Brain does not utilize low density lipoprotein-cholesterol during fetal and neonatal development in the sheep

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Abstract Several lines of evidence have suggested that central nervous system development and function depend upon a supply of cholesterol that comes from low density lipoproteins (LDL-C). These studies test this hypothesis directly by measuring in vivo the uptake of LDL-C in nine regions of the central nervous system at five different stages of development in the fetal and neonatal sheep. The concentration of LDL-C in the plasma decreased from 49 mg/dl in the fetus 90 days before birth (-90 days) to only 10 mg/dl at -13 days. By 17 days postnatal this value increased to nearly 60 mg/dl. Throughout the period of development between -90 days (very early fetus) and 17 days (late neonatal animal) the weight of the brain increased 32-fold (from 2.3 to 73.6 g) and the content of cholesterol rose 100-fold (from 8.6 to 876 mg), yet there was no detectable LDL-C uptake in any of nine areas of the central nervous system at any stage of development (clearances of <2 μl/h per g). This was true even in the -90 day fetus prior to closure of the blood brain barrier. In contrast, LDL-C clearance by the adrenal gland increased dramatically (from 91 to 348 μl/h per g) as it also did in the liver (from 36 to 85 μl/h per g) during fetal development. These studies strongly suggest, therefore, that cholesterol carried in LDL plays little or no role in the process of sterol acquisition during brain development or in cholesterol turnover in the mature central nervous system. Changes in circulating LDL-C concentration, therefore, should have no effect on brain function.—**Turley, S. D., D. K. Burns, C. R. Rosenfeld, and J. M. Dietschy.** Brain does not utilize low density lipoprotein-cholesterol during fetal and neonatal development in the sheep. *J. Lipid Res.* 1996. **37:** 1953–1961.

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Recognition that the risk of developing atherosclerosis rises as plasma LDL-cholesterol (LDL-C) concentrations increase culminated in the formulation of a national cholesterol education program that has become a catalyst for the development of a plethora of treatments for managing plasma LDL-C levels in the general population (1, 2). While the benefit of these therapies is reflected in the steady decline in mortality from atherosclerotic disease in western societies, several recent epidemiological studies have concluded that lowering plasma cholesterol levels may have deleterious effects on the central nervous system (CNS) and may increase mortality from non-cardiovascular diseases (3–6). Although other reports have refuted these findings (7), the issue remains important and controversial. These epidemiological studies, together with recent advances in the etiology of Alzheimer's disease and the Smith-Lemli-Opitz syndrome (8–10), have stimulated interest in more fully understanding the origin of the cholesterol that is used in the growth, development, and maintenance of tissues in the CNS.

During late fetal and early neonatal life the brain manifests a very high rate of cholesterol synthesis (11–13). This rate declines rapidly at about the time of weaning, and remains very low throughout adulthood (12–14). There are several lines of evidence to suggest that a high rate of cholesterol biosynthesis in early life is essential to ensure normal growth and development of the brain. This is perhaps best illustrated by the Smith-Lemli-Opitz syndrome, an autosomal recessive disorder in which a major block in the terminal step of the cholesterol biosynthetic pathway results in severe mental retardation and multiple other birth defects (9, 10). Similarly, in the "Jimpy" mouse, a mutant strain that spontaneously develops multiple neurological disorders, brain cholesterol synthesis and content are lower than in matching control animals (15, 16). More recently, a study that used [^{3}H]water to measure the absolute rate of cholesterol synthesis in the brain of neonatal rats showed that the amount of cholesterol synthesized locally was sufficient to fully account for all

Abbreviations: LDL-C, cholesterol carried in low density lipoproteins; CNS, central nervous system; LDLR, low density lipoprotein receptor; sLDL, LDL derived from sheep plasma; hLDL, LDL derived from human plasma; HDL, high density lipoprotein; TCB, tyramine cellobiose.

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the brain cholesterol deposition that occurs during that stage of their development (17).

While such experimental evidence suggests that the cholesterol requirements of the developing CNS may be largely met through de novo synthesis, certain parts of the brain may also potentially derive cholesterol from the uptake of LDL. This possibility is implied by the discoveries that apolipoprotein E mRNA is abundant in the brain of several species, and that cultured astrocytes express LDL receptor (LDLR) activity (18, 19). Furthermore, other investigators have shown significant levels of LDLR mRNA in the brains of early neonatal rabbits and rats (20, 21). It is, nevertheless, unclear whether any part of the brain actively transports LDL from plasma in the intact animal, and if it does, whether this occurs only at a certain point in the development of the fetus or neonate. Thus, the present studies were undertaken to try to resolve this very important question by attempting to detect net LDL-C uptake in several regions of the brain and by making these measurements at various times throughout the development of the CNS, from early in fetal development before the blood brain barrier has closed to late in the neonatal period when brain growth is nearly complete (22, 23).

MATERIALS AND METHODS

Animals

Timed-pregnant, cross-bred Rambouillet ewes (*Ovis aries*) carrying either single or twin fetuses, and weaned female lambs were obtained from the Department of Veterinary Sciences, University of Texas M.D. Anderson Cancer Center (Bastrop, TX). They were housed individually in a room at 18–20°C with alternating periods of light (6 AM to 6 PM) and dark (6 PM to 6 AM) and had free access to water, mineral and salt blocks, and their diets at all times. Their diet consisted of grass hay and a 1:1 mixture of 12% creep pellets and horse and mule feed (Acco Feeds Inc., Abilene, TX). The lambs in the early and late neonatal groups had unrestricted access to their dam's milk. Studies were carried out in sheep at seven stages of development. The age of the animals was determined on the basis that the average gestation period for the Rambouillet breed of sheep is 150 days (11). Hence, by setting the time of conception at -150 days, the average ages of the sheep at the various stages of development were as follows: -90 days (very early fetal), -73 days (early fetal), -41 days (mid fetal), -13 days (late fetal), 1 day (early neonatal), 17 days (late neonatal), and 105 days (weaned). The weaned lambs were separated from their dams at about 70 days. All groups were of mixed gender except the weaned lambs which were all female. All procedures were approved by the Institutional Animal Care and Research Advisory Committee.

Catheterization of fetal and neonatal sheep

As described in detail elsewhere (24) permanent catheters were placed in a femoral vein and artery of mid (-41 days) and late (-13 days) fetal sheep about 7 to 10 days before the LDL transport experiments were carried out. Catheters were also inserted into a femoral vein and artery of the ewes. In the case of the very early fetal sheep (-90 days) the catheterization was done on the morning of the transport experiments. A percutaneous jugular vein catheter was placed, and the ewe was anesthetized with intravenous ketamine hydrochloride and pentobarbital. After exteriorization of the fetus, catheters were placed in one vein and artery of the umbilical cord with the tips lying in the intra-abdominal portions. The fetus was covered with cotton pads dampened with isotonic saline, and its temperature was maintained at 36–38°C with a warming lamp. The ewe remained anesthetized with ketamine hydrochloride throughout the experiment. In the studies with the neonatal lambs, catheterization was also done on the day of the experiment. The lambs were sedated with xylazine (11) and fitted with two jugular vein catheters. They were kept sedated under warm covers for the duration of the experiment.

Preparation of labeled LDL

Sheep low density lipoproteins (sLDL) were isolated by density gradient ultracentrifugation in the density range of 1.020 to 1.055 g/ml from the plasma of donor adult animals that had been fed a cholesterol-free commercial diet and pasture. Gradient gel electrophoresis revealed apolipoprotein B-100 to be essentially the only protein in this fraction (25). The LDL was labeled with either ^{125}I-labeled tyramine cellobiose ([^{125}I]TCB) or directly with ^{131}I as described (26). These preparations were used for the measurement of the rate of total LDL clearance by all the organs. For the measurement of the rates of receptor-independent LDL clearance, hLDL (d 1.020–1.055 g/ml) was labeled with [^{125}I]TCB or ^{131}I and then reductively methylated (26). All labeled lipoprotein preparations were used within 48 h and were filtered through 0.45-μm Millex filters (Millipore, Bedford, MA) immediately before use. A preliminary experiment was carried out to determine whether, in the ovine species, sLDL labeled with [^{125}I]TCB was cleared from the circulation at the same rate as directly iodinated sLDL. As shown in **Fig. 1,** the rate of catabolism of both labeled LDL preparations was virtually identical during the period that LDL transport measurements were to be carried out. Furthermore, in more prolonged observations the fractional catabolic rates equalled about 0.75 pools/d for the two preparations.

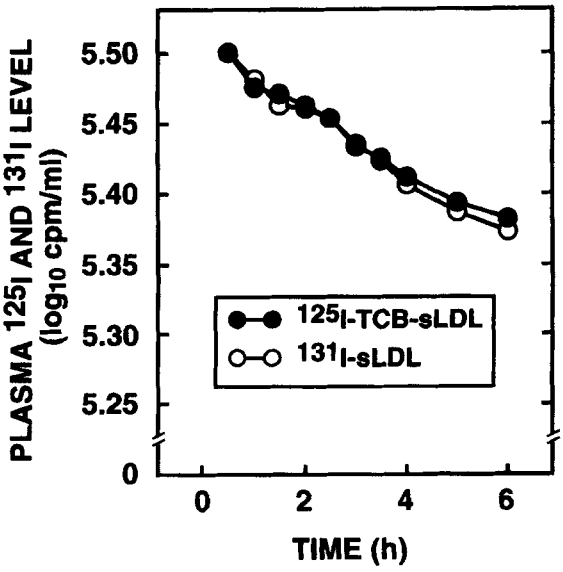

Fig. 1. Rate of catabolism of homologous LDL labeled with either ^{125}I-labeled tyramine cellobiose or ^{131}I in a young adult sheep. Low density lipoproteins were isolated from the plasma of normolipidemic donor sheep (sLDL) and labeled with either ^{125}I-labeled tyramine cellobiose (TCB), a non-degradable marker, or ^{131}I alone, as described in Materials and Methods. A mixture of both labeled sLDL preparations was administered as a single bolus to a young nonpregnant ewe, and the plasma level of each radiolabel was measured at multiple time points over the following 6 h.

Measurement of rates of total and receptor-independent LDL clearance in vivo

Blood was taken from each animal immediately before the administration of the labeled LDL for the purpose of measuring plasma total and LDL-C concentrations. In the studies with the very early fetal sheep, a bolus injection of [^{125}I]TCB-labeled LDL (either unmodified homologous or reductively methylated heterologous LDL) was given through the arterial catheter. After 10 min approximately 200 μl of blood was taken through the venous catheter. The [^{125}I]TCB-labeled LDL was allowed to circulate for a total of 2 h. Ten min before the end of this 2-h period, a bolus injection of a small volume of the appropriate ^{131}I-labeled LDL preparation was given through the arterial catheter. After the ^{131}I-labeled LDL had circulated for 10 min, the ewe was given an overdose of sodium pentobarbital through the jugular catheter (11), and, at the same time, the fetus was exsanguinated through its venous catheter. The experiments with the mid and late fetal sheep were conducted in a similar way, except that a continuous infusion of the [^{125}I]TCB-LDL was given after the bolus injection of this preparation. In addition, the infusion period for these larger fetuses lasted for a total of 5 or 6 h during which time blood samples were periodically taken through the venous catheter to monitor the plasma level of ^{125}I. At the end of this period a bolus of ^{131}I-labeled LDL was given, and 10 min later the ewes, which were fully conscious throughout the experiment, were administered an overdose of sodium pentobarbital. An incision was made in the abdomen, the uterus was opened, and the fetus was removed. The umbilical cord was clamped and the fetus was exsanguinated through both the umbilical artery and vein. The experiments with the early and late neonatal lambs were run in the same way as those with the larger fetal animals.

Organ removal and tissue analyses

Immediately after the animals were exsanguinated both adrenal glands were excised and bile was withdrawn from the gallbladder before the whole liver was removed. Except in the case of the very early fetal sheep, the excision of the brain was carried out as follows. The cranial vault was opened superiorly and the rostral spinal cord was exposed via a posterior vertebral laminectomy. Cranial nerves and vascular connections were severed, the brain and spinal cord were removed, and the brain was weighed. The cerebral hemispheres, brainstem, and cerebellum were sectioned in the coronal, horizontal, and sagittal planes, respectively. The following regions were then separated out: superior frontal neocortex, superior parietal neocortex, white matter from the corpus callosum and centrum ovale, corpus striatum, thalamus posterior to the corpus striatum, midbrain at the level of the superior colliculus, mid-portion of the basis pontis, mid-portion of the medulla oblongata, cerebellar vermis, and cervical spinal cord. In the studies with the very early fetuses, tissue was taken from only the frontal cortex, cerebellar folia and spinal cord because these were the most anatomically distinct regions from which sufficient material could be recovered. Aliquots of tissue (200 to 300 mg) from the various regions of the central nervous system, as well as from the liver, adrenal glands, and plasma, were taken for the measurement of both their ^{125}I and ^{131}I content and their total cholesterol concentration (26). From the ^{125}I and ^{131}I content of the tissue and plasma, the rates of total and receptor-independent LDL clearance were calculated. These were expressed as the μl of plasma cleared of its LDL content per h per g wet weight of tissue (μl/h per g).

Analytic procedures

Plasma total cholesterol concentrations were measured enzymatically using a kit (no. 1127578) from Boehringer Mannheim (Indianapolis, IN). Aliquots of plasma were fractionated by density gradient ultracentrifugation at densities of 1.020 and 1.063 g/ml. The apolipoprotein composition of the fraction of density 1.020–1.063 g/ml was resolved using gradient polyacrylamide gel electrophoresis (25). The total cholesterol concentration in the lipoprotein fractions, as well as in

the liver and various regions of the central nervous system, was determined by gas–liquid chromatography using stigmastanol (Sigma Chemical, St. Louis, MO) as an internal standard (27). Desmosterol, a precursor of cholesterol, appeared on the chromatograms for brain tissue from early fetal animals. This was not included in the calculation of cholesterol concentration.

Analysis of data

The data are expressed as the mean ± 1 SEM of values obtained from the number of animals indicated for each group. The data for the male and female lambs were combined because no gender-related differences in any of the metabolic parameters studied were apparent. Where relevant, the significance of differences between these mean values was tested using a two-tailed unpaired Student's *t* test.

RESULTS

The average body, brain, and liver weights at seven stages of development are given in **Table 1.** Using an average gestation period of 150 days, the age of each group of animals was set relative to the day of conception, i.e., -150 days. The ages of the sheep ranged from a very early point in gestation (-90 days) to post weaning (105 days). With the exception of the weaned animals, all groups included both males and females. Over this age span the body weights ranged from 60 g to about 30 kg. In the very early fetal animals the mass of the brain relative to body weight was 10 times greater than it was in 105 day weaned lambs. While the relative brain mass continued to change significantly after birth, this was not the case with the liver which represented approximately 2% of total body weight in the 1, 17, and 105 day animals.

The plasma cholesterol concentration changed markedly throughout development (**Fig. 2A**). As gestation progressed, the plasma LDL-C level in the fetus fell from 48.7 ± 1.9 mg/dl at -90 days to 10.1 ± 1.1 mg/dl at -13 days. After parturition, however, levels rose to 27.7 ± 2.5 and 59.3 ± 4.0 mg/dl in the 1 day and 17 day lambs, respectively, with the intake of the ewe's milk. This level decreased to 14.5 ± 1.5 mg/dl after weaning (105 days). Apolipoprotein B-100 was overwhelmingly the major apoprotein present in these plasma LDL fractions. The data in Fig. 2B illustrate the divergent nature of the ontogenic changes in the cholesterol concentration of the brain and liver in the same group of animals for which plasma LDL-C levels are shown. The values for brain cholesterol represent the average of measurements made on tissue from eight separate regions. The mean levels found in fetuses at -90 days (3.8 ± 0.1 mg/g) and -73 days (3.8 ± 0.2 mg/g) increased more than 2-fold toward the end of gestation (9.2 ± 0.5 mg/g at -13 days). There was, in turn, a further doubling of mean brain cholesterol levels by the time the lambs reached 105 days of age (17.9 ± 1.7 mg/g). In contrast, hepatic cholesterol levels remained constant at 2–3 mg/g throughout development, except in the 17-day suckled lambs where this value transiently increased to 4.2 ± 0.3 mg/g.

To determine whether functional LDL receptor activity was expressed in these organs at each stage of development, measurements were made of the rates of both total LDL clearance (using [^{125}I]TCB-labeled sLDL) and the fraction of this uptake that was receptor-independent (using [^{125}I]TCB-labeled methylated-hLDL). Total LDL clearance was measured in 3–5 animals each at -90, -41, -13, 1, and 17 days of age. The rates of receptor-independent transport were determined in three fetal sheep, two at -81 days and one at -13 days. As shown in **Fig. 3A,** in the adrenal the total clearance rate varied more than 5-fold depending on the stage of development, and there was a dramatic increase be-

TABLE 1. Body, brain, liver and adrenal weights of fetal and neonatal sheep at different stages of development

Stage of Development	Age	Number and Sex of Animals	Body Wt	Brain Wt		Liver Wt		Adrenal Wt	
				Absolute	Relative to Body Wt	Absolute	Relative to Body Wt	Absolute	Relative to Body Wt
	d		*kg*	*g*	%	*g*	%	*g*	%
A. Fertilization	-150								
B. Very early fetal	-90	1F/4M	0.06 ± 0.01	2.3 ± 0.2	3.9 ± 0.1	4.2 ± 0.2	7.2 ± 0.3	0.025 ± 0.003	0.042 ± 0.004
C. Early fetal	-73	5F/2M	0.21 ± 0.01	7.1 ± 0.5	3.5 ± 0.1	10.8 ± 0.7	5.3 ± 0.4	nm	nm
D. Mid-fetal	-41	5F/2M	1.58 ± 0.14	35.7 ± 2.6	2.3 ± 0.2	67.9 ± 8.1	4.3 ± 0.3	0.221 ± 0.030	0.011 ± 0.001
E. Late fetal	-13	5F/4M	4.07 ± 0.15	52.3 ± 1.9	1.3 ± 0.1	104.7 ± 4.8	2.6 ± 0.1	0.357 ± 0.012	0.008 ± 0.001
F. Early neonatal	1	9F/16M	4.22 ± 0.16	58.5 ± 1.3	1.4 ± 0.1	92.2 ± 5.4	2.1 ± 0.1	0.734 ± 0.031	0.017 ± 0.001
G. Late neonatal	17	9F/5M	8.98 ± 0.25	73.6 ± 1.4	0.8 ± 0.1	191 ± 13	2.1 ± 0.1	0.771 ± 0.039	0.009 ± 0.001
H. Weaned	105	6F	29.6 ± 1.1	118.2 ± 4.3	0.4 ± 0.1	514 ± 17	1.8 ± 0.1	nm	nm

These data were obtained from a series of studies involving a total of 73 animals. LDL transport experiments were performed in animals at five stages of development (B, D, E, F, and G) as described in Materials and Methods. Values represent the mean ± 1 SEM of data from the total number of males and females combined in each group; nm, not measured.

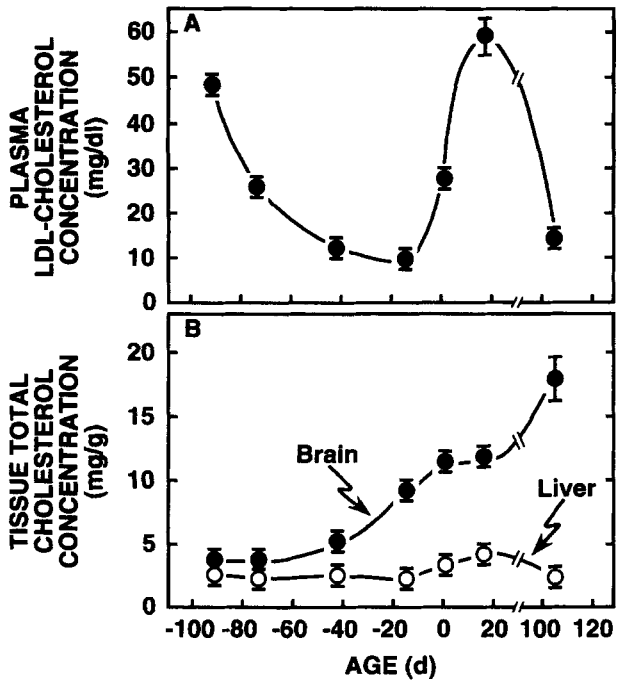

Fig. 2. Plasma LDL-C and brain and liver total cholesterol concentrations at different stages of development in the sheep. These data were obtained from the same groups of animals described in Table I. In the case of the adrenal, tissue cholesterol levels were measured in only two of these groups. Hence, these data are not shown. The time designated 0 d represents the day of birth. Values represent the mean ± 1 SEM of data from the number of animals given in Table I.

tween -41 days (63 ± 22 μl/h per g) and -13 days (261 ± 63 μl/h per g). An even higher rate was seen in the lambs 1 day after birth (348 ± 60 μl/h per g), but as they continued to suckle, adrenal LDL clearance fell (141 ± 22 μl/h per g at 17 days). The pattern of age-related changes in hepatic LDL transport (Fig. 3B) during fetal development differed from that seen in the adrenal. This was particularly evident in the animals at -41 days. Thus, while the lowest rate of clearance by the adrenal was seen at this point, the transport rate in the liver (81.0 ± 12.4 μl/h per g) had already reached about the same level that was manifest in lambs 1 day after birth (85.0 ± 12.4 μl/h per g). However, as was the case with the adrenal, the rate of clearance by the liver fell appreciably as the lambs continued to suckle (27.2 ± 4.2 μl/h per g at 17 days).

In striking contrast to the rates of transport that were manifest in the adrenal and liver, at no stage of development did total LDL clearance by the brain exceed 1 to 2 μl/h per g (Fig. 3C). Such low levels of clearance were not statistically different from zero. The levels of receptor-independent clearance found for all three organs in animals at two widely different points in fetal development are shown in Fig. 3D. These values did not vary with age, averaging about 15, 10, and 1 μl/h per g for the adrenal, liver and brain, respectively.

A final set of measurements was made in order to correlate rates of cholesterol acquisition and LDL-C uptake in nine different regions of the CNS at five different times during fetal and neonatal development. The results of these studies are summarized in **Fig. 4.** These data illustrate two important observations. First, no region of the brain transported LDL at a rate significantly different from the overall average for this organ (<1 to 2 μl/h per g). Second, despite the lack of interregional differences in LDL clearance, there were striking differences in tissue cholesterol concentration both between regions at any one time point, and within regions as a function of the animal's stage of development. All parts of the brain and the spinal cord showed higher tissue cholesterol concentrations as development progressed. In the case of the frontal and parietal cortex the concentration increased from about 5 mg/g at -41 days to approximately 9 mg/g in the 17-day-old suckled lambs. Over this same period the more myelin-rich regions like cerebral white matter and spinal cord manifested increases in concentration from about 6 and 16 mg/g, respectively, to 26 and 40 mg/g, respectively. Together, these two sets of data illustrate the massive rates of cholesterol acquisition that occur in all regions of the central nervous system under circumstances where there is no demonstrable net uptake of LDL-C.

DISCUSSION

Over the past two decades studies in both humans and a variety of animal models have yielded detailed information on the physiology of LDL metabolism and, particularly, on the central role that the liver plays in the regulation of plasma LDL-C concentrations (28–31). Nevertheless, there are several aspects of LDL-C metabolism that remain poorly understood. One of these is the question of the extent to which major organ systems may utilize LDL-C during the earlier stages of development and, particularly, during fetal differentiation. The chronically catheterized fetal sheep model provided us with a unique opportunity to define, in quantitative terms, how LDL in the unborn animal is utilized by different organs as development progresses.

In evaluating these data, two points concerning the method for measuring LDL-C transport should be emphasized. One of these relates to the assumption that the clearance rate of LDL labeled in its protein moiety faithfully reflects the movement of cholesterol carried in the same particle. Although in the transport of HDL by some organs the uptake of cholesterol becomes dissociated from that of the protein, this has never been shown to be the case with LDL (32–36). The second related point is that the clearance rates shown here

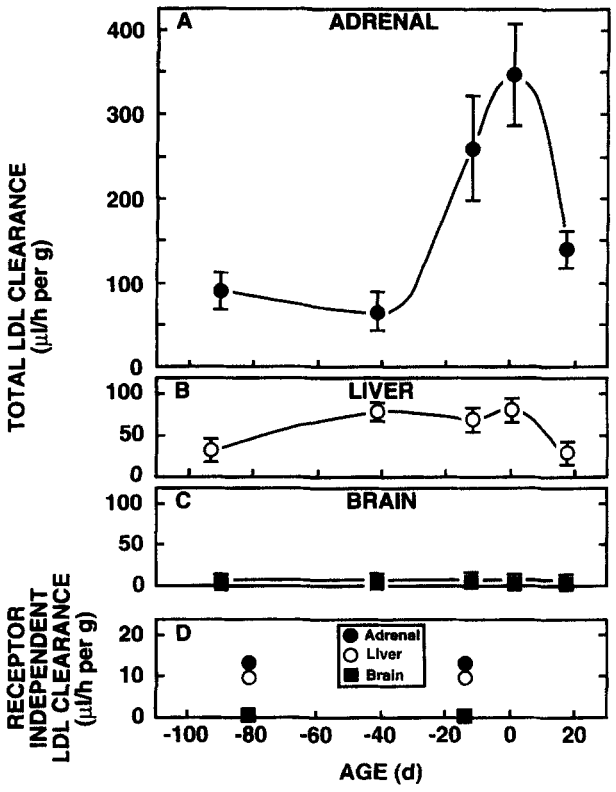

Fig. 3. Rates of total and receptor-independent LDL clearance by the adrenal, liver, and brain at different stages of development in the sheep. The rates of total clearance of LDL were measured in vivo in very early, mid, and late fetal, and in early and late neonatal sheep as described in Materials and Methods. These stages of development correspond to groups B, D, E, F, and G described in Table I. Receptor-independent LDL clearance was only measured in very early and late fetal sheep. The values for total LDL clearance represent the mean ± 1 SEM of data from the number of animals as follows: -90 d, 3; -41 d, 3; -13 d, 4; 1 d, 5; and 17 d, 5. In the case of the receptor-independent clearance measurements, the values at -81 d represent the mean of data from two lambs, while at -14 d values for a single animal are given.

reflect mass movement of LDL out of the circulation rather than isotope exchange which potentially occurs when lipoproteins bearing cholesterol labeled with either ^{3}H or ^{14}C are administered to intact animals.

Based on the data from the present experiments, two principal conclusions can be drawn regarding the metabolism of LDL by the brain, liver, and adrenal gland in this animal model. First, in the case of the brain it is apparent that at no point in development, including that time prior to closure of the blood–brain barrier (about -90 days), is LDL used as a source of cholesterol in the central nervous system (22, 23). Thus, while the concentration of cholesterol in all regions of the brain increased 3- to 8-fold, and the absolute amount of sterol increased 100-fold, over the 107-day age span that was studied (from -90 to 17 days), at no stage of growth did any region manifest total LDL clearance rates of more than 1 to 2 μl/h per g. Such low values are within the error of measurement that is inherent in this method. It should also be emphasized that these negligible rates of LDL clearance by the brain were found in the same experimental setting where very high rates of lipoprotein uptake were detected in the adrenal and liver.

The observation that brain cholesterol deposition in these lambs was fully dissociated from LDL-C in the circulation is emphasized by calculating what the LDL-C clearance rate would have to be to account for the total increment in brain cholesterol content during a particular phase of development. For example, using the data in Table 1 and in Figs. 2 and 4, it can be determined that in the 28-day period from -41 to -13 d of age, whole brain cholesterol content increased by 288 mg (10.3 mg/d). Using an average brain weight of 44 g and a mean plasma LDL-C level of 11.4 mg/dl for this 28-day interval, it can be calculated that the brain would have had to clear LDL from the plasma at a rate of about 85 μl/h per g to provide it with 10.3 mg/day of cholesterol.

Other data derived from animals and humans lacking LDLR activity further support this concept that LDL-C and LDL receptors play no role in cholesterol homeostasis within the central nervous system. In both the mouse and rabbit lacking receptors, brain development and cholesterol concentrations are no different from those values found in the appropriate control animals (13, 30, 37). In the normal adult mouse and rabbit, the rates of homologous LDL-C clearance into the brain are also <2 μl/h per g, as was found in these studies in the brain of the developing sheep (30, 37). Furthermore, in mice and rabbits lacking LDLR activity, there was no demonstrable difference in this clearance of homologous LDL-C into the central nervous system nor is there any alteration in the rate of cholesterol synthesis (13, 30). Finally, the central nervous system appears to function normally in mice, rabbits, and humans lacking functional LDL receptor activity. This finding is in contrast to the major abnormalities in brain development and function that are manifest in animals and humans that have a defect in cholesterol biosynthesis (9, 10, 38).

The second major finding of these studies relates to the profile of ontogenic changes in the receptor-dependent clearance of LDL by the liver and adrenal. While there have been several published reports describing either the levels of LDL receptor mRNA in fetal rat liver or LDL binding activity in fetal membranes (39–41), the present studies represent the first quantitative description of how receptor-dependent clearance of LDL by the liver changes during an extended period of fetal and postnatal development. The expression of functional LDLR activity in the very early fetal sheep (-90 days) was evident from the finding that the hepatic total LDL clearance rate in these animals (36 ± 7 μl/h per g) was

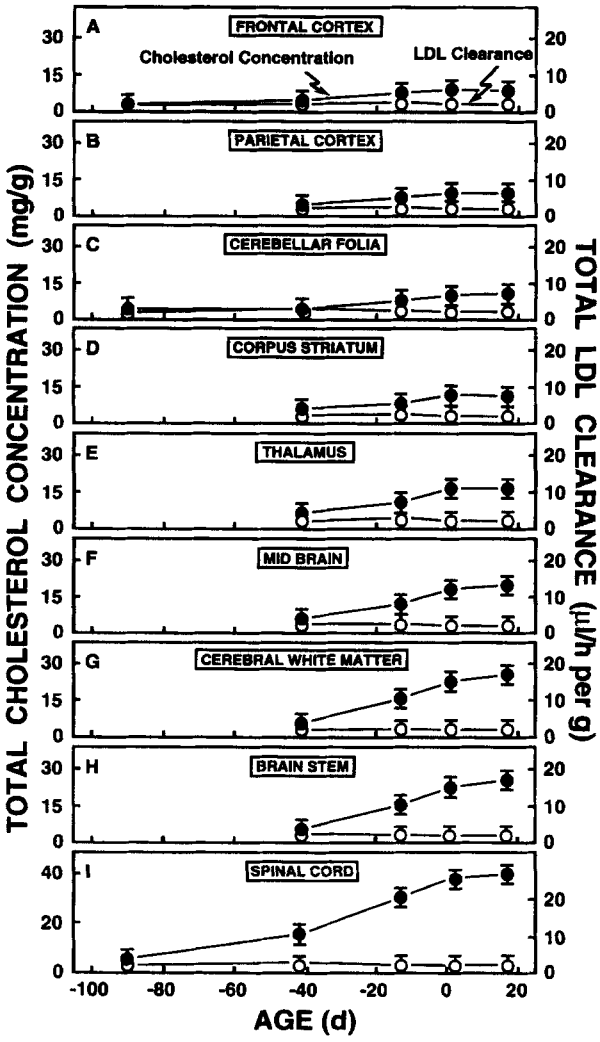

Fig. 4. Tissue total cholesterol concentrations and rates of total LDL clearance in various regions of the central nervous system at different stages of development in the sheep. These stages correspond to groups B, D, E, F, and G described in Table 1. In the case of the very early fetal sheep, tissue was taken from only three readily discernible regions of the CNS. The values represent the mean ± 1 SEM of data from the following number of animals: -90 d, 3; -41 d, 3; -13 d, 4; 1 d, 5; 17 d, 5.

more than 3-fold higher than the clearance achieved with the methylated hLDL at this stage of development (average clearance value for two animals at -81 days was 10 µl/h per g). At two points within the last 6 weeks of gestation (-41 and -13 days), the level of receptor-dependent LDL clearance by the liver reached values that were 3- to 4-fold higher than those found in the very early fetal animals.

The pattern of age-related changes in LDL clearance by the adrenal was generally similar to that for the liver, except that the acceleration of LDL uptake by the adrenal in the developing fetus occurred noticeably later. This is evident from a comparison of the total clearance rates for both organs at -41 and -13 days (Fig. 3). As receptor-independent clearance by the adrenal was only about 15 µl/h per g, the magnitude of receptor-dependent uptake by the adrenal in the late fetal (-13 days) and newborn (1 day) animals was more than 4-fold higher than it was in the liver at these time points. This dramatic induction of adrenal LDLR activity coincides with the period when plasma levels of adrenocorticotropin and cortisol increase many fold in the fetal sheep (42–44).

Two further points regarding the LDL clearance data for the organs studied here warrant emphasis. One is that the marked increase in LDL receptor activity in the liver and adrenal towards term probably largely accounted for the precipitous fall in plasma LDL-C levels that occurred during this stage of development (40, 41, 45). The other point is that any organ that needs LDL-C for utilization, either as a precursor for the production of steroid hormones or bile acids or more generally for new membrane formation, will modulate its level of receptor-dependent LDL transport to meet that particular requirement. The finding that there was no significant LDL clearance into the brain at any stage of development demonstrates that, unlike the adrenal and liver, the growth and differentiation of CNS tissue in the fetal and neonatal sheep is apparently not dependent on the uptake of LDL-C from the circulation. This is supported by the observation in neonatal rats that, at least after birth, all of the cholesterol needed for myelination is derived from local synthesis, not preformed sources (17, 46). Consistent with these reports, we have found that in early neonatal lambs the absolute rate of cholesterol synthesis in the brain varies widely from one region to another, and that those regions with the highest rates of biosynthetic activity also contain the highest concentrations of cholesterol (S. D. Turley, D. K. Burns, and J. M. Dietschy, unpublished observations). Unlike other organs that can utilize both preformed and newly synthesized cholesterol for tissue growth, the brain appears to use only cholesterol from the latter source, as the inhibition of the biosynthetic pathway in the unborn animal results in severe CNS dysfunction after birth (9, 10).

If, as these data indicate, development and function of the brain are not dependent upon circulating LDL-C, the question arises as to the function of the LDLR (and LDLR related protein) activity that has been identified on various cells within the CNS. One attractive possibility that has been postulated is that apolipoprotein E (or apolipoprotein A-I) may serve to recirculate cholesterol among the cells of the nervous system, particularly after damage to these cells, and this sterol may then be reutilized for myelination through intervention of the LDLR (or LDL related protein) (19, 47, 48). Unfortunately, in mice that lack apolipoprotein E (or apolipo-

protein A-I), not only is brain development apparently normal, but nerve regeneration after damage is also unimpaired (49, 50). Thus, it is not clear what role these receptors play within the central nervous system. Nevertheless, taken together, these various experimental results indicate that LDL-C circulating in the plasma seems to play no role in the development or maintenance of brain function. It follows, therefore, that lowering of the plasma LDL-C concentration to prevent atherosclerosis should, in itself, have no adverse effect on CNS function.

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