Dietary fatty acids can have profound effects on sterol metabolism in the adult. In the hamster, an animal that responds to dietary components in a manner similar to a human (9, 40), dietary saturated fatty acids (SFA) elevate plasma low-density lipoprotein cholesterol (LDL-C) concentrations by increasing LDL-C production rates and decreasing LDL receptor levels (52–54). In contrast, dietary polyunsaturated fatty acids (PUFA) reduce plasma LDL-C concentrations by decreasing LDL-C production rates and increasing LDL receptor levels (54). From in vitro studies, it is known that the mechanisms responsible for the various effects of PUFA include the activation of various nuclear receptors, such as the peroxisome proliferator-activated receptors (PPAR) (13, 25) and liver X receptors (LXR) (reviewed in Ref. 21), and the manipulation of levels of proteins responsible for regulation of sterol metabolism, including the sterol regulatory element binding proteins 1 and 2 (SREBPs) (reviewed in Refs. 21 and 55). One of the effects of PUFA in rapidly dividing cells in vitro is the suppression of SREBP-2 levels (55). Assuming that SREBP-2 is a major regulator of sterol synthesis (18), reduction of expression levels could lead to a suppression of sterol synthesis in rapidly dividing cells.

Although the ability to decrease sterol synthesis rates would be advantageous to the adult, suppression of sterol synthesis rates in rapidly growing embryonic or fetal tissues could be quite detrimental since cholesterol is absolutely necessary for their proper development (22, 36, 42). Fetal cholesterol is required for membrane formation, hormone synthesis, and development of the central nervous system through activation of Sonic hedgehog (34). Consequently, fetuses with disrupted cholesterol biosynthesis experience abnormal development, as demonstrated by rodents given cholesterol synthesis inhibitors (7, 35) and individuals with Smith-Lemli-Opitz syndrome (20, 38, 42). Patients with this syndrome have abnormally low tissue and plasma cholesterol concentrations and numerous congenital birth defects, including craniofacial abnormalities, limb malformation, congenital heart defects, and holoprosencephaly, in the most severe cases (22, 42).

The purpose of the present study was to determine the impact of dietary PUFA on in vivo sterol synthesis rates in a rapidly growing tissue, the fetus, by using an animal model that responds to dietary factors as do humans (40). Previous studies that examined the role of PUFA in fetal development (29) used an animal model, the rat, that has exceedingly high hepatic and whole body sterol synthesis rates compared with the human (9) and does not express some receptors for cholesterol; placenta; yolk sac; fetus; gestation
PUFA, specifically the PPARs, during early/midgestation (2, 5). Also, we proposed to elucidate the mechanism responsible for the response to PUFA in the fetal tissues. Hampsters were fed diets enriched in medium-chain triglycerides (MCT) as a control group and either PUFA or SFA. Both male and female hamsters were used. Sterol synthesis rates were measured in the adult male hamster liver, because of its sensitivity to various dietary components (9, 40), and in the fetus and extraembryonic fetal tissues. Dietary PUFA resulted in an ~60% decrease in sterol synthesis rates in the adult liver. In contrast, fetal sterol synthesis rates were not suppressed by PUFA fed to the pregnant dam. The lack of effect was due to a lack of PUFA enrichment of the fetal fatty acid pool or to a lack of expression of fatty acid receptors in the hamster fetus. Interestingly, the adult could be converted to also become nonresponsive to dietary PUFA by creating a net negative sterol balance across the hepatocyte. Thus the cause of the dysregulation of sterol synthesis rates in the fetus could be at least partially due to a negative sterol balance across the fetus.

MATERIALS AND METHODS

Animals and diets. Male and female hamsters (Charles River Laboratories, Kingston, NJ) were housed in colony cages and exposed to a 14-h light/10-h dark cycle before and during the studies. Male hamsters were fed diets ad libitum for 3 wk before each study, and female hamsters were fed diets ad libitum for 3 wk before mating and throughout gestation. Pregnant dams were studied at 11 and 14 days of gestation; the hamster has a gestational period of 15.5 days (51).

Two different sets of diets were used for the studies. For the first set of diets, adult males and females were fed diets consisting of ground rodent chow (7102CM; Harlan Teklad, Madison, WI) mixed with 15% (wt/wt) dietary oil and 0.05% vitamin E (ICN Biomedicals, Aurora, OH) and containing an inherent cholesterol concentration of 0.001% (wt/wt); all ground rodent chow contains 0.001–0.01% inherent levels of cholesterol. Three different oils were added to the diets. The control diet contained MCT oil (Mead Johnson, Evansville, IN) comprised mainly of caprylic acid (8:0) and capric acid (10:0). MCT was fed to animals as the control diet to J have an equal distribution of calories between fat, protein, and carbohydrate in all three diets and 2) because the addition of MCT oil to chow has been shown to have no effect on sterol synthesis rates in vivo compared with diets containing 100% plain chow and thus makes an appropriate control when studying the effects of oil vs. no oil (52). Experimental diets contained either corn oil as a source of PUFA, comprises primarily of linoleic acid (18:2), or hydrogenated coconut oil as a source of SFA, comprised primarily of lauric acid (12:0). For the second set of diets, males were fed 15% oil and 0.05% vitamin E plus 3% (wt/wt) cholestyramine (a gift from Dr. Deborah Diersen-Schade, Mead Johnson Nutritional). The cholestyramine powder was mixed with the ground chow before the addition of the oils. All diets were made fresh every 2–3 wk and stored under nitrogen at 4°C. The Institutional Animal Care and Use Committee of the University of Cincinnati approved all protocols.

In vivo sterol synthesis rates. Pregnant female and adult male hamsters were injected intraperitoneally with 50 mCi \( ^{3}H \) \( ^{2}O \) (3, 8, 51). After 1 h, animals were anesthetized and exsanguinated, and adult livers, placentas, yolk sacs, and fetuses were isolated. After saponification of tissues, the digitonin-precipitable sterols were isolated and assayed for \( ^{3}H \) (8). The rates of sterol synthesis are presented as nanomoles of \( ^{3}H \) \( ^{2}O \) incorporated into sterol per hour per gram of tissue.

Cholesterol concentrations. Placentas, yolk sacs, and fetuses were isolated and saponified. The mass of cholesterol was measured by gas liquid chromatography (GLC) using stigmastanol as an internal standard (46, 51). Data are presented as milligrams of cholesterol per gram of tissue.

Tissue fatty acid compositions. Maternal livers, placentas, yolk sacs, and fetuses at 11 days gestation were isolated and pooled. Lipids were extracted, extracts were dried under \( N_{2} \) and saponified, and the fatty acids were methylated (30). Fatty acids were separated by GLC (41) and are presented as a percentage of total fatty acid content.

Immunodetection of PPAR proteins. At 11 days of gestation, fetuses were isolated from the extraembryonic tissue and snap frozen in liquid nitrogen. At 14 days of gestation, fetuses were isolated and the fetal peritoneal organs were removed and snap frozen in liquid nitrogen. The tissues were stored at \(-70^\circ C\) for 1–2 wk. PPAR protein expression was determined according to Kremarik-Bouillaud et al. (26). Proteins (200 \( \mu\g\)) were separated by electrophoresis in an 8% SDS-PAGE gel and transferred to nitrocellulose. Membranes were incubated with antibodies directed against either PPARα, -β, or -γ (Santa Cruz Biotechnology sc-9000, sc-7197 and sc-7196, respectively) at dilutions of 1:200 (α and γ) or 1:500 (β). The proteins were detected by incubation with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Boehringer-Mannheim, Indianapolis, IN) and visualized with ECL Plus (Amersham Pharmacia, Piscataway, NJ).

Statistics. Data are presented as means ± SE. Differences between mean values of MCT-fed animals and the PUFA- or SFA-fed groups were tested for statistical significance (\( P < 0.05 \)) by using the two-tailed unpaired Student’s \( t \)-test.

RESULTS

PUFA decrease the expression of enzymes and proteins necessary for cholesterol synthesis in vitro and during acute feedings (23, 55, 56). To test the role of chronically fed PUFA on sterol synthesis rates in vivo in an animal with hepatic sterol synthesis rates that parallel the human (9), male hamsters were fed diets enriched in MCT, PUFA, or SFA, and hepatic sterol synthesis rates were measured. In vivo hepatic sterol synthesis rates were decreased 58% (\( P < 0.05 \)) in hamsters fed PUFA and in the presence of a very low inherent level of dietary cholesterol compared with hamsters fed MCT (Fig. 1). Dietary SFA had no effect on hepatic sterol synthesis rates compared with hamsters fed MCT.

Before the studies in which dams were fed MCT, PUFA, or SFA, tests were completed to determine which level of oil could be added to diets to ensure normal fetal development. Females were fed 0, 5, 10, and 15% MCT before and during gestation. At 11 days of gestation, fetal weights and cholesterol concentrations were measured. There was no difference in the weight or cholesterol concentration in fetuses of dams fed any diet (data not shown). For the remainder of the studies, females were fed 15% dietary oil.
Sterol synthesis rates and concentrations were measured initially in fetuses of dams fed MCT, PUFA, and SFA before and throughout gestation. In early/midgestation, sterol synthesis rates were greatest in the yolk sac (2,673 ± 237 nmol/h per gram), next greatest in the fetus (774 ± 47 nmol/h per gram), and lowest in the placenta (315 ± 18 nmol/h per gram) of MCT-fed dams. In contrast to rates in the adult, dietary PUFA had no effect on sterol synthesis rates in any of the fetal tissues (Fig. 2, A–C). As with PUFA, dietary SFA had no effect on sterol synthesis rates in the placenta and fetus. However, in contrast to PUFA, sterol synthesis rates were reduced 22% (*P < 0.05) in the yolk sac of dams fed SFA compared with MCT-fed dams (Fig. 2B). Tissue cholesterol concentrations were similar regardless of dietary oil fed to the dam (Fig. 2, D–F). By late gestation, sterol synthesis rates decreased 76% in the yolk sac and 53% in the fetus of control dams and remained relatively constant in the placenta (Fig. 3, A–C) compared with tissues at midgestation. Neither dietary PUFA nor SFA had an effect on sterol synthesis rates in the fetal tissues, including the yolk sac, at this late stage of gestation. As one might expect, cholesterol concentrations were similar in all fetal tissues from dams fed any of the diets (Fig. 3, D–F); cholesterol
concentration increased \( \approx 25\% \) in the placentas and yolk sacs as gestation progressed.

The lack of effect of dietary PUFA on fetal sterol synthesis rates could be due to the fact that 1) the fetus did not become enriched in the maternal dietary fatty acids, 2) PUFA receptors were not expressed during gestation in the hamster since they were not expressed in midgestation of the mouse or rat (2, 5), or 3) the fetus has a dysregulation of sterol synthesis compared with the adult in steady state. Each of these three mechanisms was examined sequentially.

The fatty acid compositions of fetal and maternal tissues were analyzed first to determine if the fetal tissues became enriched in maternal dietary fatty acids (Table 1). Only the most prevalent fatty acids are represented. The maternal livers of control dams contained primarily palmitic (16:0) and stearic acids (18:0), whereas the fetuses had primarily the 16:0 fatty acid. When dams were fed PUFA, the relative amount of the linoleic acid (18:2) increased by 33% compared with amounts in MCT-fed dams. The fetuses in the PUFA-fed dams had a 44% increase in the enrichment.

Table 1. Fatty acid composition of maternal and fetal tissues

<table>
<thead>
<tr>
<th>Fatty acid chain length</th>
<th>Liver</th>
<th>Placenta</th>
<th>Yolk Sac</th>
<th>Fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCT</td>
<td>PUFA</td>
<td>SFA</td>
<td>MCT</td>
</tr>
<tr>
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<td>0.5</td>
<td>2.6</td>
<td>0.9</td>
</tr>
<tr>
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<td>26.2</td>
<td>25.5</td>
<td>28.7</td>
<td>26.0</td>
</tr>
<tr>
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<td>21.2</td>
<td>17.5</td>
<td>17.6</td>
<td>20.2</td>
</tr>
<tr>
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<td>17.8</td>
<td>23.7</td>
<td>17.2</td>
<td>18.6</td>
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</tbody>
</table>

Female hamsters were fed diets containing 15% (wt/wt) medium-chain triglyceride oil (MCT; control), polyunsaturated fatty acids (PUFA; corn oil), or saturated fatty acids (SFA; HCO) for 3 wk before gestation and throughout gestation until the day of the study. At 11 days gestation, the maternal liver, placenta, yolk sac, and fetus were removed and fatty acids were separated by GLC. Data are presented as % fatty acid of total fatty acid composition. Each value represents percentage for data pooled from 4 animals.
of the 18:2 fatty acid compared with fetuses of dams fed MCT. In livers of SFA-fed dams, there were 333 and 10% increases in the myristic (14:0) and palmitic acids, respectively, compared with MCT-fed dams. Correspondingly, the fetuses of dams fed SFA had a 59% increase of the 14:0 fatty acid. Thus the fetuses did become enriched in maternal dietary fatty acids.

PUFA can affect the nuclear receptors PPAR and LXR and modify processing of SREBPs in the adult (21). Both the LXR and SREBPs are expressed by midgestation in other rodent models (10, 49, 50), whereas PPARs are not expressed in fetuses from midgestation in the mouse and rat (2, 5). Therefore, the expression profiles of all three PPARs (α, β, and γ) were examined in fetuses of the hamster at mid- and late gestation.Contrary to what occurs in mouse and rat, PPAR isoforms were expressed by midgestation in the hamster and were still present at late gestation (Fig. 4).

The third possible mechanism for the lack of effect of PUFA in the fetus is that there is a dysregulation of sterol synthesis in the fetus compared with the adult. Previous studies have shown that the responsiveness of an animal to dietary cholesterol can be changed by manipulating sterol balance across the hepatocyte (Refs. 43 and 44 and Woollett and Dietschy, unpublished data). To test if a difference in sterol balance could change the responsiveness of an animal to dietary PUFA, adult male hamsters were placed into a negative sterol balance by feeding animals cholestyramine plus a control oil (MCT), PUFA, or SFA (43); adult male hamsters were studied because we demonstrated that sterol synthesis rates would become suppressed in these animals when fed PUFA (Fig. 1). All animals fed cholestyramine had a marked increase (>3.5-fold) in hepatic sterol synthesis rates, regardless of dietary fatty acid. Furthermore and even more importantly, dietary PUFA did not result in a decrease in hepatic sterol synthesis rates compared with rates in the MCT-fed animals when cholestyramine was added to the diets (Fig. 5).

**DISCUSSION**

These studies are the first to show that sterol synthesis rates in adult male livers and fetal tissues respond differently to PUFA. When the adult liver and fetal tissues of the hamster are enriched with PUFA, hepatic sterol synthesis rates in the adult decrease ~60%, whereas fetal tissue synthesis rates remain unchanged. This lack of effect of dietary PUFA implies a difference in the regulation of sterol synthesis in fetal tissues compared with adults. Several mechanisms could be involved in the inability of PUFA to regulate sterol synthesis rates in fetal tissues.

First, for fatty acids to have an effect within a tissue, the fatty acid must become incorporated into the lipid pool of the tissue. Even though the fetus does not come in direct contact with the maternal circulation, there are various fatty acid transporters within the placenta that will transport fatty acids to the fetus. In fact, the preferred substrate for these placental fatty acid transporters are PUFA (11, 16) because ~50% of the fatty acids within the developing brain consist of the long-chain, maternal-derived PUFA (15). Similar to that which occurs in the rat (29), there was a significant increase in the enrichment of fetal linoleic acid when dams were fed PUFA-containing diets; the degree of enrichment within the hamster fetus was slightly greater than that which occurs in the liver of the pregnant dam. Thus the lack of effect of PUFA on sterol synthesis rates was not due to a lack of enrichment of PUFA in fetal tissues.

Second, for the PUFA to exert an effect, the receptors or transcription factors that PUFA affect in adult cells or cell culture must be expressed in the fetus. Recently, PUFA have been shown to activate the nuclear receptors PPAR and LXR as well as affect the processing of SREBP. Because it is known that LXR and SREBP are expressed in murine fetal tissues during gestation (10, 49, 50) and that PPARs are not expressed in early/midgestation fetal tissues of the mouse and rat (2, 5), it was necessary to determine whether the PPARs are expressed in the hamster fetus. A lack of PPARs in the fetus could explain the lack of effect of PUFA. In contrast to other rodents, the hamster fetus expresses all three isoforms of the PPARs by midgestation. Thus the lack of effect of PUFA on fetal sterol synthesis rates is not due to a lack of expression of the PPARs. The various roles of the different forms of the PPARs in the fetus are unknown; murine fetal growth can continue in the absence of PPARα (27), possibly as the result of PPARβ having similar, redundant functions to PPARα (31), and is abnormal in the absence of PPARγ (1). It should be noted that these results do not rule out the

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**Fig. 4.** Peroxisome proliferator-activated receptor (PPAR) expression in the fetus at 11 and 14 days gestation. Female hamsters were fed diets containing 15% (wt/wt) MCT oil, PUFA, or SFA for 3 wk before gestation and throughout gestation until the day of the study. At 11 and 14 days gestation, fetuses and fetal peritoneal organs, respectively, were removed and evaluated for the expression of PPARα, β, and γ by immunoblot analysis. Bands correspond to 3 pooled samples. The apparent molecular weights of the immunoreactive bands correspond to published sizes for the PPAR isoforms (52–56 kDa) and are shown in relation to the 47-kDa band of a molecular weight marker.
possibility that these receptors are not active in the fetus or are under different regulation. Also, other receptors or transcription factors that PUFA affect may not be expressed in the fetal period.

Third, the sterol balance of the fetus or fetal tissues could be different from that of the control adult. A neutral sterol balance is depicted by an equal amount of cholesterol leaving and entering the regulatory pool of cholesterol (or the body) and is indicative of a steady state, whereas a negative sterol balance is one in which there is more cholesterol leaving vs. entering the regulatory pool of cholesterol (or the body). In the adult, a net negative sterol balance can be induced with bile acid sequestrates, such as cholestyramine, and is denoted by high hepatic sterol synthesis rates (43, 44). The fetus is unique in that it is growing rapidly and has a high requirement for cholesterol; thus it is not in a steady state as is the adult. During this rapid growth rate, if the fetus uses cholesterol as soon as it is synthesized or accrued for membranes, hormones, or signaling molecules, then the fetus could be in a negative sterol balance. Since the fetal sterol synthesis rate and requirement for sterol are both high, we hypothesize that the fetus and extraembryonic fetal tissues exist in a negative sterol balance, whereas the control adult is in a neutral sterol balance.

Previous studies (9, 43, 44) have shown that the responsiveness of an animal to dietary components is at least partially dependent on sterol balance. Most of the previous studies have examined the effect of exogenous cholesterol on sterol balance. For example, a male hamster that responds to dietary cholesterol can be converted to one that does not respond simply by putting the animal into a net negative sterol balance (Refs. 43 and 44 and Woollett and Dietschy, unpublished data). Another indication of how an animal will respond to dietary cholesterol is the rate of sterol synthesis within the liver. Animals that have higher baseline synthesis rates seem to be less responsive to exogenous sterol (9). Rats have markedly elevated hepatic sterol synthesis rates compared with the hamster (9) and are much more unresponsive to dietary cholesterol (17). Additionally, cynomolgus monkeys with higher hepatic sterol synthesis rates are more unresponsive to exogenous cholesterol compared with counterparts with lower hepatic sterol synthesis rates (47).

The fact that one could convert the male hamster that responds to dietary PUFA into one that does not respond simply by changing net sterol balance implies that responsiveness to exogenous factors may not be limited to just dietary cholesterol but may include other compounds that could affect sterol balance, such as fatty acids (6). Further support for the lack of effect of PUFA during a negative sterol balance is that nonpregnant females have different sterol synthesis rates when fed 15% MCT or PUFA (908 ± 59 vs. 612 ± 110 nmol/h per gram for MCT- vs. PUFA-fed females; P = 0.07, n = 3). However, in late gestation, when females are in a negative sterol balance, sterol synthesis rates are similar in pregnant dams fed either diet (3,311 ± 990 vs. 3,568 ± 256 nmol/h per gram for MCT- vs. PUFA-fed females; n = 3). The differences in hepatic sterol synthesis rates and responsiveness to cholesterol could be at least part of the explanation for any differences in responsiveness to dietary fatty acids obtained between different rodent models (24, 56) or studies done in the same animal species, including humans, but fed different inherent levels of cholesterol (14, 28, 39).

A change in the sterol balance across the adult liver results in changes in expression patterns of various proteins, including the SREBPs (32, 37). Under normal conditions, cholesterol synthesis is regulated primarily by SREBP-2 and fatty acid synthesis is regulated by SREBP-1c and -1a; however, when expressed at elevated levels, SREBP-1c can affect sterol synthesis rates (18). The SREBP levels can be made unresponsive to regulation under different conditions, such as overexpression of the SREBP cleavage-activating protein (SCAP) or continuous processing of SREBP to the mature form (19, 57, 58). In fact, when processing of SREBP was mutated to become continuous, the ability of PUFA to reduce SREBP-activated gene expression was lost (55).

Even though it is possible that SREBP activation and expression are different during times of negative or neutral sterol balance, recent studies (4, 12, 33, 48) have suggested that factors in addition to SREBP levels may be involved in the regulation of lipid synthesis within tissues. In one such study (12), hamsters were fed various fatty acids, and fatty acid synthesis rates, SREBP-1 mRNA, and mature SREBP-1 levels were measured. The authors found a suppression of fatty acid synthesis in animals fed PUFA with no change in SREBP-1 mRNA or mature protein levels. In a second recent study (48), a novel isoform of SREBP-2 isolated from spermatogenic cells was found not to be sensitive to sterol regulation. This protein is synthesized as a soluble transcription factor that is constitutively active and bypasses the sterol-regulated proteolytic activation of SREBP.
Interestingly, at 11 days of gestation there was a slight decrease in sterol synthesis rates in the yolk sacs of dams fed saturated fatty acids. This appeared to be a transient effect because there was no difference in synthesis rates by 14 days of age. Because the cholesterol concentration of the tissue was similar to those measured in tissues of dams fed other fatty acids, the slight decrease could have occurred to regulate differences in uptake of exogenous maternal lipoproteins (51).

In conclusion, these data demonstrate that PUFA enrichment inhibits sterol synthesis rates in the adult male liver but not in the fetus, indicating a difference between fetal and adult regulation of sterol synthesis rates. The mechanism for this difference appears to be at least partly due to a negative sterol balance that exists across the fetus, or possibly any rapidly growing tissue. These are important data since the consumption of PUFA has increased recently as the result of dietary recommendations by the American Heart Association. An inhibition of sterol synthesis rates by PUFA in the fetus of women consuming a PUFA-enriched cholesterol-lowering diet would have severe consequences due to the essential requirement of cholesterol for normal development. Thus it is a benefit for fetal development that fetal sterol synthesis rates are unresponsive to regulation by dietary PUFA.

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DISCLOSURES

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REFERENCES


