fichter, KJD Ashbourne-Excoffon, CW Sensen, S Scherer, S Mott, M Denis, D Martindale, J Frohlich, K Morgan, B Kiip, S Pinstone, JJP Kastelein, J Genest Jr. and MR Hayden: Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nature Genet* 22, 336-345 (1999)

87. Rust, S, M Rosier, H Funke, J Real, Z Amoura, JC Piette, JF Deleuze, HB Brewer, N Duverger, P Denefle and G Assmann: Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nature Genet* 22, 352-355 (1999)

88. Langmann, T, J Klucken, M Reil, G Liebisch, MF Luciani, G Chimini, WE Kaminski and G Schmitz: Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): Evidence for sterol-dependent

regulation in macrophages. *Biochem Biophys Res Commun* 257, 29-33 (1999)

89. Christiansen-Weber, TA, JR Voland, Y Wu, K Ngo, BL Roland, S Nhuyen, PA Peterson and W-P Fung-Leung: Functional loss of *ABCA1* in mice causes severe placental malformation, aberrant lipid distribution, and kidney glomerulonephritis as well as high-density lipoprotein cholesterol deficiency. *Am J Path* 157, 1017-1029 (2000)

90. Raabe, M, LM Flynn, CH Zlot, JS Wong, MM Veniant, RL Hamilton and SG Young: Knockout of the abetalipoproteinemia gene in mice: Reduced lipoprotein secretion in heterozygotes and embryonic lethality in homozygotes. *Proc Natl Acad Sci USA* 95, 8686-8691 (1998)

91. Farese, RV, Jr. , SL Ruland, LM Flynn, RP Stokowski and SG Young: Knockout of the mouse apolipoprotein B gene results in embryonic lethality in homozygotes and protection against diet-induced hypercholesterolemia in heterozygotes. *Proc Natl Acad Sci USA* 92, 1774-1778 (1995)

92. Homanics, GE, TJ Smith, SH Zhang, D Lee, SG Young and N Maeda: Targeted modification of the apolipoprotein B gene results in hypobetalipoproteinemia and developmental abnormalities in mice. *Proc Natl Acad Sci USA* 90, 2389-2393 (1993)

93. Gregg, RE and JR Wetterau: The molecular basis of abetalipoproteinemia. *Curr Opin Lipidol* 5, 81-86 (1994)

94. Herz, J, DE Clouthier and RE Hammer: LDL receptor-related protein internalizes and degrades uPA-PAI-1 complexes and is essential for embryo implantation. *Cell* 71, 411-421 (1992)

95. Willnow, TE, J Hilpert, SA Armstrong, A Rohlmann, RE Hammer, DK Burns and J Herz: Defective forebrain development in mice lacking gp330/megalin. *Proc Natl Acad Sci USA* 93, 8460-8464 (1996)

96. Rigotti, A, BL Trigatti, M Penman, H Rayburn, J Herz and M Krieger: A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc Natl Acad Sci USA* 94, 12610-12615 (1997)

97. Chiang, C, Y Litingtung, E Lee, KE Young, JL Corden, H Wesphal and PA Beachy: Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* 383, 407-413 (1996)

98. Hammerschmidt, M, A Brook and AP McMahon: The world according to hedgehog. *Trends Genet* 13, 14-21 (1997)

99. Goodrich, LV and MP Scott: Hedgehog and patched in neural development and disease. *Neuron* 21, 1243-1257 (1998)

100. Rubenstein, JLR and PA Beachy: Patterning of the embryonic forebrain. *Curr Opin Neurobiol* 8, 18-26 (1998)

101. Muenke, M and PA Beachy: Genetics of ventral forebrain development and holoprosencephaly. *Curr Opin Genet Dev* 10, 262-269 (2000)

102. Pepinsky, RB, C Zeng, D Wen, P Rayhorn, DP Baker, KP Williams, SA Bixler, CM Ambrose, EA Garber, K Miatkowski, FR Taylor, EA Wang and A Galdes: Identification of a palmitic acid-modified form of human Sonic hedgehog. *J Biol Chem* 273, 14037-14045 (1998)

103. Chen, Y and G Struhl: Dual roles for Patched in sequestering and transducing Hedgehog. *Cell* 87, 553-563 (1996)

104. Stone, DM, M Hynes, M Armanini, TA Swanson, Q Gu, RL Johnson, MP Scott, D Pennica, A Goddard, H Phillips, M Noll, JE Hooper, F de Sauvage and A Rosenthal: The tumour-suppressor gene *patched* encodes a candidate receptor for Sonic hedgehog. *Nature* 384, 129-134 (1996)

105. Osborne, TF and JM Rosenfeld: Related membrane domains in proteins of sterol sensing and cell signaling provide a glimpse of treasures still buried within the dynamic realm of intracellular metabolic regulation. *Curr Opin Lipidol* 9, 137-140 (1998)

106. Weed, M, S Mundlos and BR Olsen: The role of sonic hedgehog in vertebrate development. *Matrix Biol* 16, 53-58 (1997)

107. Roessler, E, E Belloni, K Gaudenz, P Jay, P Berta, SW Scherer, LC Tsui and M Muenke: Mutations in the human *Sonic Hedgehog* gene cause holoprosencephaly. *Nature Genet* 14, 357-360 (1996)

108. Gofflot, F, M Kolf-Clauw, F Clotman, C Roux and JJ Picard: Absence of ventral cell populations in the developing brain in a rat model of the Smith-Lemli-Opitz syndrome. *Am J Med Genet* 87, 207-216 (1999)

109. Incardona, JP, W Gaffield, RJ Kapur and H Roelink: The teratogenic *Veratrum* alkaloid cyclopamine inhibits Sonic hedgehog signal transduction. *Development* 125, 3553-3562 (1998)

110. Guy, RK: Inhibition of sonic hedgehog autoprocessing in cultured mammalian cells by sterol deprivation. *Proc Natl Acad Sci USA* 97, 7307-7312 (2000)

111. Kelley, RL, E Roessler, RC Hennekam, GL Feldman, K Kosaki, MC Jones, JC Palumbos and M Muenke: Holoprosencephaly in RSH/Smith-Lemli-Opitz syndrome: Does abnormal cholesterol metabolism affect the function of *Sonic Hedgehog*? *Am J Med Genet* 66, 478-484 (1996)

112. Miguel, SG and S Abraham: Effect of maternal diet on fetal hepatic lipogenesis. *Biochim Biophys Acta* 424, 213-234 (1976)

113. Robertson, AF and H Sprecher: A review of placental lipid metabolism and transport. *Acta Paediatr Scand* 183, 3-18 (1968)

114. Wolf, G: Nutritional and hormonal regulation of fatty acid synthase. *Nutr Rev* 54, 122-123 (1996)

115. Schoonjans, K, B Staels and J Auwerx: Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* 37, 907-925 (1996)

116. Braissant, O, F Foufelle, C Scotto, M Dauca and W Wahli: Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR--alpha, -beta, and -gamma in the adult rat. *Endocrinology* 137, 354-366 (1996)

117. Wahli, W, O Braissant and B Desvergne: Peroxisome proliferator-activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more... *Chem Biol* 2, 261-266 (1995)

118. Kliewer, SA, BM Forman, B Blumberg, ES Ong, U Borgmeyer, DJ Mangelsdorf, K Umesono and RM Evans: Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci USA* 91, 7355-7359 (1994)

119. Knipp, GT, B Liu, KL Audus, H Fujii, T Ono and MJ Soares: Fatty acid transport regulatory proteins in the developing rat placenta and in trophoblast cell culture models. *Placenta* 21, 367-375 (2000)

120. Dutta-Roy, AK: Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta. *Am J Clin Nutr* 71, 315S-322S (2000)

121. Gurr, M: Fats. In: Human nutrition and dietetics. Eds: Garrow, JS, James, WPT, Churchill Livingstone, Edinburgh, 77-102 (1993)

122. Haggarty, P, K Page, DR Abramovich, J Ashton and D Brown: Long-chain polyunsaturated fatty acid transport across the perfused human placenta. *Placenta* 18, 635-642 (1997)

123. Lopaschuk, GD, RL Collins-Nakai and T Itoi: Developmental changes in energy substrate use by the heart. *Cardiovasc Res* 26, 1172-1180 (1992)

124. Cunnaae, SC, CR Menard, SS Likhodii, JT Brenna and MA Crawford: Carbon recycling into de novo lipogenesis is a major pathway in neonatal metabolism of linoleate and a-linoleate. *Prostaglandins Leukot Essent Fatty Acids* 60, 387-392 (1999)

125. Hull, D: Total fat metabolism. In: Fetal physiology and medicine. Eds: Beard, RW, Nathanielz, G, W.B. Saunders Co., London, 105-120 (1976)

Send correspondence to: Dr Laura A. Woollett Department of Pathology and Laboratory Medicine, University of Cincinnati, 231 Albert B. Sabin Way, Cincinnati, OH 45267-0529, Tel: 513-558-3969, Fax: 513-558-2141, E-mail: woollela@email.uc.edu