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THE LEVELS OF PLASMA CHOLESTEROL IN THE HUMAN FETUS THROUGHOUT GESTATION

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SUMMARY

The present study was undertaken to define the umbilical cord plasma concentrations of cholesterol throughout human gestation. Mixed arterial and venous cord plasma samples obtained from abortuses of women undergoing elective abortion or from infants of women who underwent spontaneous premature vaginal delivery, and from infants of women who delivered vaginally at term were assayed for cholesterol by a micro-enzymatic method. No cases that involved any maternal or fetal complications (other than prematurity) were included in this study. Early in gestation (10-16 weeks post-conception), the total cholesterol level in cord plasma was 85.4 ± 30.7 mg/dl (mean ± SD), n = 68, with the cholesterol levels in some samples falling within the range of those of adults. Between 16.5 and 20 weeks post-conception, the umbilical cord plasma cholesterol level declined to 39.9 ± 21.0 mg/dl, n = 19 ($P < 0.001$). The cholesterol concentration in umbilical cord plasma then rose to 67.8 ± 24.0 mg/dl, n = 17, ($P < 0.001$) between 26.5 and 32 weeks of gestation. Thereafter, a second decline in the umbilical cord plasma cholesterol level occurred, with the values at 32.5-36 weeks being 58.4 ± 13.6 mg/dl (n = 16), and at 36.5 to 40 weeks post-conception (term) being 51.4 ± 11.5 mg/dl, n = 44 ($P < 0.01$ vs. 26.5-32 wks). We suggest that the observed changes in fetal cholesterol levels could be related to alterations during development in the rates of lipoprotein-cholesterol biosynthesis and subsequent clearance from plasma by the fetal adrenals wherein cholesterol is used as substrate for steroid biosynthesis.

INTRODUCTION

A definition of the genetic and nutritional regulation of cholesterol synthesis and metabolism is believed to be of signal importance in the development of rational approaches in the prevention of arteriosclerotic cardiovascular disorders. Generally the levels of cholesterol in plasma of adult humans are higher than those in plasma of other mammalian species with the exception of aquatic mammals and hibernators (7,22). These differences are due primarily to higher levels of low density lipoprotein (LDL) in humans; in man LDL is the principal lipoprotein for delivery of cholesterol to extrahepatic tissues (18). The levels of cholesterol, and in particular those of LDL-cholesterol, in cord blood of human newborns are much lower than those of adults (10,15,16). However, the levels of cholesterol in plasma rise rapidly during the first few weeks of postnatal life (11,12,14,31) and continue to rise progressively throughout adulthood into middle age (19). It has been presumed that the rise in cholesterol levels after birth was due to the commencement of oral feeding and thus was attributable to dietary cholesterol. As a straightforward extrapolation of these findings, it might have been anticipated that during human fetal development, plasma cholesterol levels early in gestation would be lower than those found at term and would rise progressively throughout intrauterine life. However, Ginsberg and Zetterstrom (12) and Anderson and Friis-Hansen (2) found that cholesterol levels in fetal plasma before 37 weeks of gestation were higher than those present after this time of gestation.

Several investigators (4,10,15,16) have attempted to ascertain whether levels of cholesterol and lipoproteins in cord plasma of term newborns were predictive of a predisposition to hypercholesterolemia or cardiovascular disease in later life. Such efforts have met with little success. One reason for this failure is that the steroidogenic activity of the fetal adrenals appears to be an important determinant of the levels of LDL-cholesterol in human fetal plasma. The human fetal adrenals synthesize large quantities of steroid hormones, particularly dehydroisoandrosterone sulfate (DS) which, in turn, serves as precursor for placental estrogen formation (27). One major source of precursor for human fetal adrenal steroidogenesis appears to be LDL-cholesterol in fetal plasma (5,6,23). We have shown previously that an inverse relationship exists between the steroidogenic activity of the fetal adrenals and the concentration of total- and LDL-cholesterol in the fetal plasma of term infants born of normal and complicated pregnancies (23). Furthermore, the aforementioned rapid rise in plasma cholesterol during the first few weeks of postnatal life is correlated well with the rapid involution of the fetal zone of the adrenal cortex (26,28). It is likely, therefore, that the rate of steroidogenesis by the human fetal adrenal glands is an important determinant of the levels of cholesterol in human fetal plasma. Conversely, the availability of circulating cholesterol in the fetus may play an important role in pregnancy-maintenance, by means of serving as substrate for adrenal steroidogenesis, in addition to its importance in the orderly growth and development of the fetus. For these reasons we sought, in the present study, to define the developmental changes in the levels of cholesterol in human fetal plasma throughout normal gestation.

MATERIALS AND METHODS

The study group consisted of abortuses of women undergoing elective abortion for socio-economic reasons (< 22 weeks post-conception), infants of women who underwent spontaneous premature vaginal delivery, and infants of women who delivered vaginally at term. No cases that involved complications of delivery (other than prematurity), maternal illness, fetal abnormalities, or multiple births were included in this study. All newborns were judged to be normal based upon the findings of a thorough examination at delivery and abortuses were judged to be normal based upon findings at autopsy. Umbilical cord blood samples of abortuses were obtained in accordance with the Donors Anatomical Gift Act of the State of Texas after consent in writing from the women to be aborted was obtained. A consent form and and protocol approved by the Human Research Review Committee of the University of Texas Health Science Center at Dallas, Dallas, Texas was used in the case of umbilical cord blood samples from newborns.

Umbilical cord blood samples (fetal portion of cord) were obtained within 1-3 minutes after delivery with the exception of a few instances wherein blood was obtained from fetuses within 20 minutes after abortion hysterectomy. In order to prevent possible contamination of fetal blood by maternal blood, the umbilical cord was doubly clamped, rinsed, severed, and blood was then collected directly by capillary flow into heparinized microhematocrit tubes (Scientific Products) and centrifuged in a micro-capillary centrifuge (IEC Model MB). Otherwise, blood was drained from the umbilical cord into tubes that contained EDTA as anticoagulant. Plasma was collected following centrifugation. All plasma samples were stored at 4 °C and were analyzed within one week of collection. The analysis for total cholesterol was conducted by use of a Beckman Cholesterol Analyzer 2 (Beckman Instruments, Inc.), according to a modified microenzymatic method based upon oxygen consumption (3). With this method, only 5-10 µl of plasma is required. Normally, samples were analyzed in duplicate, but sometimes, as in many cases in the samples of the < 22 weeks gestational age group, it often was possible to analyze only a single aliquot because of the small volume of plasma collected. No systematic difference in cholesterol level was noted between blood samples collected in heparinized tubes and those collected in tubes containing EDTA. The inter-assay coefficient of variation for cholesterol levels in a pool of cord plasma was 4.1%. The intraassay coefficient of variation for plasma samples with cholesterol concentrations ranging from 40 to 200 mg/dl was found consistently to be less than 10%. As a test of the accuracy of the method, a pool of cord plasma derived from normal newborns delivered of normal mothers at term was assayed with this technique and also assayed by another laboratory using a modification of the Liebermann-Burchard method (30). The cholesterol levels that we obtained with the Beckman Cholesterol Analyzer 2 and the microenzymatic method (48.7 ± 4.7 mg/dl, mean ± SD) were similar to those obtained using this other method (49.9 ± 0.7 mg/dl).

Data for several gestational ages (weeks post-conception) were grouped and were analyzed using the Mann-Whitney U test. Differences between groups were considered to be significant at $P < 0.05$.

RESULTS

The data obtained are illustrated graphically in Figure 1. The somewhat unexpected finding was that an apparently biphasic pattern exists in the levels of cholesterol in fetal plasma during gestation. The most striking finding was that in early gestation, high levels of cholesterol were present in fetal plasma, the values in some cases being within the range of values for cholesterol in plasma of adult humans. Between the gestational ages of 10 and 16 weeks post-conception, the total cholesterol concentration in umbilical cord plasma (n = 68) was 85.4 ± 30.7 mg/dl (mean ± SD), with the cholesterol concentrations in some individual samples being as great as 140 mg/dl. At later gestational ages the levels of cholesterol were signficantly less ($P < 0.001$), viz., 39.9 ± 21.0 mg/dl between 16.5 and 20 weeks post-conception (n = 19). Still later in gestation, the plasma cholesterol levels were higher, viz., 67.8 ± 5.8 mg/dl, n = 17, between 26.5 and 32 weeks post-conception ($P < 0.03$). From 30 weeks of gestation through term, the plasma cholesterol concentrations were again lower. The cholesterol levels at 32.5-36 weeks of gestation (n = 16), were 58.4 ± 13.6 mg/dl, and at 36.5 to 40 weeks (n = 44), were 51.4 ± 11.5 mg/dl, a value significantly lower ($P < 0.03$) than that observed between 26.5 and 32 weeks of gestation.

DISCUSSION

In the current study, we found marked fluctuations in fetal plasma cholesterol levels during gestation. Although a potential influence of the mode of delivery on the observed levels of cholesterol during various gestational periods cannot be totally ruled out, we propose that the ontogenetic changes in the levels of total cholesterol in fetal plasma likely are, in part, reflective of the relative rates of synthesis and utilization of cholesterol by the fetal liver and adrenal, respectively. We previously have shown that of the various organs in the human fetus, the liver and adrenal synthesize cholesterol at the highest rate (Carr and Simpson, unpublished observations), whereas the adrenal appears to utilize plasma cholesterol, in the form of LDL, at a rate greater than any other tissue (6). It follows therefore, that the high concentration of cholesterol in fetal plasma in early gestation may be due to a rapid rate of lipoprotein-cholesterol biosynthesis in the fetal liver, which, like the fetal adrenal, is extremely active in the synthesis of cholesterol (13,29). Since the umbilical cord was carefully cleansed prior to blood sampling, it is not likely that direct contamination of fetal blood by maternal blood occured during these studies.

Coincident with the initial fall in cholesterol levels during the period of 12 to 20 weeks post-conception, there is a 10-fold increase in the size of the fetal adrenal (28). The fetal adrenal takes up and degrades LDL-cholesterol (6) and the liberated cholesterol is used in the synthesis of steroid hormones, most notably DS (5). Thus, the rapid growth of the adrenal and the attendant increase in steroidogenesis could account for the depletion of plasma cholesterol at mid-gestation. In support of this hypothesis is the finding that the plasma cholesterol levels in prematurely delivered anencephalic newborns, in whom the adrenal is atrophied and secretes small quantities of steroid (23,24), are significantly greater than in normal fetuses during this period of development (Parker et al., unpublished observations). Following a nadir at 16-20 weeks of gestation, the plasma cholesterol levels rise through about 32 weeks post-conception. The weight of the fetal liver triples between 20 and 32 weeks and thereafter the rate of growth of the fetal liver declines (25). Thus, the rate of synthesis of lipoprotein-cholesterol during this gestational period likely exceeds its rate of utilization by the fetal adrenal.

The second decrease in fetal plasma cholesterol levels is that which occurs near term. A similar decline in plasma cholesterol levels near term has been documented by others (2,12). This progressive fall in fetal plasma cholesterol might be explained by the accelerated utilization of cholesterol by the fetal adrenals that are enlarging rapidly at this stage of gestation, a time when the rate of growth of the fetal liver is declining. Coincident with the decline in fetal plasma cholesterol levels during the last few weeks of gestation there is a marked increase in the level of estriol in maternal plasma (9,17). Estriol is synthesized in the placenta principally from fetal plasma 16α-hydroxydehydroisoandrosterone sulfate which, in turn, is formed from DS secreted by the fetal adrenal (27). Consistent with the hypothesis that the progressive decline in fetal plasma cholesterol levels near term is due to accelerated clearance of LDL-cholesterol through fetal adrenal utilization in steroid formation are the findings of Parker et al. (23). They found that the concentrations of total and LDL-cholesterol in plasma of term newborns were related inversely to the umbilical cord plasma concentration of the fetal adrenal estrogen precursor, i.e., DS. In addition, exceptionally high levels of cholesterol and very low levels of DS were found in plasma of anencephalic newborns delivered at term (23,24). An analogous situation has been observed in the case of adults. Leitchter and Daughaday reported the finding of massive steroid secretion and severe hypocholesterolemia in a

woman with a large, benign adrenal adenoma (21). After removal of the tumor, cholesterol levels promptly returned to normal. Conversely it was shown by Illingworth et al. (20) that ACTH-stimulated cortisol and C_{19}-steroid secretion were impaired in a person with abetalipoproteinemia.

The cholesterol concentrations in umbilical cord plasma of normal newborns near term that we found in the current study are lower than those reported by other investigators (4,10,11,14,15). Although it is known that the several methods for cholesterol determination are variably affected by many factors (18), we conclude that the difference between our results and those of others is not attributable wholly to methodological differences. The newborns from whom plasma was obtained in this study as well as their mothers were screened carefully to exclude any abnormal newborn and any mothers with medical complications. Others have reported unexplained elevations in cholesterol and triglyceride levels in newborns delivered of women with certain complications of pregnancies (1,8) and in newborns of women treated with betamethasone (2). Furthermore, we have shown previously that maternal chronic hypertension, pregnancy-induced hypertension, and diabetes are associated with elevations in cord plasma levels of total and LDL-cholesterol, presumably as the result of depressed utilization of LDL-cholesterol by the fetal adrenal as steroid substrate (23,24). The blood samples obtained from newborns between gestational ages 24 and 36 weeks were from pregnancies delivered after the spontaneous onset of premature labor in otherwise normal pregnant women. This group of newborns was chosen since, as cited above, it is known that the plasma cholesterol values are altered in newborns of mothers with complications of pregnancy that threaten fetal well-being. We cannot be sure that spontaneous premature labor is not associated with alterations in fetal plasma cholesterol levels. However from the findings of this study it is clear that the cholesterol levels in infants born prematurely are lower than in those electively delivered prematurely because of maternal disease (Parker et al., unpublished observations).

In summary, cholesterol levels in human fetal plasma early in gestation are extremely high, compared to those found at term. Indeed the concentration of cholesterol early in gestation is similar to that found in adults. Subsequently cholesterol levels in fetal plasma fall until 20 weeks post-conception. This nadir in fetal plasma cholesterol levels is followed by a secondary rise and thence a second decline beginning at about 30 weeks post-conception and persisting until term. These changes in fetal plasma cholesterol levels may be explained, in part, by alterations in the rate of biosynthesis of lipoprotein-cholesterol, presumably in the fetal liver, and by the rate of LDL clearance from plasma principally by the fetal adrenal wherein cholesterol is utilized as substrate for the biosynthesis of large amounts of steroids, particularly DS.

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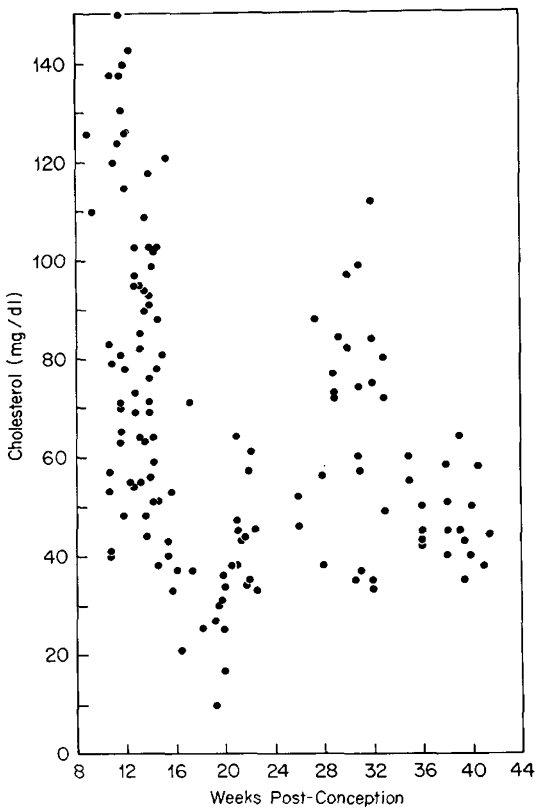

Fig. 1 Total plasma cholesterol levels in human umbilical cord plasma throughout gestation. The plasma cholesterol levels in individual samples are plotted as a function of post-conception gestational weeks. Plasma cholesterol was assayed as described in the text.

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