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Inability to fully suppress sterol synthesis rates with exogenous sterol in embryonic and extraembyronic fetal tissues

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SUMMARY

The requirement for cholesterol is greater in developing tissues (fetus, placenta, and yolk sac) as compared to adult tissues. Here, we compared cholesterol-induced suppression of sterol synthesis rates in the adult liver to the fetal liver, fetal body, placenta, and yolk sac of the Golden Syrian hamster. Sterol synthesis rates were suppressed maximally in non-pregnant adult livers when cholesterol concentrations were increased. In contrast, sterol synthesis rates were suppressed only marginally in fetal livers, fetal bodies, placentas, and yolk sacs when cholesterol concentrations were increased. To begin to elucidate the mechanism responsible for the blunted response of sterol synthesis rates in fetal tissues to exogenous cholesterol, the ratio of sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP) to Insig-1 was measured in these same tissues since the ratio of SCAP to the Insigs can impact SREBP processing. The fetal tissues had anywhere from a 2- to 6-fold greater ratio of SCAP to Insig-1 than did the adult liver, suggesting constitutive processing of the SREBPs. As expected, the level of mature, nuclear SREBP-2 was not different in the fetal tissues with different levels of cholesterol whereas it was different in adult livers. These findings indicate that the suppression of sterol synthesis to exogenous sterol is blunted in developing tissues and the lack of response appears to be mediated at least partly through relative levels of Insigs and SCAP.

Keywords

cholesterol; Smith-Lemli-Opitz; HMG-CoA reductase; fetus; SREBP-2; IUGR

INTRODUCTION

During embryonic and fetal development, there is substantial requirement for cholesterol due to the rapid cell division that occurs to maintain significant growth. The necessity of synthesized cholesterol by the fetus and/or embryo is depicted best by fetuses and/or embryos unable to synthesize cholesterol. There are 7 known defects in the sterol biosynthetic pathway, all of which will lead to congenital malformations [1–3]. The most common of the defects is

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the Smith-Lemli-Opitz syndrome (SLOS). Individuals with this syndrome have mutations in 3β-hydroxysterol Δ^7-reductase (DHCR7) and convert minimal to no 7-dehydrocholesterol to cholesterol, depending on the type of mutation [4,5]. The deficiency of cholesterol can lead to a wide range of congenital defects, ranging from the mild (minor physical abnormality with behavioral and learning disabilities) to the severe (lethal with multiple major congenital anomalies) [6,7]. Not surprising, the severity of the syndrome is correlated with circulating sterol concentrations [8].

The fetus and/or embryo has two sources of cholesterol: endogenous and exogenous [9–11]. The endogenous sterol is synthesized de novo. As might be expected of a compound essential for growth, fetal sterol synthesis rates are relatively elevated [12–14]. Since the fetus does not come in direct contact with the maternal circulation, its exogenous source of cholesterol originates in the placenta and yolk sac and is secreted and/or effluxed to the fetal circulation [9]. The cholesterol secreted and/or effluxed is that which was synthesized in the placenta and yolk sac and/or that which was taken up from the maternal circulation by the placenta and yolk sac, transported across cells and secreted and/or effluxed. Though both endogenous and exogenous sources make significant contributions to the fetal cholesterol, it is likely that a greater percentage of fetal sterol originates from de novo synthesis [15,16].

The synthesis of cholesterol is a multi-step pathway with the rate-limiting step occurring with the conversion of HMG-CoA to mevalonate, a reaction catalyzed by HMG-CoA reductase (HMGR). HMGR is regulated at several levels and by multiple effectors, including nonsterol intermediates as well as cholesterol [17,18]. The major transcription factor that elicits the sterol-mediated response is the sterol regulatory element-binding protein-2 (SREBP-2) [19–21]. There are three SREBPs, SREBP-1a, SREBP-1c, and SREBP-2. The SREBPs are synthesized as inactive, precursor proteins which are inserted into the ER membrane where they bind to the SREBP cleavage-activating protein (SCAP). Under conditions of depleted cholesterol, SCAP escorts the SREBPs to the Golgi. Once in the Golgi, two different proteases cleave the SREBPs to their mature forms. The SREBPs are translocated to the nucleus where they bind to sterol regulatory elements within promoter regions of HMGR and other proteins involved in the maintenance of sterol balance such as HMG-CoA synthase and the LDL receptor [19]. When cellular cholesterol is in excess, SCAP undergoes a conformational change that allows it to bind to Insig-1 and -2 [22–24]. Since the Insigs are resident membrane proteins of the ER, the SCAP:SREBP complex is retained within the ER and the SREBPs are not processed to their mature forms [22–24]. This processing of the SREBPs can be affected markedly by changing the ratio of SCAP to the Insigs [25,26]. Though SREBP-2 drives sterol synthesis in adult tissues, SREBP-1a drives sterol synthesis in cell culture and when overexpressed [27].

Though the regulation of sterol biosynthesis has been studied extensively in adult tissues, very little research has examined its regulation during development. To gain a better understanding of the regulation of sterol biosynthesis during this rapid time of growth, the relationship between cholesterol concentrations and sterol synthetic rates in the fetal liver and body, the placenta, and the yolk sac were compared to the same relationship in the non-pregnant adult liver of the Golden Syrian hamster. Hamsters were used in these studies because 1) hamsters are readily responsive to dietary cholesterol [14,28], 2) embryonic and extraembryonic fetal tissue cholesterol concentrations can be readily changed in hamsters [29] and 3) the gestational age of hamsters is very precise [29]. We found that sterol synthesis rates were maximally suppressed in the adult liver but only marginally suppressed in each of the extraembryonic and embryonic fetal tissues in the presence of excess tissue cholesterol. The ratios of SCAP mRNA to Insig-1 mRNAs were also markedly different between the adult and fetal tissues, and were 2–6-fold greater in the fetal tissues. Thus, we hypothesize that the differences in responsiveness

of sterol synthesis rates to cholesterol concentrations between adult and fetal tissues are at least partly due to constitutive processing of SREBP-2.

MATERIAL AND METHODS

Animals and diets

Male and female hamsters (Charles River Laboratories, Inc.) weighing between 110 and 120 g were maintained in a temperature and humidity controlled room and fed a pelleted chow (diet #7012, Harlan Teklad). After at least one week of acclimation, females were split into two different groups. In one group, females were fed diets consisting of 0, 0.05, 0.10, 0.15, 0.20, or 0.25 % added cholesterol (wt/wt) in ground chow (#7012, Harlan Teklad); diets had an inherent cholesterol concentration of 0.00013% (wt/wt). After 3 weeks, studies were performed in the non-pregnant females; livers from non-pregnant females were used instead of livers from pregnant females due to the marked and potentially confounding effects of pregnancy on the maternal liver [30]. In the second group, females were fed diets consisting of 0, 0.12, 0.5, or 2.0% added cholesterol (wt/wt), levels that were known to affect whole fetus sterol synthesis, in ground chow for 3 weeks and mated [29]. At 11.5 days post-conception (dpc), studies were performed; hamsters have a gestational period of 15.5 days.

Sterol synthesis rates

Animals were injected with 50 (pregnant) or 15 (non-pregnant) mCi of $[^3H]H_2O$ (GE Healthcare). After 1 h, adult livers, placentas, yolk sacs, fetal livers, and fetal bodies were collected, saponified, and digitonin-precipitable sterols (DPS) were isolated [31,32]. The rates of sterol synthesis are expressed as the nmol of $[^3H]H_2O$ incorporated into DPS per h per g tissue. Synthesis rates in fetal livers and bodies were corrected for the time it took for $[^3H]H_2O$ to equilibrate between the fetus and dam [31,33].

Cholesterol concentrations

Tissues were saponified in alcoholic base. Cholesterol was extracted with petroleum ether, and concentrations were determined by gas liquid chromatography using stigmastanol (Sigma) as an internal standard [34].

Immunoblots

Fetal and adult livers, fetal bodies, placentas, and yolk sacs were homogenized. Nuclei and microsomes were isolated as described [35,36], except samples were stored in 10% glycerol. Protein concentrations were measured by the Lowry assay, and equal amounts of protein of like samples pooled. Proteins were separated on Bio-Rad 4–15% Tris-HCL Ready gels under denaturing conditions, and transferred to nitrocellulose membranes. Blots containing membrane-bound proteins were pre-incubated in a blocking buffer containing 5% dry milk and 0.1% Tween. Blots of nuclei were incubated with anti-SREBP-2 IgG (a gift from Dr. J. Horton, University of Texas Southwestern Medical Center) followed by an incubation with donkey anti-rabbit secondary antibody (GE Healthcare). Chemiluminescence from ECL Plus was detected by a Storm 450 Phosphoimager, and band densities were quantitated by Image J software. Blots were stripped with Restore Western Blot Stripping Buffer [37] and reprobed with CREB (Cell Signaling Technology, Inc.), to standardize protein loading. Blots of microsomes were incubated with anti-Insig-1 (Santa Cruz Biotechnology) or anti-SCAP (Santa Cruz Biotechnology) followed by incubations with appropriate secondary antibodies. Bands were detected, blots stripped, and reprobed with β-actin (Bioscience Research Reagents). Pooled samples were analyzed at least twice (n=3–4 per set).

Real time PCR

Samples of adult, neonatal, and fetal livers, fetal bodies, placentas, and yolk sacs were collected. Tissue RNA was isolated using TRIazol [38] and stored in FORMAzol®. RNA was treated with RNase-free DNase I and reverse transcribed to cDNA by SuperScript II reverse transcriptase using random hexamers. PCR assays were performed on a Bio-Rad iCycler iQ real-time PCR Detection System with SYBR green as our fluorophore. A serial dilution of a randomly-picked sample was used to generate a standard curve for each gene examined. This standard curve was used to calculate the relative levels of mRNA for the gene of interest and the reference/housekeeping gene (cyclophilin). Primers used were modified from those previously reported (Table I; SCAP, Insig-1, and cyclophilin) [39–41] or were similar to those previously reported (SREBP-1a) [42].

Statistical analyses

Data are presented as means ± SEM. Linear regression models were run treating dietary cholesterol as the independent variable and cholesterol concentration as the dependent variable. Only the first three data points were used to determine if the slope of the line was significantly different from zero since the first three data points correspond to the dietary concentration we are most interested in. Plus, inclusion of all points will skew results and end in a positive line due to the marked changes that occur with higher cholesterol concentrations. Student's t-tests were conducted when comparing mean levels between two groups. When increasing amounts of cholesterol were fed, cholesterol concentrations or synthesis rates were compared between livers of dams fed 0 versus 0.05% cholesterol, 0.05 versus 0.10% cholesterol, 0.10 versus 0.15% cholesterol, etc. In fetal tissues, comparisons were between 0 and 0.12% cholesterol, 0.12 and 0.50% cholesterol, and 0.5 and 2.0% cholesterol. Significance was considered $P<0.05$.

RESULTS

The goal of these studies was to delineate differences in regulation of sterol biosynthesis between adult and fetal tissues and to begin to elucidate the mechanism(s) responsible for any differences in regulation. We studied the adult liver, the fetal liver and body, the placenta, and the yolk sac. In the adult non-pregnant female, diets were fed that contained from 0 to 0.25% added cholesterol. Hepatic cholesterol concentrations were 1.93 ± 0.10 mg/g in dams fed ground chow (Fig. 1A, left panel). Cholesterol concentrations did not increase in livers of dams fed 0.05% or 0.10% added cholesterol. In support of this, the slope of the line using cholesterol concentrations in dams fed 0, 0.05, and 0.10% cholesterol as the dependent variable and dietary cholesterol as the independent variable was not different from zero (Table II). Hepatic cholesterol concentrations increased 2.2-fold, 4.3-fold and 6.2-fold in livers of females fed 0.15, 0.20, or 0.25% cholesterol ($P<0.05$), respectively, as compared to those fed no added cholesterol. Even though hepatic cholesterol concentrations were not increased when hamsters were fed 0.05% cholesterol, sterol synthesis rates decreased 51% (Fig. 1A, right panel; $P<0.05$). Rates were not significantly different with 0.10% dietary cholesterol but decreased further with 0.15% dietary cholesterol ($P<0.05$) and reached a nadir of maximal suppression when 0.20 or 0.25% cholesterol was fed ($P<0.05$).

To study the relationship between cholesterol concentrations and sterol synthesis rates in fetal tissues, females were fed diets which would result in increased cholesterol concentrations in the embryonic and extraembryonic fetal tissues [29]; maternal cholesterol concentrations increased 15–300% with dietary cholesterol [29]. Cholesterol concentrations in tissues of dams fed 0.12% cholesterol were not significantly different from concentrations in tissues of dams fed no added cholesterol. However, concentrations were increased in the fetal livers (Fig. 1B, left panel), fetal bodies (Fig. 1C, left panel), placentas (Fig. 1D, left panel), and yolk sacs (Fig. 1E, left panel) of dams fed 0.5% cholesterol. Importantly, when cholesterol concentrations

were plotted as the dependent variable and dietary cholesterol as the independent variable, the slopes of all the lines were significantly different from zero, or at least tended to be different from zero (Table II). Sterol synthesis rates were also measured in these same fetal tissues. Synthesis rates decreased in each of the fetal tissues of dams fed 0.12 or 0.5% cholesterol ($P<0.05$). Synthesis rates were suppressed to a nadir in the fetal livers (Fig. 1B, right panel) and bodies (Fig. 1C, right panel) of dams fed 0.5% cholesterol. The nadir in these fetal tissues (≈500 nmol/h per g) was much greater than the nadir in the adult liver (18 nmol/h per g). Synthesis rates were also suppressed to a nadir in the placentas (Fig. 1C, right panel) of dams fed 0.5% cholesterol, though the nadir of this tissue was ≈200 nmol/h per g. Rates did not reach a nadir in the yolk sac but decreased continuously in yolk sacs (Fig. 1E, right panel) of dams fed increasing amounts of cholesterol.

To better understand the differences in regulation between the adult liver and the fetal tissues (fetal liver and body, placenta, and yolk sac), relative sterol synthesis rates were plotted against relative cholesterol concentrations in each tissue; sterol synthesis rates and cholesterol concentrations in hamsters fed no added cholesterol were set at 100% and all other values presented as a percentage of the original rates or concentrations. As seen in Figure 2 and Table II, sterol synthesis rates decreased prior to an increase in cholesterol concentrations in the adult liver. In contrast, rates and concentrations changed simultaneously in the fetal liver and body, the placenta, and the yolk sac. To determine if the differences in response were merely due to a relatively smaller increase in cholesterol concentrations in the fetal tissues, the relative sterol synthesis rates of tissues with ≈2-fold increase in cholesterol concentration were compared (Fig. 3). Regardless of the fact that all tissues presented in this figure had ≈2-fold increase in cholesterol concentrations, sterol synthesis rates were suppressed to 8% of original rates in the adult liver whereas rates were suppressed to only 29 to 59% in fetal tissues.

The question now becomes - why are the fetal tissues less responsive to exogenous sterol as compared to the adult tissue? We hypothesized that the blunted response to exogenous sterol in the fetal tissues was the result of constitutive processing of SREBP-2 due to different ratios of SCAP to the Insigs [25,26] and/or higher SREBP-1a levels [27]. To test this hypothesis, SCAP and Insig-1 mRNA levels were measured in the adult livers and the fetal tissues; only Insig-1 was measured in these studies since Insig-2 is not expressed in the rodent liver until after parturition [43]. The relative amounts of Insig-1 mRNA were 11.6% ($P<0.001$) in the fetal liver, 23.1% ($P<0.001$) in the placenta, and 50.2% ($P=0.01$) in the yolk sac, as compared to amounts in the adult liver (Fig. 4A). The amounts of SCAP mRNA were 98.2% (P=ns) in the fetal liver, 125.4% (P=ns) in the placenta, and 40.4% ($P=0.057$) in the yolk sac, as compared to amounts in the adult liver (Fig. 4A). The ratio of SCAP to Insig-1 (Fig. 4B) was 5.8-fold greater in the fetal liver ($P=0.006$), 2.8-fold greater in the yolk sac ($P=0.037$), and 1.8-fold greater in the placenta (P=ns) as compared to the adult liver. Additionally, the relative mRNA levels in whole fetuses of dams fed 0 and 2% cholesterol were similar: Insig-1 (0.24±0.01 vs 0.21±0.01 for 0 and 2% cholesterol, respectively) and SCAP (0.35±0.04 and 0.32±0.01 for 0 and 2% cholesterol, respectively). Protein levels of Insig-1 and SCAP in relation to β-actin were measured as well (Fig. 5). For Insig-1, very little protein was detected for the fetal body and fetal liver. The amount of Insig-1 with respect to β-actin expression in the extraembryonic tissues were ≈62% of that in the adult liver. For SCAP, there were relatively similar amounts to slightly more SCAP in relation to β-actin in the fetal liver (128%), fetal body (116%), placenta (102%), and yolk sac (98%) as compared to adult liver. The relative levels of SREBP-1a mRNA levels were measured in the adult and fetal livers as an alternative mechanism for a lack of change in sterol synthesis rates. Interestingly, the adult liver (1.95 ±0.08) had more SREBP-1a mRNA as compared to the fetal liver (1.17±0.17).

Finally, we wanted to measure the relative levels of mature, nuclear SREBP-2 protein levels in the fetal tissues with different levels of cholesterol. As expected in tissues with constitutive

processing, protein expression levels were similar between fetal tissues of dams fed 0 or 2% cholesterol (Fig. 6A). Also expected, was a lack of effect of diet on HMGR or HMG synthase in the fetal tissues (data not shown). Not surprisingly, adult livers with a 2-fold increase in cholesterol concentration (0.15% dietary cholesterol) demonstrated ≈45% decrease in mature, nuclear SREBP-2 protein levels (Fig. 6B).

DISCUSSION

The regulation of sterol synthesis is complex and multivalent [17,18]. This complex regulation allows for a greater than 200-fold variation in sterol synthesis rates to occur over relatively short periods of time [18]. This ability to finely regulate sterol production is beneficial to the adult tissues where the need for cholesterol is low, and excess sterol can lead to cell death and/or acceleration of disease processes. In fetal tissues, the ability to rapidly suppress and activate sterol synthesis rates may not be advantageous since the need for cholesterol is considerable and constant. In the current studies, cholesterol-induced suppression of sterol synthesis rates was blunted in the fetal tissues as compared to adult tissues. First, sterol synthesis rates were suppressed maximally in adult livers (18 nmol/h per g) whereas sterol synthesis rates were suppressed only marginally in each of the embryonic and extraembryonic fetal tissues (187–644 nmol/h per g). Second, cholesterol concentrations increased in fetal tissues simultaneously with a decrease in synthesis rates whereas cholesterol concentrations increased in the adult liver only when synthesis rates were suppressed maximally. While the relationship between synthesis rates and cholesterol concentrations in the adult liver have been described previously [44], these are the first to demonstrate a simultaneous change when synthesis is only marginally suppressed. Simply put, in the adult liver, synthesis rates are suppressed to compensate for exogenous sterol and concentrations increase only when synthesis rates can not be reduced further. In contrast, sterol synthesis rates in fetal tissues can not be suppressed significantly and so concentrations increase.

Several mechanisms exist which could be responsible for the apparent dysregulation of sterol synthesis in the fetal tissues and possibly other rapidly growing tissues, including constitutive processing of SREBP-2, decreased degradation of SREBP-2, and decreased degradation of HMGR.

Constitutive processing of SREBP-2

The hallmark of constitutive processing of the SREBPs is elevated sterol synthesis rates, even in the presence of increased cholesterol concentration. Others have demonstrated that the processing of SREBP-2 can be manipulated by changing the amount of SCAP relative to the amount of Insigs in cells. When SCAP was overexpressed, SREBP-2 was constitutively processed, even in the presence of sterol [26]. Likewise, overexpression of the Insigs resulted in maintaining SREBP-2 in the Golgi [25]. Based on our data, specifically the fact that the SCAP to Insig-1 ratio is ≈2–6-fold greater in the fetal tissues as compared to the adult liver and the fact that nuclear, mature SREBP-2 levels do not decrease in fetal tissues with increased cholesterol concentrations whereas levels do change in adult tissues with similar fold increases in cholesterol concentration, at least part of the reason for the lack of change in sterol synthesis rates in fetal tissues could be due to constitutive processing of SREBP-2. The ratio of SCAP to Insig-1 was greater in all fetal tissues, though the greatest difference (≈6-fold) occurred between the fetal and adult livers. It appeared that differences in protein may have been even greater than differences in mRNA levels. Why might the Insig-1 levels, especially protein, be so low in the fetal tissues in comparison to the adult liver? Either the relatively different expression levels of SCAP or Insig-1 between fetal tissues and adult liver is hard-wired to ensure high sterol synthesis rates or other effectors are involved in their regulation. Since insulin can affect Insig levels [45], it is possible that the low levels of insulin in the rodent fetus

[43] can impact upon Insig expression levels. This would not explain why Insig-1 levels are low in the placenta and yolk sac since these tissues come in contact with the maternal circulation, though it has been hypothesized that all tissues are insulin resistant during gestation [46,47]. Also, Insig-1 is stabilized when sterols are present [22,23]. If there is no abundance of sterols due to their requirement for membrane formation during rapid growth, Insig-1 may be ubiquintated and degraded rapidly during the fetal period as seen by low protein levels [48], supporting our hypothesis that the fetus is in a negative sterol balance [49]. Regardless, other factors besides the ratio of SCAP to Insig-1 must be involved in responsiveness of sterol synthesis rates to exogenous sterol since a correlation did not exist between the ratio of mRNA of these two proteins and the degree of suppressed synthesis rates in the fetal liver, placenta, and yolk sac.

SREBP-2 degradation

The lack of marked change in sterol biosynthesis in the fetal tissues could also be due to an inability to degrade SREBP-2. SREBP-2 is degraded via the ubiquitin-proteosome pathway after its phosphorylation and ubiquination [50–52]. Though the ubiquitin-proteosome system for degradation is active during development [53,54], it seems that the SREBPs would not be readily modified and/or degraded during this time of rapid growth since sterol synthesis rates remain relatively active in the presence of cholesterol. Lack of SREBP-2 degradation may be the result of a change in proteosome activity [55] or rate of de-ubiquitination [41].

HMGR degradation

Finally, the blunted effect of exogenous sterol in the fetal tissues could be the result of an inability to degrade HMGR. As with the SREBPs and other proteins, HMGR is targeted for degradation once it is ubiquitinated [41,56]. While HMGR can be ubiquinated in the presence of sterol [41], it is further ubiquinated in the presence of both sterol plus geranylgeraniol, implying involvement of a geranylgeranylated protein [57], or plus lanosterol [58]. These more recent data support the observations that maximal suppression of sterol synthesis rates occurs only in the presence of cholesterol plus a mevalonate-derived product [18,59], which may be absent in a tissue in a negative sterol balance and/or proliferating rapidly as during development [49]. Additionally, a lack of HMGR degradation in the fetal tissues could be due to the fact that HMGR degradation is accelerated by binding to Insig-1 [41]. The relatively low levels of Insig-1 mRNAs we detected in the early/mid fetal tissues would favor a lack of HMGR ubiquitination and therefore a lack of degradation of HMGR.

Summary

To summarize, it appears that there is a dysregulation of sterol synthesis rates in the embryonic and extraembryonic fetal tissues. The dysregulation might occur to ensure a steady supply of cholesterol for the essential growth- and development-related processes that take place in these tissues during gestation. The lack of regulation may occur through multiple processes, including constitutive processing of SREBP-2.

What are some of the cholesterol-dependent growth- and development-related processes that take place in the fetus and/or placenta? The most noted growth-related role of cholesterol is that it is a major component of cellular membranes. As might then be expected in persons unable to synthesize significant amounts of cholesterol, SLOS infants and children have diminished growth rates [6], which can be improved with dietary cholesterol [60–62]. It would be expected that fetal growth rates are also reduced. Fetal growth rates could be further impacted upon if placental growth rates were altered; a small placenta often times lead to placental insufficiency, the leading cause of intrauterine growth restriction (IUGR) [63–65]. The ability to maintain placental growth rates through a constant supply of membrane components could be a novel way to ensure adequate placental, and thereby fetal, growth rates.

In addition to its role as a membrane substrate, cholesterol and closely related sterols can affect various signaling events. First, the sterols activate Sonic hedgehog (Shh) [66,67]. Shh is a key patterning protein involved in forebrain as well as limb and organ development [68]. A lack of Shh activity, possibly due to low levels of sterols [69], leads to a number of congenital defects in mice and humans alike [66–72]. Second, changes in cholesterol concentrations could affect composition of membrane microdomains where abundant signaling originates. While it has been demonstrated that signaling will change in membranes of SLOS individuals [73,74], due at least partly to a redistribution of proteins into various microdomains [75], the full extent of a change in signaling during development is unknown. Thus, a steady supply of cholesterol during development through relatively unresponsive, elevated sterol synthesis rates aids in the maintenance of a number of sterol-dependent developmental processes and growth.

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Abbreviations

SLOS
: Smith-Lemli-Opitz syndrome

DHCR7
: 3β-hydroxysterol Δ^7-reductase

HMGR
: HMG-CoA reductase

SREBP
: sterol regulatory element binding protein

SCAP
: SREBP cleavage-activating protein

DPS
: digitonin-precipitable sterols

Shh
: Sonic hedgehog

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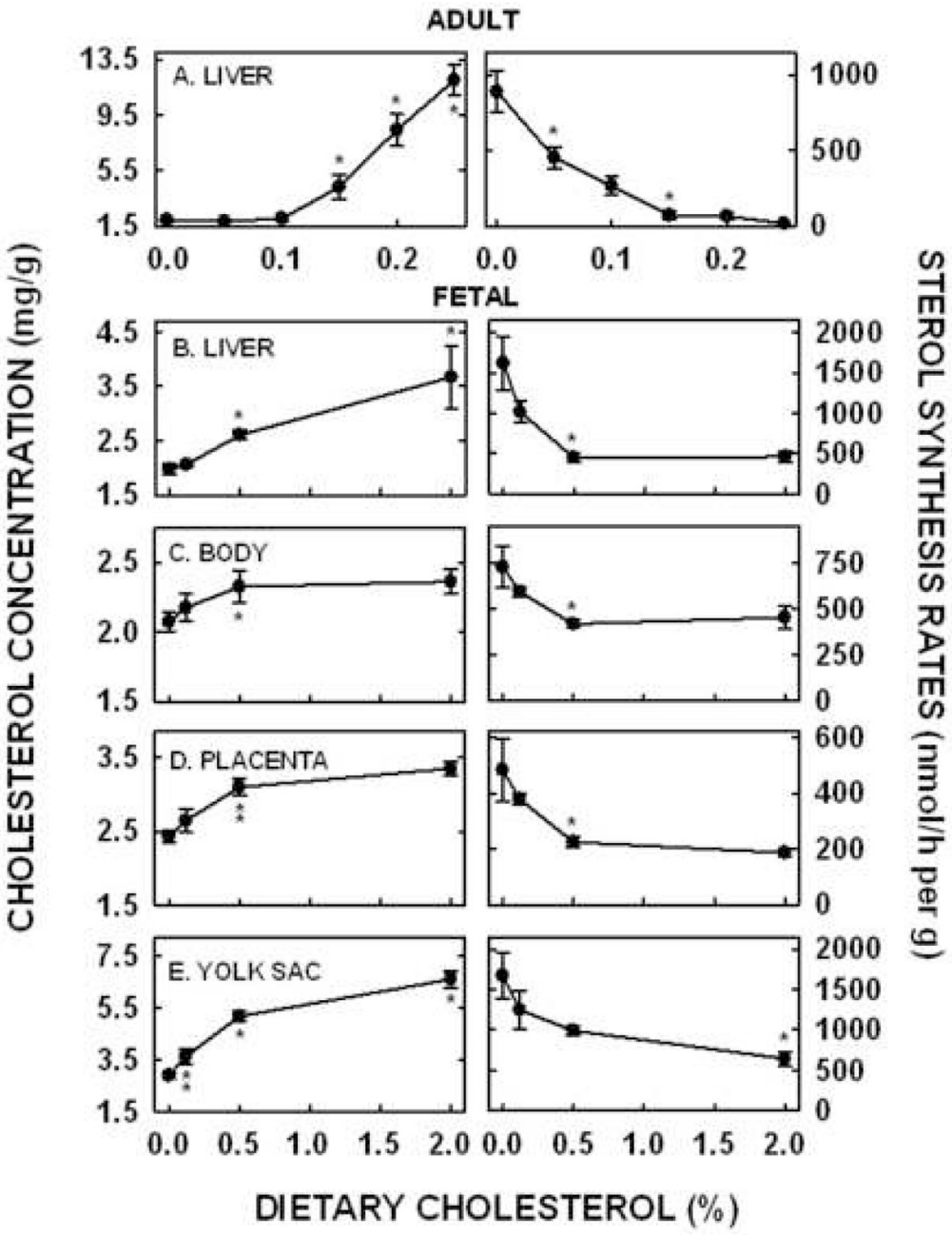

Figure 1.

Hepatic cholesterol concentrations and sterol synthesis rates in tissues of pregnant and non-pregnant females fed increasing amounts of cholesterol. Non-pregnant females were fed 0, 0.05, 0.10, 0.15, 0.20, and 0.25% (wt/wt) added cholesterol. Hepatic cholesterol concentrations (A, left panel) were measured by GLC and sterol synthesis rates (A, right panel) were measured in vivo using [^{3}H]H_2O. Data are presented as means ± SEM (n=3–6). Pregnant females were fed 0, 0.12, 0.50, and 2.00% (wt/wt) added cholesterol prior to and during pregnancy. Cholesterol concentrations (left panel) and sterol synthesis rates (right panel) were measured in fetal livers (B), fetal bodies (C), placentas (D), and yolk sacs (E) of dams at 11.5 dpc. Data are presented as means ± SEM (n=3–6). * depicts significant differences ($P<0.01$) or ** depicts

trends to be significantly different ($0.05<P<0.07$) between values from tissues of dams fed consecutive amounts of cholesterol, i.e. dams fed 0.10 versus 0.15% cholesterol.

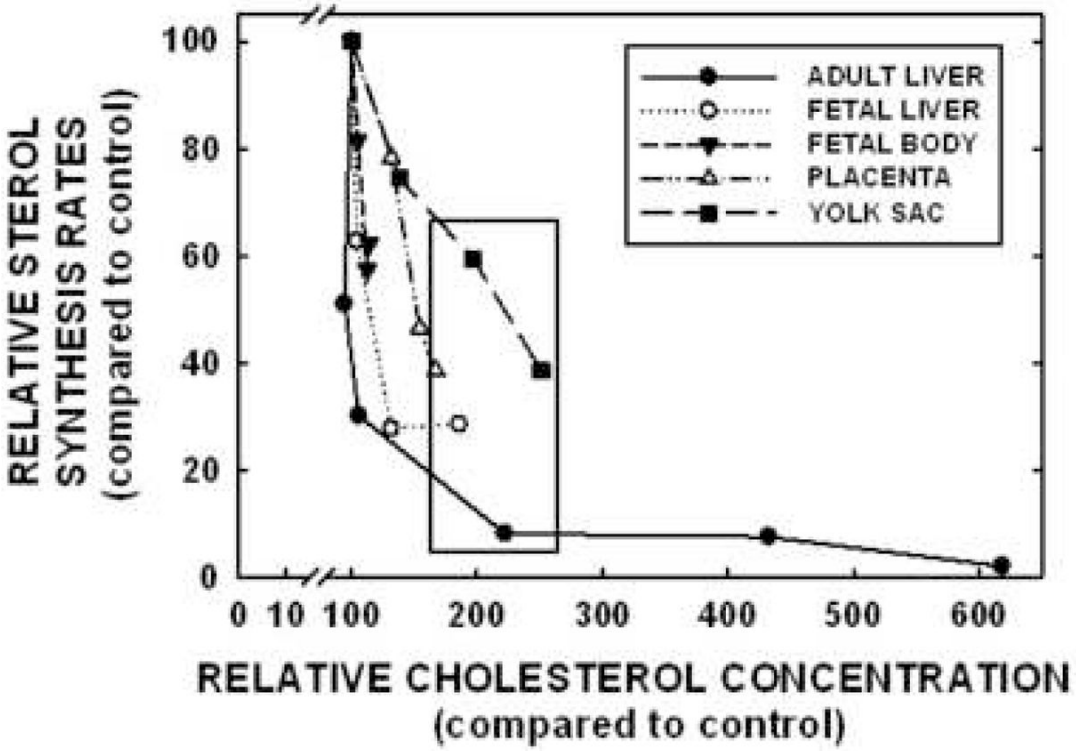

Figure 2.
Relationship between sterol synthesis rates and cholesterol concentrations in the adult liver and fetal tissues. Sterol synthesis rates and cholesterol concentrations in tissues of dams fed no added cholesterol were set to 100%; data originated in Figure 1. Relative changes in synthesis rates and cholesterol concentrations in tissues of dams fed cholesterol were calculated and plotted. The box depicts tissues with ≈2-fold increases in cholesterol concentrations.

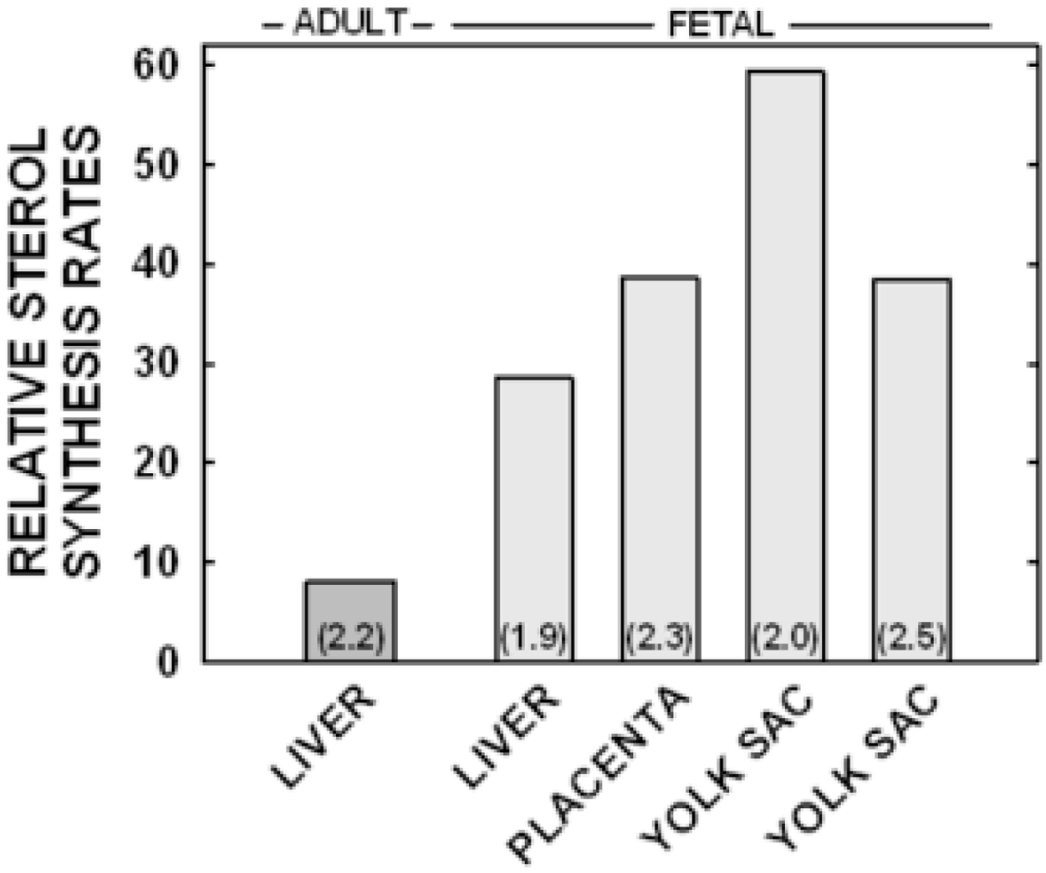

Figure 3.

Sterol synthesis rates in tissues with increased cholesterol concentrations. Relative sterol synthesis rates from tissues with ≈2-fold increase in cholesterol concentration were plotted; data corresponds to the box in Figure 2. The fold increase of cholesterol concentrations in tissues of non-pregnant and pregnant dams fed cholesterol compared to values in non-pregnant and pregnant dams fed no cholesterol are in parentheses. Note that there are two values for yolk sacs corresponding to yolk sacs of dams fed 0.5 and 2.0% cholesterol.

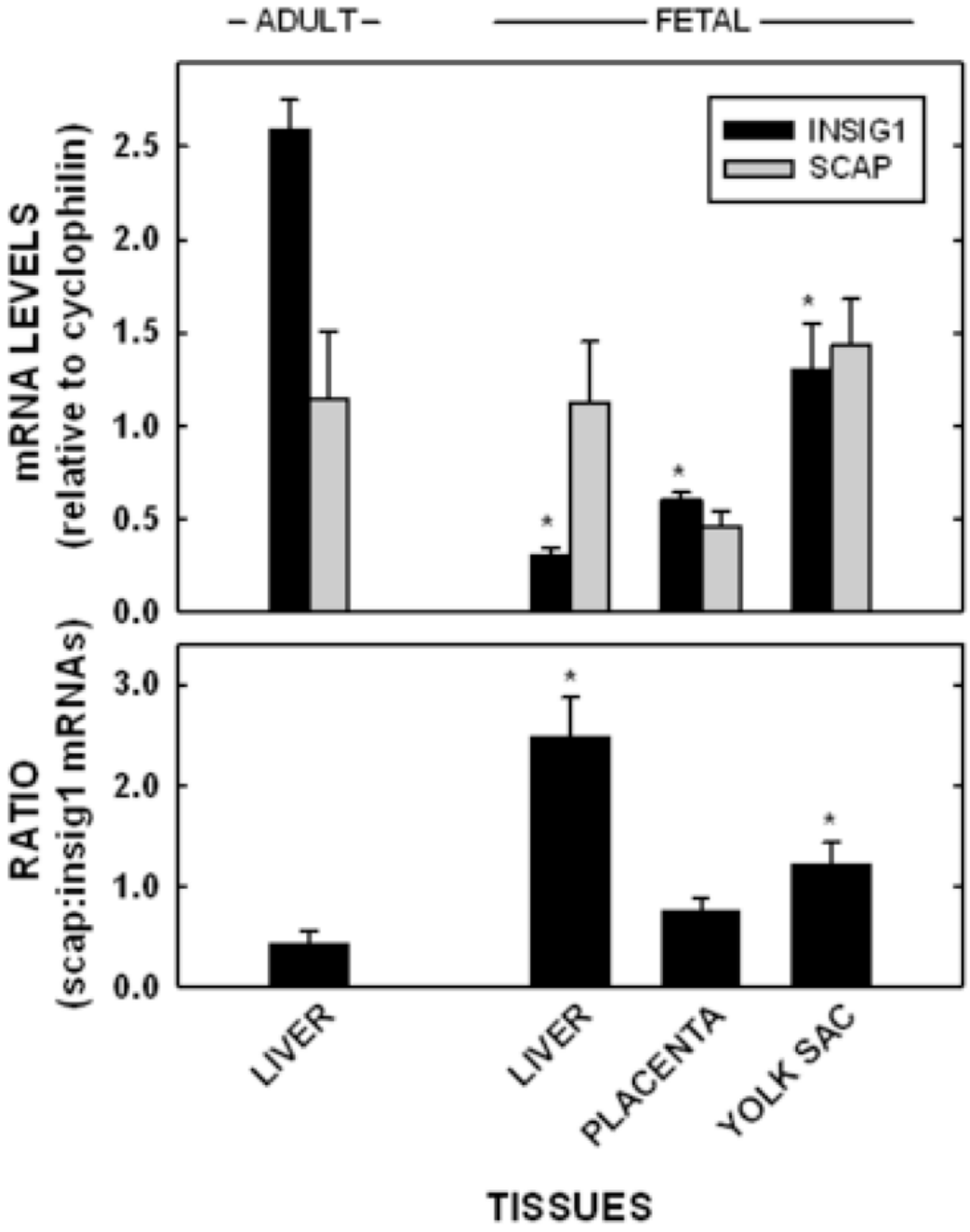

Figure 4.

Insig-1 and SCAP mRNA levels in the adult liver and fetal tissues. Tissues were collected from non-pregnant and pregnant (11.5 dpc) females fed no added cholesterol. The Insig-1 and SCAP mRNA levels were measured by real time PCR and presented as means ± SEM (n=3–4) using cyclophilin as our reference/housekeeping gene (A). The ratio of SCAP:Insig-1 mRNA levels were calculated and presented (B). In both panels, * depicts significant differences from the adult liver ($P<0.05$).

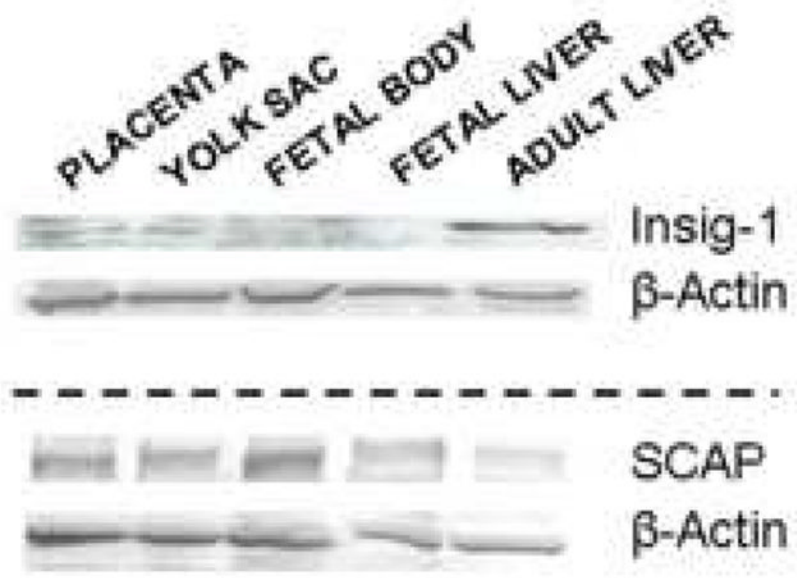

Figure 5.
Insig-1 and SCAP expression levels in embryonic and extraembryonic fetal tissues and adult livers. Fetal tissues were collected at 11.5 dpc and adult livers were collected 3 weeks after being fed ground chow. β-Actin was used to equalize protein loading.

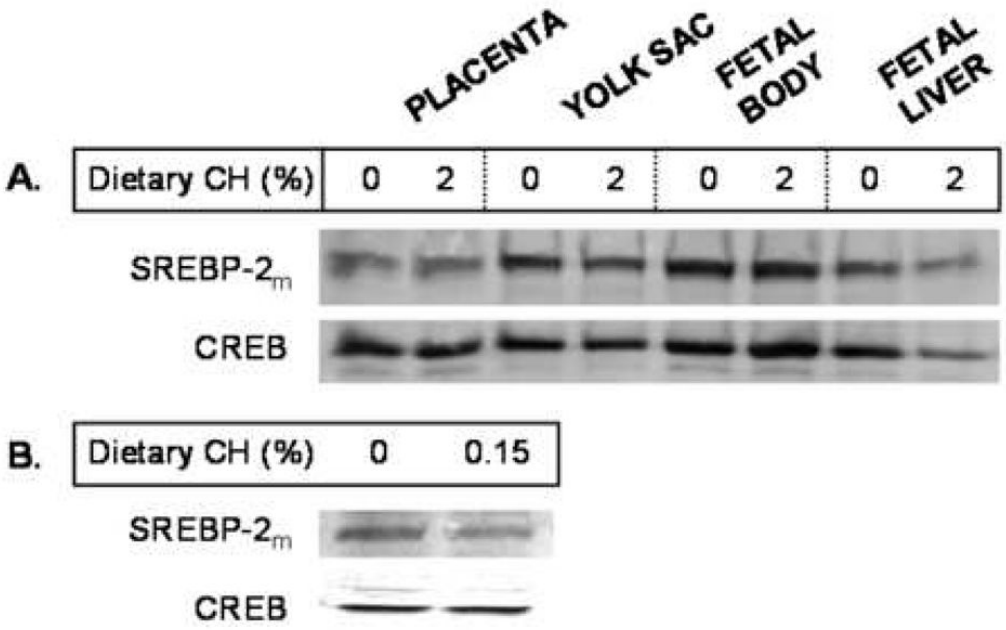

Figure 6.

Mature SREBP-2 (SREBP-2_m) expression levels in nuclei of embryonic and extraembyronic fetal tissues and adult livers with different cholesterol concentrations. Embryonic and extraembryonic fetal tissues were collected from females treated as described in Figure 1 except only 0 and 2% cholesterol was fed (A). At 11.5 dpc, tissues were collected and assayed for mature, nuclear SREBP-2 in the nuclei using CREB to equilibrate protein loading. Livers from non-pregnant females fed 0.15% cholesterol for 3 weeks were also assayed for mature, nuclear SREBP-2 in the nuclei (B).

TABLE I

Nucleotide sequences of hamster-specific primers used for quantitative real time PCR

mRNA	Sequences of forward and reverse primers (5′-3′)
Insig-1	CCCAGATTTCTTCTATATTCG
	CCCATAGCTAACTGTCGTCC
SCAP	CTGCTGCTACCCTCTGCTGAAG
	CTTGTTTGTGGTCAGAGTC
Cyclophilin	ATTCATGTGCCAGGGTGGTGACT
	TCAGTCTTGGCGGTGCAGAT

TABLE II

Cholesterol concentrations as dependent variables in the adult liver and fetal tissues

Tissue	Slope	SE	P value vs. 0
Adult liver	1.11	1.84	0.555
Fetal liver	1.29	0.20	<0.001
Fetal body	0.54	0.27	0.063
Placenta	1.28	0.39	0.007
Yolk sac	4.38	0.73	<0.001

Slopes were determined using values obtained in the livers of non-pregnant females fed 0, 0.05, and 0.10% cholesterol or in tissues of pregnant dams fed 0, 0.12, and 0.5% cholesterol.