Origin of cholesterol in the fetal Golden Syrian hamster: contribution of de novo sterol synthesis and maternal-derived lipoprotein cholesterol

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Abstract A fetal hamster increases in mass almost 100-fold in the third trimester of gestation. During this 5.5-day period, the acquisition of over 4 mg of cholesterol is required for normal development. The purpose of the present studies was to determine the potential source(s) of this fetal sterol. Rates of cholesterol synthesis in the whole fetus were measured initially. Synthesis rates in the whole fetus increased linearly from 10 days (≈25 nmol sterol/h) through 13.5 days of gestation (≈400 nmol sterol/h). During the last 1.5 days of intrauterine development, rates remained constant. Even though the synthesis rates were relatively elevated, as compared to those in an adult, the amount of cholesterol synthesized was about half of that accrued. When synthesis rates in all of the fetal tissues were summed, however, a majority of the sterol in the fetus could now be accounted for. During this same time when the fetus was accumulating 4 mg of cholesterol, the placenta and yolk sac increased in cholesterol content by 2.5 mg, indicating the need for a second source of sterol for fetal tissue development. Two other sources of sterol for these tissues were found to be maternal low density and high density lipoprotein (LDL and HDL, respectively). In fact, more than 0.9 mg of cholesterol was taken up during the third trimester as LDL. To summarize, a majority of cholesterol in the fetus could be accounted for by synthesis in all fetal tissues. Additionally, a significant amount of cholesterol was taken up as maternal-derived LDL and HDL by these same tissues.—**Woollett, L. A.** Origin of cholesterol in the fetal Golden Syrian hamster: contribution of de novo sterol synthesis and maternal-derived lipoprotein cholesterol. *J. Lipid Res.* 1996. **37:** 1246–1257.

Supplementary key words fetal development • pregnancy • gestation • cholesterol synthesis • low density lipoprotein • high density lipoprotein

During development, the fetus grows at a rate unparalleled at any other stage of life. In order for this to occur, a significant amount of sterol is required for membrane synthesis, maintenance of membranes, hormone synthesis, and bile acid synthesis (1). The fetus has two possible sources of cholesterol, as do infants, children, and adults. The first source is endogenous, and it constitutes cholesterol synthesized within the fetus or that synthesized in the whole body of the post-partum individual. Cholesterol also could originate from an exogenous source. Infants, children, and adults consume their exogenous source of sterol. In the fetus, this cholesterol would correspond to any source of sterol that has been transferred by way of the placenta and yolk sac to the fetus, and could include cholesterol synthesized within these other fetal tissues and/or cholesterol derived from the maternal circulation in the form of lipoproteins.

With the exception of the liver, brain, and endocrine glands, most organs contain 1.5–2.0 mg of cholesterol per g of tissue (2). Thus, in the late-term fetal hamster, rat, and human that each weighs 3 g, 6 g, and 4000 g, respectively, at least 4.5 mg, 9 mg, and 6000 mg of cholesterol would be required for growth and development during gestation, which is a major amount of sterol to be accrued over a relatively short period of time. Previous studies have demonstrated an elevated rate of sterol synthesis when activities were normalized to 100 g body weight during gestation as compared to adult life (3–9), and thus de novo synthesis most likely contributes a majority of the sterol deposited.

In addition to the fetus, the placenta and yolk sac also require a significant amount of sterol for membrane formation and for hormone production. In the human, the placenta increases on average 570 g in mass (10) during the course of pregnancy which corresponds to an additional 1140 mg of cholesterol required for tissue growth alone (11). As might be expected in tissues of

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; DPS, digitonin-precipitable sterols; apoB-100, apolipoprotein B-100; TCB, tyramine cellobiose; LDL-C and HDL-C, cholesterol carried in LDL and HDL, respectively; apoA-I, apolipoprotein A-I; VLDL, very low density lipoprotein; apoE, apolipoprotein E; LRP, LDL receptor-related protein; gp330, glycoprotein 330.

fetal origin, sterol synthesis rates are greater per g placenta and yolk sac than those in most maternal tissues (5, 12). The newly synthesized cholesterol in these tissues could be used either for membrane formation or be transported across the placenta to the fetus through a not yet defined pathway. This sterol would most likely not be used for hormone production as the reproductive steroid hormones appear to be derived preferentially from cholesterol carried in maternal lipoproteins (13, 14) which bind to these fetal tissues and are internalized both in vitro and in vivo (5, 15–17).

A number of studies have attempted to quantitate the proportion of fetal cholesterol originating from de novo synthesis as compared to the amount derived from maternal sources. Two different types of experimental approaches have been applied to investigate this question. In one, the amount of radiolabeled maternal lipoprotein that crosses the placenta and enters the fetus is examined (16–21). The values for contribution of maternal cholesterol to the fetus in these studies has varied from essentially 0% to 65%, and is still under much debate. The second type of study more directly estimates the requirement for exogenous sterol in the fetus in that the absolute amount of sterol synthesized in the fetus is measured in vivo (5). From these more direct studies, rates of synthesis in all the fetal tissues in the rat were found to be great enough to account for the cholesterol accumulated during intrauterine development (5). While the rat fetal tissues may have sufficient endogenous cholesterol to fully support growth and development of the fetus, the indirect studies described above would imply that in some other species, a portion of fetal sterol may originate from the maternal circulation.

Thus, the overall objective in the present study was to examine all of the potential sources of cholesterol in the fetus of the Golden Syrian hamster, an animal that regulates cholesterol metabolism similar to that found in humans. The hamster was chosen for the model in these studies for several reasons. First, rates of sterol synthesis are relatively low in both the hamster and human, with a majority of the synthesis occurring in extrahepatic tissues (2). Second, hamsters respond to dietary lipid treatments similar to humans (22–24). Third, and possibly most important, during the third trimester of gestation, both the hamster and human become hypercholesterolemic, with the main increase occurring in the low density lipoprotein (LDL) fraction (25–29). Thus, using the hamster as the model for humans, the three specific objectives of these studies will be: *1)* to characterize and quantitate growth and development of all fetal tissues, including the fetus, the placenta and the yolk sac, with a focus on cholesterol acquisition; *2)* to measure the absolute rates of sterol synthesis in all fetal tissues; and *3)* to determine the clearance rates of maternal-derived lipoprotein cholesterol, specifically LDL and high density lipoprotein (HDL), in these same tissues.

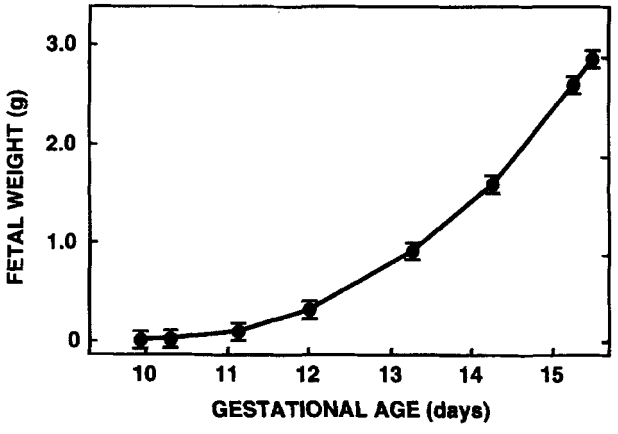

Fig. 1. Fetal weight as a function of gestational age in time-mated hamsters. Dams were killed and several fetuses were removed from three different dams and weighed. Values presented are the averaged means of the fetuses ± 1 SEM (n = 3). The SEM was 1–2% of the meaned weights.

MATERIALS AND METHODS

Animals and diets

Male and non-pregnant female Golden Syrian hamsters (*Mesocricetus auratus*) (Charles River Laboratories, Montreal, Canada), weighing 100 and 90 g, respectively, were housed on wood shavings in colony cages and subjected to 12 h of darkness and 12 h of light. The room in which the animals were housed was temperature- and humidity-controlled. Animals were fed ad libitum a pelleted cereal-based diet (Rodent Diet no. 7001) (Teklad Premier Laboratory Diets, Madison, WI). After 1 week, the diet of the animals was changed to a ground Wayne Lab Blox (no. 8604) (Teklad) which had an inherent cholesterol and triacylglycerol content of 0.02% (wt/wt) and 5% (wt/wt), respectively. Two weeks after the ground diet was begun, the animals were mated according to the following protocol. At noon, each male hamster was separated into an individual cage. A few hours prior to the beginning of the dark cycle, one female was placed into each cage with a male and remained there until noon the next day. This protocol was repeated over a 4-day period, which corresponds to the length of the estrus cycle of the hamster (30). After the 4-day mating period, males were removed. Females remained housed individually until the time of the study. Pregnant dams were studied 13.5 and 15.5 days after the males and females were initially placed together, in the middle of the dark phase. With this approach, a continuous spec-

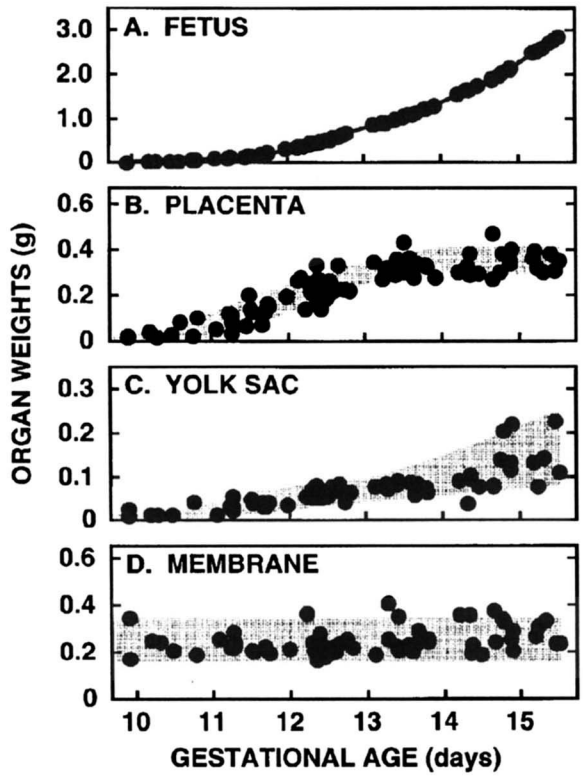

Fig. 2. Weight of the fetus, the placenta, the yolk sac, and the uterine membrane in dams 9.9 through 15.5 days into gestation. The fetus (A) was initially isolated from the placenta (B), the yolk sac (C), and the uterine membrane (D) at 9.9 days gestation when the fetus weighed 0.030 mg. Each data point represents an average of two different fetuses, placentas, yolk sacs, and membranes within one pregnant dam.

trum of fetal ages from 9.9 to 15.5 days of gestation was obtained.

Timed pregnant females also were purchased from Charles River Laboratories (Kingston, NY) and were used only when specified. Animals were shipped within 2–5 days of mating. Upon arrival, dams were placed in individual cages and were fed ground Wayne Lab Blox (no. 8604). The benefit of these animals was that the exact hour when the dams became impregnated was known. Experiments with all animals were approved by the Institutional Animal Care and Research Advisory Committee.

Generation of fetal hamster growth curve

Pregnant dams were purchased from Charles River Laboratories. Dams were sacrificed at 9.2, 9.9, 10.3, 11.1, 12.0, 13.3, 14.3, 15.3, and 15.5 days into gestation. At each time point, three dams were sacrificed. Three to four fetuses from each dam were isolated and weighed. The means ± 1 SEM of the averaged fetal weights were plotted as a function of age.

Determination of tissue weights and cholesterol concentration and content

The fetus, the placenta, and the yolk sac were initially separable from one another for analyses when the fetus weighed 0.030 g which occurred at 9.9 days after mating. The uterine membrane surrounding the fetal unit, including the decidua, was isolated also. The amnion was combined with the yolk sac in all studies. The weights of the fetus, the placenta, the yolk sac, and the membrane were determined throughout fetal development. These same tissues were saponified, and the amount of cholesterol in each tissue was measured by GLC using stigmastanol as an internal standard (31). Data are presented as both cholesterol concentration (mg/g tissue) and cholesterol content (mg/organ).

Determination of rates of sterol synthesis in vivo

Pregnant females were injected intravenously with 100 mCi of ^3H_2O. After 60 min, dams were anesthetized and blood was collected by exsanguination from the

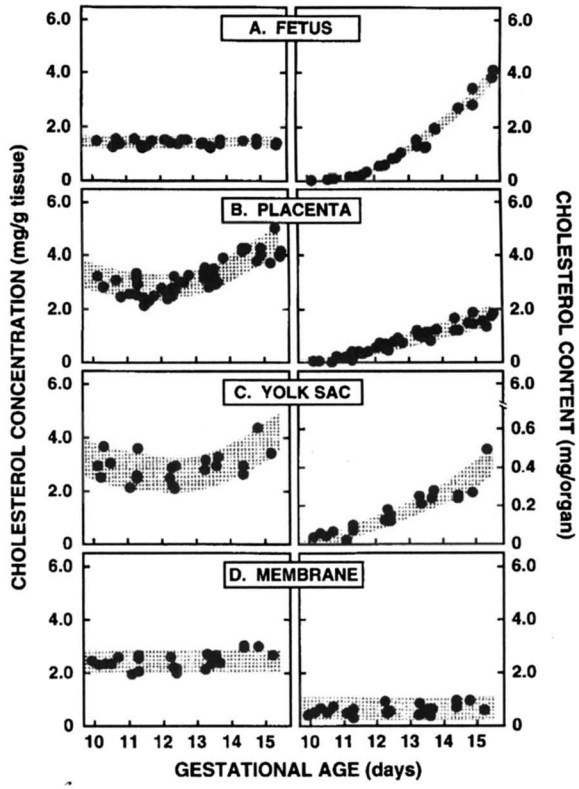

Fig. 3. Cholesterol concentration and content in the fetus, the placenta, the yolk sac, and the uterine membrane from dams 9.9 through 15.5 days into gestation. Concentration (mg/g tissue) is shown on the left vertical axis of the figure in the fetus (A), the placenta (B), the yolk sac (C), and the uterine membrane (D). Cholesterol content (mg/organ) is presented on the right vertical axis of the figure in the same tissues. One fetus, placenta, yolk sac, or uterine membrane was isolated from each pregnant dam studied and is presented as an individual data point.

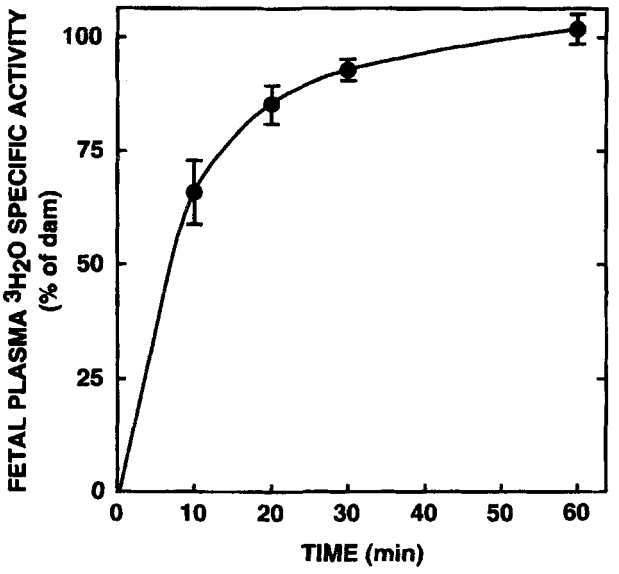

Fig. 4. Plasma specific activity of ^3H_2O in fetuses relative to pregnant dams over a 60-min period. Hamsters 14.5 days into gestation were administered 100 mCi ^3H_2O. At 10, 20, 30, and 60 min after injection of the radiolabeled water into the female, animals were exsanguinated and plasma was collected from the abdominal aorta of the female and after decapitation of the fetus. Plasma from 3–4 fetuses was pooled. Two, three, or four dams and their fetuses were evaluated at each time point. Data are presented as fetal specific activity divided by maternal specific activity. Specific activity of ^3H_2O is calculated by dividing the dpm per µl plasma by the nmol water per ml of water times the ml of water per ml of plasma.

abdominal aorta. Fetal tissues were isolated rapidly; the tissues collected were the fetus, the placenta, the yolk sac, and the uterine membrane. Tissues were saponified and digitonin-precipitable sterols (DPS) were isolated and assayed for ^3H content (5, 32). The loss of DPS through this extraction and isolation procedure was also determined. The rates of synthesis are presented as nmol ^3H_2O incorporated into sterol per h per g tissue or per h per organ. Additionally, synthesis rates were converted to µg of cholesterol synthesized each day knowing 1.45 carbon atoms were incorporated into DPS for each ^3H atom (2, 32) and that the sterol isolated was cholesterol (5).

To determine the specific activity of ^3H_2O in the plasma of the fetus in relation to that in the pregnant dam in the 60-min study, pregnant hamsters were purchased from Charles River Laboratories and at 14.5 days into gestation were injected intravenously with 100 mCi of ^3H_2O. Dams were anesthetized and exsanguinated at 10, 20, 30, and 60 min after the injection of ^3H_2O. Each fetus was isolated quickly and blood was collected after decapitation. Approximately 100 µl of plasma was collected from 3–4 fetuses. Plasma was isolated and dpm of ^3H was determined in the pooled fetal plasma and the plasma of the dam. To calculate the specific activity of ^3H_2O, the dpm of ^3H in 100 µl of plasma was divided by the nmol of water times the ml of water per ml of plasma (5). In the fetus, 0.96 ± 0.01 ml of water was present in each ml of plasma, whereas in the dam, the value was 0.90 ± 0.01 ml. These values are very similar to those found previously in the rat dam and fetus (5).

Determination of clearance of maternal-derived LDL in vivo

The clearance of LDL was measured as previously described (22, 23, 33). Briefly, LDL was isolated from donor hamsters fed a low fat, low cholesterol diet in the density range of 1.020 to 1.055 g/ml. Apolipoprotein B-100 (apoB-100) was the predominant apolipoprotein present in this density fraction (34). The LDL was labeled with ^{125}I-tyramine cellobiose (TCB) (35) or ^{131}I (36). The ^{125}I-TCB-labeled LDL was used to determine the uptake of the LDL, whereas the ^{131}I was used to correct for plasma remaining in tissues. A femoral vein catheter was placed into the female through which a bolus of ^{125}I-TCB-labeled LDL was given that was equal to the amount of LDL cleared from the plasma over an hour. The bolus was followed by a 4-h infusion to maintain a constant specific activity of plasma LDL. Ten min prior to completion of the infusion, the ^{131}I was injected via the catheter. Animals were anesthetized, exsanguinated and tissues were excised rapidly. The fetus, the placenta, the yolk sac, and the surrounding membrane were assayed for ^{125}I and ^{131}I. Using the dpm of ^{125}I and ^{131}I in the plasma of the dam, the rates of LDL clearance by the various tissues were calculated. Data are presented as µl of plasma cleared of its LDL content per h per g tissue or per h per organ.

Determination of uptake of maternal-derived LDL-cholesterol in vivo

The uptake of LDL-cholesterol (LDL-C) by the various tissues was determined by multiplying the rate of LDL clearance times the concentration of circulating LDL-C in the pregnant dam; plasma was collected from the dam and the amount of cholesterol in the LDL fraction (1.020 to 1.063 g/ml) was quantitated.

Determination of clearance of maternal-derived HDL in vivo

Clearance of HDL was performed similarly to that of LDL. Briefly, the HDL was isolated from donor hamsters fed a low fat, low cholesterol diet. Lipoproteins used for this preparation were those present in the range of 1.070 to 1.210 g/ml. In the hamster, apolipoprotein A-I (apoA-I) is essentially the only protein present in this fraction (34). The HDL was labeled and the experiment was performed as described above. Choles-

terol carried in the HDL fraction was that in the density range of 1.063 to 1.21 g/ml.

RESULTS

Fetal tissues were examined initially at 9.2 days into gestation. At this stage of development, the fetus was indistinguishable from the yolk sac or the uterine membrane plus decidua. It was not until 9.9 days into gestation that the fetuses, weighing 0.030 g, could be separated from these other fetal tissues. Within 2.5 days of the initial separation, the fetuses increased in mass over 18-fold to 0.55 g. In the next, and final, 3 days of gestation the weights of the fetuses increased another 2.30 g corresponding to a 4.2-fold increase in mass. The fetal weights from these animals were plotted as a function of gestational age to generate a current growth curve (**Fig. 1**).

The weights of the fetuses used in the studies were superimposed onto the growth curve described in Fig. 1 to determine the gestational age of the experimental pups (**Fig. 2A**). As the fetus was growing in mass, so were the placenta and yolk sac, though not at such a rapid rate. Initially, when the fetus weighed 0.030 g, the placenta weighed 0.020 g (Fig. 2B) and the yolk sac weighed 0.015 g (Fig. 2C). Throughout the third trimester, the placenta increased in mass 18-fold to ≈0.35 g while the mass of the yolk sac was elevated 12-fold to ≈0.18 g. The fourth tissue examined in these studies was the uterine membrane plus decidua (Fig. 2D). At the beginning of gestation, this tissue weighed 0.25 ± 0.07 g and, thus, made up a significant proportion (79%) of the fetal unit. By the time of parturition these tissues had not increased in mass and constituted only 7% of the whole unit.

As the fetal tissues grew and developed, their requirements for cholesterol appeared to change as demonstrated in their concentration and content of cholesterol (**Fig. 3**). As seen in panel A, the concentration of cholesterol present in the whole fetal animal remained constant at 1.48 ± 0.02 mg/g as the fetus increased in mass from 0.03 to 2.85 g. However, when presented on a whole tissue basis, the amount of cholesterol increased from ≈0.04 to 4.16 mg in a manner that replicated the increase in mass of the fetus. Unlike the fetus, in which the concentration of cholesterol remained constant throughout gestation, the concentration of cholesterol in the placenta increased from ≈3.1 to 4.5 mg/g (Fig. 3B). Cholesterol content increased even more dramatically than did concentration in this tissue (≈0.07 to 1.82 mg). In the yolk sac, the concentration of cholesterol at 10 days of gestation was approximately the same as that in the placenta and increased slightly, whereas, similar to the fetus and placenta, the amount of cholesterol in the whole yolk sac rose dramatically from ≈0.05 to 0.40 mg as gestation progressed. Finally, the cholesterol concentration (2.54 ± 0.07 mg/g) and content (0.66 ± 0.04 mg) in the uterine membrane and decidua was approximately the same throughout development.

The origin of this recently acquired cholesterol, whether it be from cholesterol synthesized within the various fetal tissues or from cholesterol taken up as maternal-derived lipoproteins, was determined in the remainder of the studies. Cholesterol synthesis was measured first, using ^3H_2O as the radiolabeled marker. In contrast to maternal tissues, plasma water of the pregnant female does not equilibrate rapidly with the water of the fetus and fetal-derived placenta and yolk sac because the maternal blood has to pass through the trophoblasts to reach these fetal tissues (5, 37, 38). When the equilibration of ^3H_2O in the fetal hamster was compared to that in the pregnant dam, it was found that over a 60-min period, the specific activity of ^3H_2O in the fetus was 81.4% of that in the dam (**Fig. 4**). This value

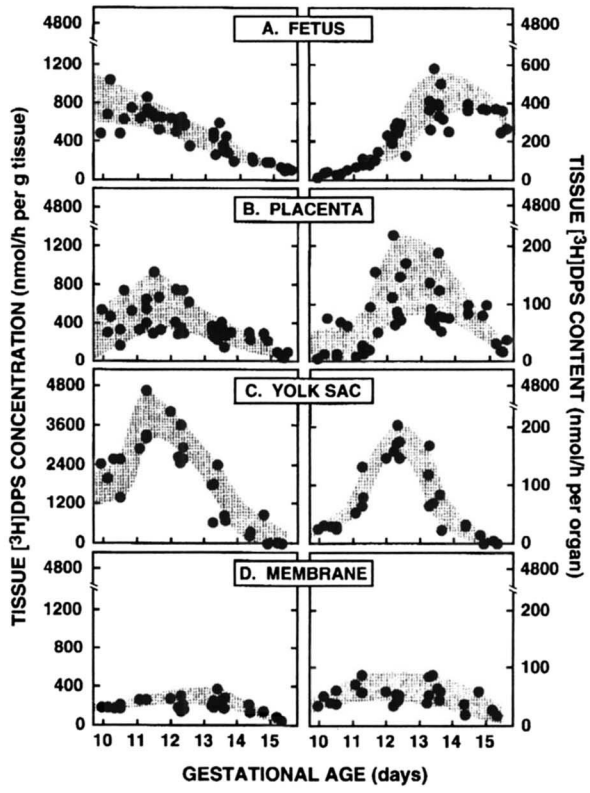

Fig. 5. Content of newly synthesized cholesterol in the fetus, the placenta, the yolk sac, and the uterine membrane from 9.9 to 15.5 days gestation. The left vertical axis represents the nmol of ^3H_2O that is incorporated into digitonin-precipitable sterols (DPS) per h per g tissue in the fetus (A), the placenta (B), the yolk sac (C), or the uterine membrane (D). The right vertical axis represents the amount of ^3H_2O that is incorporated into DPS per h per organ. One fetus, placenta, yolk sac, or uterine membrane was isolated from each pregnant dam studied and is presented as an individual data point.

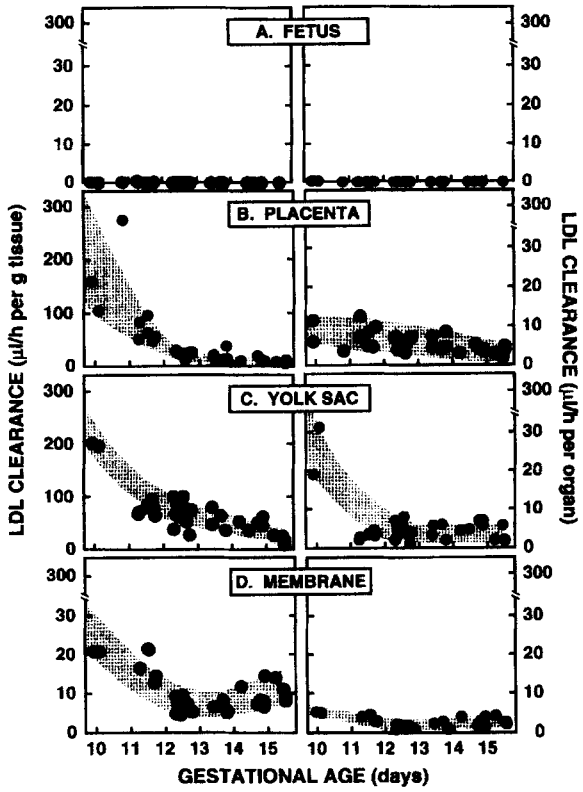

Fig. 6. Clearance of LDL from plasma by the fetus, the placenta, the yolk sac, and the uterine membrane in dams 9.9 to 15.5 days into gestation. Clearance rates on the left vertical axis are expressed as µl of plasma cleared of its LDL per h per g tissue in the fetus (A), the placenta (B), the yolk sac (C), or the uterine membrane (D). Rates are expressed as µl LDL cleared per h per organ on the right vertical axis in these same tissues. One fetus, placenta, yolk sac, or uterine membrane was isolated from each pregnant dam studied and is presented as an individual data point.

was used to calculate specific activity of ^3H_2O, and thus sterol synthesis, in the fetus, the placenta and the yolk sac, from values of plasma ^3H_2O specific activities in the dam.

Sterol synthesis rates were examined within the fetus itself, as well as the other fetal tissues and the uterine membrane. All data are presented as nmol cholesterol synthesized in each g of tissue as well as that in the whole organ because these tissues are increasing in mass at the same time they are developing. In the fetus, rates of sterol synthesis, on a per g basis, were the greatest in the smallest-sized fetus at ≈800 nmol/h per g (**Fig. 5A**). As the fetus matured, the amount of sterol synthesized decreased to ≈100 nmol/h per g just prior to birth. When these same data were expressed on a whole fetus basis, the rates of synthesis were lowest in the smallest fetus (≈25 nmol/h) and increased up to ≈400 nmol/h as the fetus grew. At 13.5 days into gestation, however, rates leveled off even though the fetus was still accruing cholesterol. At the time of parturition, rates fell to ≈250 nmol/h. In the placenta (Fig. 5B), the amount of cholesterol synthesized per g in the 10-day-old pre-term animal was ≈300 nmol/h per g, increased to ≈600 nmol/h per g by 11.5 days of age and then decreased to ≈100 nmol/h per g by birth. When the whole placenta was examined, synthesis increased from ≈30 to 120 nmol/h as the tissue grew in mass and as rates per g tissue were elevated. Similar to the fetus, rates decreased as parturition drew near. Even though synthesis in the fetus and placenta were relatively elevated, they were not as high as those in the yolk sac (Fig. 5C). In the yolk sac, synthesis rates were ≈1800 nmol/h per g in an animal 10 days into gestation. As gestation progressed to 12 days post-conception, rates increased further to values greater than 4000 nmol/h per g. In the later stages of pregnancy, however, sterol synthesis fell dramatically. Even though the amount of sterol synthesis per g yolk sac was higher than the other tissues, values were much lower in the whole tissue and increased to a maximum of only ≈180 nmol/h because of the small size of the yolk sac. In contrast to the fetal tissues, rates of synthesis in the maternal uterine membrane (Fig. 5D) were moderate throughout the whole gestational period.

Studies were next performed to quantitate the amount of maternal-derived cholesterol, in the form of maternal lipoproteins that were taken up by the fetal tissues. The clearance of LDL was examined first. In the fetus (**Fig. 6A**), rates of clearance of apoB-100-labeled LDL were less than 1 µl/h when presented as either per g fetus or per whole fetus. In the placenta, clearance rates per g tissue were much greater than rates in the fetus at ≈210 µl/h per g. As gestation continued, rates fell to progressively to ≈12 µl/h per g placenta. In the whole placenta, rates were initially low (≈9 µl/h) and decreased slightly. Similar to the placenta, clearance rates in the yolk sac fell from ≈225 to 20 µl/h per g during the third trimester. When tissue mass was accounted for, rates in the yolk sac decreased from only ≈25 to 4 µl/h. Finally, the membrane cleared ≈25 µl/h per g early in the third trimester. By the time of parturition, ≈10 µl of LDL was cleared per h in each g of this tissue. This same pattern was found when clearance rates were determined in the whole membrane.

From these measurements it was possible to determine the absolute rate of uptake of LDL-C. To determine uptake rates (µg LDL-C/h per g tissue), the clearance rates of the various tissues in each dam were multiplied by the plasma LDL-C concentration (**Fig. 7**) in that same animal. Similar to the human (26–29, 39), a slight decrease in maternal plasma LDL-C levels occurred early in the pregnancy, followed by a dramatic increase in plasma LDL-C concentration from ≈15 to 110 mg/dl. The result of multiplying the clearance rates

of LDL (Fig. 6) by plasma LDL-C concentrations (Fig. 7) appears in **Fig. 8.** In the fetus, the amount of LDL-C taken up as intact LDL was essentially zero at any age (Fig. 8A). In the placenta (Fig. 8B), ≈30 μg of LDL-C was taken up per h by each g placenta at 10 days into gestation. Uptake decreased by 11.5 days of age to ≈11 μg/h per g and remained as such throughout the third trimester. However, because the mass of this tissue expanded 18-fold during this period, the amount of LDL-C taken up by the whole placenta actually increased from ≈0.5 to 7 μg/h. Just prior to parturition, a decrease in uptake occurred. The uptake of LDL-C per g of yolk sac was initially the same as that of the placenta. In contrast to the placenta, rates increased to ≈60 μg/h per g before decreasing just prior to birth. As a result of the increase in uptake in each g of yolk sac and an increase in tissue mass, uptake rates increased almost 20-fold throughout gestation from ≈0.4 to 7 μg/h. Finally, LDL-C was taken up in each g, as well as the whole uterine membrane, in increasing amounts as pregnancy progressed.

To continue the examination of maternal-derived lipoprotein uptake by the fetal tissues, HDL clearance rates were determined in the final set of experiments. Cholesterol from HDL is taken up by tissues through two different processes (40): first, by the removal of the whole lipoprotein particle and second, by the transfer the cholesteryl ester out of the HDL. In the present report, the clearance of the whole lipoprotein particle by the tissue was studied in animals 12 and 15 days into gestation. However, because cholesteryl ester uptake rates were not determined in the present study, it cannot be assumed that the whole lipoprotein particle was internalized and degraded. The clearance rates of HDL (**Table 1**) were on average greater than those for LDL. Similar to LDL, the fetus had no significant clearance of the protein-labeled HDL particle, and rates were highest in the yolk sac at both 12 and 15 days into gestation.

DISCUSSION

Cholesterol deposition in the body is directly related to growth in that cholesterol is an integral part of membranes in all tissues. When the growth rate of an animal is minimal, as in adulthood, rates of synthesis and uptake of LDL-C remain relatively low and constant (41, 42). Conversely, when body mass is increasing rapidly, such as during infancy and childhood, cholesterol is synthesized and/or taken up by tissues, as LDL-C, at a relatively higher rate than that found in the adult (41, 42). The greatest increase in body mass, however, occurs during fetal development. A fetal hamster will increase in mass almost 100-fold in just the 5.5-day third trimester alone, whereas, a suckled hamster will increase in mass 8-fold in 21 days (L. Woollett, unpublished observation). This expansion in new fetal tissue corresponds to an elevation in cholesterol content from 0.045 to 4.12 mg, not including the amount of sterol converted to bile acids.

It has been shown in a number of species that sterol synthesis per 100 g body weight is elevated during gestation as compared to a neonate or an adult animal, and may be great enough to account for all of the sterol deposited during this stage of life (3–9). To determine whether the amount of sterol synthesized was as great as that accumulated throughout gestation in the fetal hamster, the nmol of ^3H_2O incorporated into cholesterol per h in each fetus (Fig. 5) was converted to the μg of cholesterol formed during the entire 5.5-day period (32). From these calculations, it was found that only ≈40% of the mass amount of cholesterol deposited in the fetus could be accounted for by de novo sterol synthesis. However, when synthesis rates in all of the fetal tissues plus the uterine membrane and decidua were combined, most of the cholesterol deposited in the fetus could now be explained. These results are nearly the same as those found previously in the rat when nmol ^3H_2O incorporated into sterol per h per g fetus (5) was converted to μg sterol per third trimester in the whole fetus. Unfortunately, these studies in both the hamster and rat can be used only to determine whether the rates of synthesis were great enough to account for the amount of sterol accrued and cannot be used to determine whether the newly synthesized sterol in the placenta, yolk sac, and uterine membrane was indeed the exogenous source of sterol for the fetus.

During this same stage of life in which the fetus was increasing exponentially in mass, the placenta and yolk sac were also growing rapidly. Associated with their

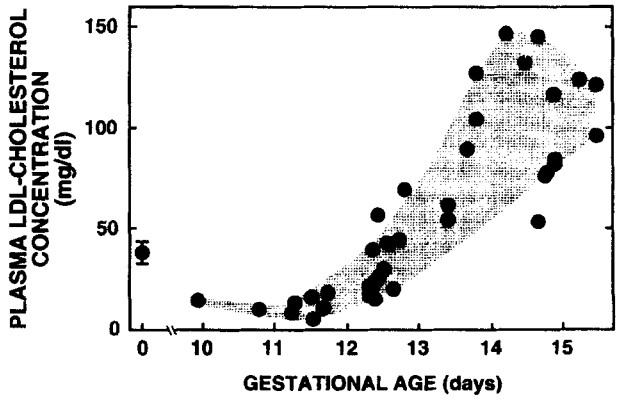

Fig. 7. Plasma LDL-C concentration in pregnant hamsters 9.9 to 15.5 days into gestation. The concentration of cholesterol was defined as that in the density range of 1.020–1.063 g/ml. In this range, apoB-100 is the predominant apolipoprotein. Each data point represents an individual dam, except for the non-pregnant female value which includes three animals.

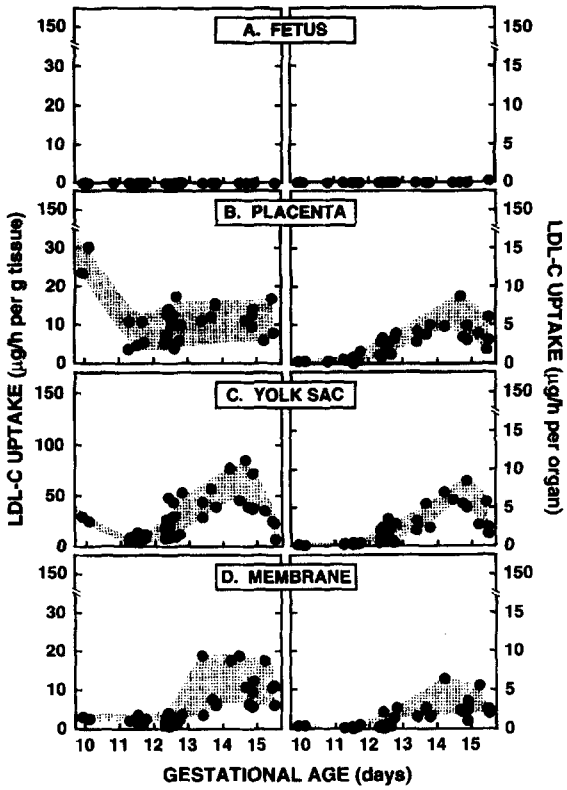

Fig. 8. Uptake of LDL-C from maternal plasma by the fetus, the placenta, the yolk sac, and the uterine membrane in dams 9.9 to 15.5 days into gestation. Uptake rates were calculated by multiplying clearance rates described in Fig. 6 by the LDL-C concentration of the pregnant dams presented in Fig. 7. Rates of uptake are presented as μg LDL-C taken up per h per g tissue on the left vertical axis in the fetus (A), the placenta (B), the yolk sac (C), and the uterine membrane (D). The right vertical axis presents data as μg LDL-C taken up per h per organ. One fetus, placenta, yolk sac, or uterine membrane was isolated from each pregnant dam studied and is presented as an individual data point.

increase in mass was an addition in cholesterol content of almost 2.5 mg. Assuming that the newly synthesized sterol in the placenta and yolk sac was transported and utilized by the fetus, then these two fetal-derived tissues would need another source of cholesterol to support their growth and development, as well as hormone production. From previous data, it appeared that LDL could be an excellent source of sterol for these tissues in that this lipoprotein has been shown to be taken up by the placenta and yolk sac, both in vitro and in vivo (5, 13–17). In the present studies, clearance of LDL from the plasma was relatively high in the placenta and yolk sac early into the third trimester and fell dramatically as gestation progressed. The initial explanation for this decrease was that these tissues were developing and differentiating, resulting in a decrease in the amount of LDL receptor they expressed. However, at the same time that the clearance rates were falling, the concentration of LDL-C in the maternal circulation was increasing. As plasma LDL-C concentration increased further and clearance rates became more depressed, a sequential increase in the amount of LDL-C taken up per g tissue occurred. The elevation in uptake was augmented even more when the increase in tissue mass was combined with the elevation in uptake of LDL-C per g tissue. It is presently unknown whether the elevation in uptake was due solely to the elevation in maternal LDL-C concentration, which would be expected in a tissue with a large receptor-independent uptake component, or whether receptor-mediated uptake, presumably via the LDL receptor, changed in response to either tissue development or to an alteration in the balance of cholesterol across the tissues. When the absolute amount of LDL-C taken up by these tissues was calculated for the full third trimester, ≈360 μg of cholesterol was taken up by the placenta, ≈300 μg was taken up by the yolk sac, and ≈250 μg was removed by the uterine membrane and decidua, which added up to over 900 μg of cholesterol in this 5.5-day period. Even though this is a significant amount of sterol, it still is less than the amount of cholesterol that the placenta and yolk sac had accumulated during the third trimester.

Another possible exogenous source of sterol for the placenta and yolk sac present in the maternal circulation is cholesterol carried in HDL. Steroidogenic tissues have been shown to take up a large amount of HDL-C, presumably for steroid hormone synthesis (43). Additionally, HDL has been shown to bind to human placental membranes (44). Thus, it seems plausible that the placenta, an endocrine-like tissue, and possibly the yolk

TABLE 1. Clearance rates of HDL by fetal tissues and the uterine membrane of the Golden Syrian hamster at 12 and 15 days into gestation

Organ	Clearance Rates
	μl/h per g tissue
12 days pregnant	
Fetus	0.1 ± 0.1
Placenta	28.6 ± 6.0
Yolk Sac	524.8 ± 45.2
Uterine membrane	10.3 ± 1.5
15 days pregnant	
Fetus	0.1 ± 0.1
Placenta	40.9 ± 4.0
Yolk Sac	234.0 ± 39.4
Uterine membrane	19.9 ± 2.4

Clearance rates were determined using a primed-continuous infusion of ^{125}I-tyramine cellobiose-labeled HDL as described in Methods. Plasma HDL-C concentrations of the dams were 25 mg/dl at 12 days and 59 mg/dl at 15 days into gestation. Values represent means ± 1 SEM for four 12-day pregnant and three 15-day pregnant animals.

sac, could also remove HDL. When measured in vivo, clearance rates were higher for HDL than for LDL in both the placenta and yolk sac even though clearance rates in the liver late into gestation were 2.2-fold greater for LDL than HDL (40 ± 5 vs. 18 ± 4 μl/h per g, respectively). Because the concentration of HDL-C increased throughout the third trimester from 25 to 59 mg/dl, the decrease in clearance could be the result of the change of the concentration of HDL-C in the maternal plasma. However, it is also possible that the decrease in clearance was due to developmental changes not yet examined. Without clearance rates of cholesteryl ester-labeled HDL, however, the exact amount of cholesterol taken up as HDL in these studies cannot be calculated as was done with LDL. Nevertheless, if the assumption were made that all HDL cleared was internalized and degraded, then HDL could have contributed 260 ± 27 μg cholesterol per day early into the third trimester and 537 ± 77 μg per day late into gestation. It appears that HDL could potentially contribute a significant proportion of the cholesterol required by the fetal tissues.

Recent data also suggest that other receptors of the LDL receptor family (45), including the very low density lipoprotein (VLDL) receptor, the LDL receptor-related protein (LRP), and glycoprotein 330 (gp330), may be involved in the uptake of maternal lipoproteins by the placenta and yolk sac. The VLDL receptor, responsible for removal of VLDL and apolipoprotein E (apoE)-containing lipoproteins, is highly expressed in placental trophoblasts (46), and expression is elevated as gestation progresses (47). Also found in trophoblasts is LRP (48, 49), which is involved in the clearance of apoE-containing lipoproteins such as chylomicron remnants (50). While the function of these receptors in the placenta is not fully known at the present time, they could be supplying the fetal tissues with fatty acids and sterol for energy and to be used in membrane formation (47, 51, 52), and as such, they may be essential for development of the fetus. In contrast to the placenta, gp330 is highly expressed in the visceral yolk sac (53, 54). This lipoprotein receptor removes apoB-100- (55) and apoE-containing lipoproteins (56) and may have been partially responsible for the removal of LDL by this tissue in the present study. The elevated uptake rates of both HDL- and LDL-C in the yolk sac of the hamsters, in combination with the presence of clathrin-containing coated pits in that tissue (57), imply that the yolk sac may have additional receptors to aid in removal of maternal lipoproteins. Thus, the protein-labeled LDL and HDL did not cross into the fetus, but instead were internalized by the placenta and yolk sac, where the apolipoproteins were most likely hydrolyzed, similar to what occurs with receptor-mediated endocytosis in other tissues (58).

Besides having the ability to remove lipoproteins, the yolk sac synthesizes numerous proteins, including apoB-100, apoE, and apoA-I (59–62). The yolk sac also synthesizes VLDL- and LDL-like particles containing the various apolipoproteins (60–62). These results, combined with those from the present studies, would indicate that the yolk sac and/or placenta aids in the transport of sterol and fatty acids to the fetus via these lipoprotein-like particles. Further support for this is that abnormal development occurred in fetal mice lacking apoB-100 (63, 64). The occurrence of the embryonic lethality may have been the result of the inability of the fetal-derived yolk sac to form lipoproteins destined for the fetus (64). As previously stated, this study cannot determine the source of the sterol transported to the fetus, whether it be that synthesized within the placenta and yolk sac or that taken up from the maternal circulation.

The placenta and yolk sac have another unique role in that these two tissues separate and protect the fetus from various nutrients circulating in the maternal plasma, such as cholesterol carried in lipoproteins. For example, in the present study, a 10-fold increase in plasma LDL-C concentration occurred within the third trimester of the pregnant dam, leading to a dramatic increase in uptake of LDL-C by the placenta and yolk sac. This elevation in uptake was accompanied by a simultaneous decrease in synthesis in both fetal tissues. The result of these two metabolic changes was an elevation in cholesterol concentration in the placenta and yolk sac, whereas cholesterol concentration in the fetus remained constant. Similarly, when pregnant animals were chronically fed cholesterol, enough to fully suppress cholesterol synthesis in the maternal liver, synthesis rates did not change significantly in the fetus (7, 65).

The present studies have tremendous implications for treatment of pregnancy-associated hypercholesterolemia. Because coronary artery disease is the leading cause of death in females, as well as males, it is theorized that a reduction in the hypercholesterolemia associated with pregnancy could have long term benefits to the female (28, 66–68). While the increase in plasma cholesterol levels cannot be prevented with dietary treatment, it can be curtailed (66, 67). However, before any further attempts are made to depress the plasma cholesterol response to pregnancy, more information about the relationship between maternal cholesterol levels and fetal tissues needs to be obtained because maternal cholesterol is required by the whole fetal unit, including the fetus, placenta, and yolk sac, for normal development and functioning of these tissues. It is presently unknown how manipulation of the maternal cholesterol concentration in the plasma will influence sterol metabolism in the fetus.

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