Inverse Relation Between Low-Density Lipoprotein-Cholesterol and Dehydroisoandrosterone Sulfate in Human Fetal Plasma

Abstract. *A striking inverse correlation was found in umbilical cord plasma between the concentrations of dehydroisoandrosterone sulfate and low-density lipoprotein (LDL)-cholesterol but not high-density lipoprotein-cholesterol or very low density lipoprotein-cholesterol. Dehydroisoandrosterone sulfate is a major secretory product of the human fetal adrenal and the principal precursor of placental estrogen production. The data suggest that the concentrations for LDL-cholesterol in fetal plasma are influenced by the rate of utilization of LDL-cholesterol by the fetal adrenal for steroidogenesis and are not necessarily related to a genetic predisposition for hypercholesterolemia or other lipoprotein disorders.*

The monitoring of estrogen concentrations, particularly estriol, in maternal blood or urine is widely used to evaluate fetal well-being in high-risk pregnancies. However, the mechanisms that control the levels of estriol in the maternal circulation are not entirely clear. Plasma estriol concentrations usually fall precipitously just before or shortly after fetal death, and in many pregnancies the level of estriol in the maternal compartment correlates well with fetal well-being (*1*). In the placenta, estriol is synthesized from the C_{19}-steroid 16α-hydroxydehydroisoandrosterone sulfate (16α-OHDS) which, in turn, arises by 16α-hydroxylation, principally in fetal liver, of dehydroisoandrosterone sulfate (DS), the major secretory product of the fetal adrenal (*2*). At term, the rate of DS production in the fetus is 100 to 200 mg per day (*2, 3*), a rate of secretion that is nearly one order of magnitude greater than that of the adrenals of adults at rest (*4*). Plasma estriol levels are frequently low in women whose fetuses are presumed to be "stressed," that is, fetuses that are hypoxic because of decreased uteroplacental blood flow, as in pregnancies complicated by chronic or pregnancy-induced hypertension, severe forms of diabetes mellitus, and other poorly defined conditions that cause fetal growth retardation (*1*). The enzymatic reactions that lead to the conversion of DS to 17β-estradiol and of 16α-OHDS to estriol, except in rare instances of placental sulfatase deficiency, are not rate limiting in the formation of estrogens by the placenta. Presumably, therefore, the rate of placental estrogen production is related directly to the rate of DS biosynthesis in the fetal adrenals. If this is, indeed, the case, then unlike the stressed adult, the fetus presumed to be stressed must show depressed rather than activated adrenal steroid secretion, with DS being especially affected.

In studies of the regulation of human fetal adrenal steroidogenesis in vitro, we used organ culture as a model system and observed that when fetal adrenal tissue was stimulated by adrenocorticotropic hormone (ACTH), lipoproteins were required for maximum steroid biosynthesis (*5*). In particular, we found that low-density lipoprotein (LDL) was the plasma lipoprotein most effective in supporting fetal adrenal steroidogenesis. High-density lipoprotein (HDL) was much less effective, and very low density lipoprotein (VLDL) was without effect on fetal adrenal steroidogenesis (*5*). We also found that the human fetal adrenal has a high capacity to take up and degrade ^{125}I-labeled LDL (*6*). Moreover, ACTH, the obligatory trophic agent in adrenal steroid hormone biosynthesis, caused a striking increase in the rate of degradation of ^{125}I-labeled LDL (*6*). The uptake and degradative processes in the fetal adrenal were similar to those described previously (*7, 8*) for steroidogenic as well as non-steroid-producing tissues and involved binding of the LDL to high-affinity receptors on the cell surface, followed by internalization and degradation of the lipoprotein. It is in this way that the cholesterol contained in the lipoprotein becomes available to serve as precursor for steroid hormone biosynthesis.

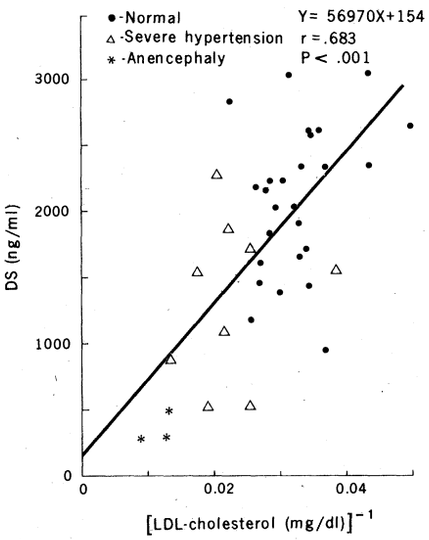

Fig. 1. Relation between the concentrations of LDL-cholesterol and DS in individual samples of umbilical cord plasma. The concentration of DS and the reciprocal of the LDL-cholesterol concentration for individual plasma samples from normal infants of normal mothers, from newborns of mothers with severe pregnancy-induced or chronic hypertension, and from anencephalic newborns are presented. The concentrations of LDL-cholesterol and DS in newborns of mothers with mild pregnancy-induced hypertension were not included in this analysis since these values did not differ significantly from those of the normal group.

The concentrations of lipoproteins, particularly LDL, in umbilical cord plasma of newborn humans are much reduced compared to those in the plasma of adults. Typically, the concentration of LDL-cholesterol in umbilical cord plasma is 30 mg/dl, compared to 125 mg/dl in normal adults (*9*). After birth the plasma concentration of LDL rises dramatically, reaching a value of 92 mg/dl within 1 year. Many investigators have sought to ascertain whether the concentrations of lipoprotein-cholesterol in cord plasma of newborns can be correlated with the occurrence of familial hypercholesterolemia or other lipoprotein disorders (*9*). Generally, the results have been negative. For example, in a screening study of 2000 newborns, only 1 of 53 whose LDL-cholesterol concentration in umbilical cord plasma exceeded the 90th percentile for normal newborns was found subsequently to be a heterozygote for familial hypercholesterolemia when tested at 1 year of age (*10*). Therefore, the factors influencing the levels of lipoproteins in cord plasma at birth are still unknown.

Since LDL-cholesterol is preferentially used by the human fetal adrenal for steroidogenesis in vitro, we reasoned that LDL-cholesterol is probably used in vivo as well. If the rate of utilization of LDL-cholesterol was such that only 50 percent (75 mg/day) of the fetal adrenal steroids were derived from LDL-cholesterol circulating in fetal plasma, then the LDL in fetal plasma would turn over at least two times per day in order to supply cholesterol only to the adrenal. Such a turnover (200 percent of the plasma pool per day) is vastly in excess of that observed in the adult (44 percent of the plasma pool per day) (*11*). Therefore, the LDL in human fetal plasma may be regulated largely by the rate of uptake and utilization of this lipoprotein for steroidogenesis by the fetal adrenal. To test this hypothesis, we compared the concentrations of lipoprotein-cholesterol and DS in the umbilical cord plasma of newborns. Lipoprotein-cholesterol and DS were quantified by methods previously described (*12*); the data were analyzed by an unpaired, two-tailed *t*-test.

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For these studies we used newborns from the following groups: (i) normal infants born of mothers with uncomplicated pregnancies; (ii) infants born of mothers with mild pregnancy-induced hypertension; (iii) infants born of mothers with severe pregnancy-induced or chronic hypertension; and (iv) anencephalic newborns. Our results are presented in Table 1. In normal infants born of normal mothers, the value for LDL-cholesterol in the cord plasma (31.1 ± 1.2 mg/dl) was similar to that found by many other investigators (*9*). The value for DS in the same infants was 2012 ± 125 ng/ml. The values for LDL-cholesterol and DS in infants born to mothers with mild pregnancy-induced hypertension were similar to those in normal infants born to normal mothers. Concentrations of estriol in women with mild pregnancy-induced hypertension are ordinarily normal or even slightly elevated (*13*). However, in the infants of this study who were born to women with severe pregnancy-induced or chronic hypertension, the concentration of DS in the umbilical cord plasma was significantly reduced compared to that in normal infants of normal mothers ($P < .01$). On the other hand, the concentration of LDL-cholesterol in the cord plasma of such infants (48.6 ± 4.1 mg/dl) was significantly elevated ($P < .001$) compared to the levels in normal newborns of normal mothers. Conversely, the concentrations of HDL- and VLDL-cholesterol in newborns whose mothers had severe hypertension were not significantly different from those found in normal newborns of normal mothers.

Maternal estrogen levels are depressed (*14*) and the fetal adrenal is markedly atrophic (*15*) in pregnancies with an anencephalic fetus. We measured DS and lipoprotein-cholesterol in cord plasma of three anencephalic newborns. The mean level of DS in the umbilical cord plasma of the anencephalic newborns was reduced to about 15 percent (357 ng/ml) of that found in normal newborns. Correspondingly, there was a significant increase in the mean concentration of LDL-cholesterol in the plasma (88 mg/dl) of the anencephalic newborns ($P < .001$, compared to normals). Once again, the concentrations of HDL were not different from those of normal newborns of normal mothers. Because the plasma sample obtained from the anencephalic newborns was small, the concentrations of VLDL were below the limits of detection. Not only was there an inverse relationship for the plasma levels of LDL and DS in umbilical cord plasma when expressed for these patient groups, but these values correlated inversely when evaluated on an individual basis and subjected to linear regression analysis (Fig. 1).

Table 1. Concentrations of dehydroisoandrosterone (DS) and lipoproteins (expressed as concentrations of lipoprotein-cholesterol) in umbilical cord plasma. The results are presented as means ± standard error.

Subject classification	Number of newborns	DS (ng/ml)	Lipoproteins (mg cholesterol/dl)		
			LDL	HDL	VLDL
Normal	26	2012 ± 125	31.1 ± 1.2	24.2 ± 1.0	2.3 ± 0.3
Mild pregnancy-induced hypertension	5	1838 ± 315	29.5 ± 3.9	27.6 ± 3.9	4.4 ± 1.9
Severe pregnancy-induced or chronic hypertension	9	1321 ± 205*	48.6 ± 4.1†	22.4 ± 3.0	3.9 ± 1.3
Anencephaly	3	357 ± 70†	88.2 ± 6.3†	27.3 ± 8.2	Undetectable

*$P < .01$. †$P < .001$, when compared to normal.

The striking inverse correlation observed between the umbilical cord plasma concentrations of LDL-cholesterol and of DS suggests that LDL in human fetal plasma is regulated by the rate of utilization of LDL by the fetal adrenal. This process can be envisioned as follows: under normal circumstances, when the fetal adrenal is actively synthesizing and secreting the placental estrogen precursor DS, the concentrations of LDL in the fetal plasma are maintained at a low steady-state level. However, when the rate of fetal adrenal steroid secretion is depressed, as in pregnancies with an anencephalic fetus or in those complicated by severe maternal hypertension, concentrations of estriol in the maternal plasma are abnormally low (*1*, *14*), and the concentrations of LDL in the fetal plasma are increased. Such an increase in LDL would be expected to occur when utilization of LDL for steroidogenesis by the fetal adrenal is impaired. When betamethasone, an inhibitor of both maternal and fetal pituitary secretion of ACTH, is given to pregnant women, the concentration of cholesterol in umbilical cord plasma increases (*16*). Others have found that when fetal well-being is compromised the concentrations of LDL in umbilical cord plasma are frequently increased (*10*, *17*). This, together with our results, suggests that the concentrations of estriol in maternal plasma and of LDL in the cord blood of newborns are related by a common factor, namely, the rate of utilization of LDL by the fetal adrenal. The relation between these two parameters is an inverse one. Thus, we believe that these results indicate that the concentrations of LDL in the umbilical cord plasma of newborns are not accurate predictors of heterozygous familial hypercholesterolemia or other forms of hyperlipidemia. Rather, the concentrations of LDL in fetal plasma probably reflect the activity of the fetal adrenal and thus are regulated by the rate of LDL biosynthesis and by the rate at which the fetal adrenal uses LDL-cholesterol to synthesize C_{19}-steroids which are utilized by the placenta for estrogen biosynthesis.

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References and Notes

1. J. W. Goldkrand, *Obstet. Gynecol.* **52**, 264 (1978); I. R. Sarda and R. H. Gorwill, *ibid.* **49**, 546 (1977); J. H. Duenhoelter, P. J. Whalley, P. C. MacDonald, *Am. J. Obstet. Gynecol.* **125**, 889 (1976); U. Goebelsman, R. K. Freeman, J. H. Mestman, R. M. Nakamura, B. A. Woodling, *ibid.* **115**, 795 (1973); L. Nachtigall, M. Bassett, U. Hogsander, M. Levitz, *ibid.* **101**, 638 (1968); V. A. Frandsen and G. Stakemann, *Acta Endocrinol. (Copenhagen)* **44**, 196 (1963).
2. P. K. Siiteri and P. C. MacDonald, *J. Clin. Endocrinol. Metab.* **26**, 751 (1966).
3. H. H. Simmer, W. E. Easterling, R. J. Pion, W. J. Dignam, *Steroids* **4**, 125 (1964).
4. R. E. Peterson, *J. Clin. Invest.* **39**, 320 (1960).
5. E. R. Simpson, B. R. Carr, C. R. Parker, Jr., L. Milewich, J. C. Porter, P. C. MacDonald, *J. Clin. Endocrinol. Metab.* **49**, 146 (1979); B. R. Carr, C. R. Parker, Jr., L. Milewich, P. C. MacDonald, E. R. Simpson, J. C. Porter, Program, 26th Annual Meeting of the Society for Gynecologic Investigation, San Diego, California (1979), p. 76.
6. E. R. Simpson and B. R. Carr, Program, 61st Annual Meeting of the Endocrine Society, Anaheim, California (1979), p. 218; B. R. Carr, J. C. Porter, P. C. MacDonald, E. R. Simpson, in preparation.
7. J. L. Goldstein and M. S. Brown, *J. Biol. Chem.* **249**, 5153 (1974); M. S. Brown, P. T. Kovanen, J. L. Goldstein, *Recent Prog. Horm. Res.* **35**, 215 (1979).
8. C. A. Winkel, J. R. Gilmore, J. C. Porter, E. R. Simpson, Program, 26th Annual Meeting of the Society for Gynecologic Investigation, San Diego, California (1979), p. 68; C. A. Winkel and E. R. Simpson, Program, 61st Annual Meeting of the Endocrine Society, Anaheim, California (1979), p. 79; C. A. Winkel, J. M. Snyder, P. C. MacDonald, E. R. Simpson, in preparation.
9. R. R. Fredricks, S. R. Srinivasan, L. S. Webber, M. C. Ruth, G. S. Berenson, *Pediatr. Res.* **12**, 858 (1978); C. J. Glueck, P. S. Gartside, R. C. Tsang, M. Meillies, P. M. Steiner, *Metabolism* **26**, 347 (1977); C. J. Glueck, M. J. Mellies,

R. C. Tsang, P. M. Steiner, *Pediatr. Res.* **11**, 957 (1977).
10. T. J. C. Boulton, I. H. Craig, G. Hill, *Acta Paediatr. Scand.* **68**, 363 (1979).
11. D. W. Bilheimer, N. J. Stone, S. M. Grundy, *J. Clin. Invest.* **64**, 524 (1979).
12. Cord plasma levels of cholesterol in low-density, high-density, and very low density lipoprotein were determined according to the techniques of the National Institutes of Health, Manual of Laboratory Operations Lipid Research Clinics Program [*Lipid and Lipoprotein Analysis*, U.S. Department of Health, Education, and Welfare, Publ. No. 75-628 (1974)]. The levels of DS in umbilical cord plasma were determined by radioimmunoassay [J. E. Buster and G. E. Abraham, *Anal. Lett.* **5**, 543 (1972)].
13. L. Nachtigall, M. Bassett, U. Hogsander, M. Levitz, *Am. J. Obstet. Gynecol.* **101**, 638 (1968).
14. L. Miyakawa, I. Ikeda, A. Nakayama, M. Makyama, *J. Endocrinol.* **69**, 291 (1976).
15. K. Benirschke, *Obstet. Gynecol.* **8**, 412 (1956).
16. G. E. Andersen and B. Friis-Hansen, *Acta Paediatr. Scand.* **66**, 355 (1977); *Pediatrics* **62**, 8 (1978).
17. ———, *Acta Paediatr. Scand.* **65**, 369 (1976); H. A. Cress, R. M. Shaher, R. Laffin, K. Karpowicz, *Pediatr. Res.* **11**, 19 (1977).
18. We thank J. Sonenthal and J. Morrison for technical assistance and M. Arnold for editorial assistance. We also thank J. Buster for the generous gift of the DS antiserum. This research was supported, in part, by NIH grants 1-RO1-HD13234, AG-00306, and HD-11149.

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Opiate Analgesia: Evidence for Mediation by a Subpopulation of Opiate Receptors

Abstract. *Naloxazone, a hydrazone derivative of the opiate antagonist naloxone, has a high affinity for opiate receptor binding sites. Naloxazone injections reduce opiate receptor binding to extensively washed mouse brain membranes for more than 24 hours, suggesting that the effect is irreversible. High-affinity binding sites are abolished by this treatment, whereas low-affinity sites are unaffected. Naloxazone treatment blocks the analgesic effects of morphine for at least 24 hours but does not prevent death from high doses of morphine. Thus analgesic but nonlethal opiate effects may be mediated by the high-affinity subpopulation of opiate receptors.*

Various pharmacological (*1*) and biochemical (*2*) studies support the existence of multiple opiate receptors. Distinct high- and low-affinity opiate receptor binding sites have been reported (*2*). Sodium selectively inhibits opiate agonist binding by abolishing high-affinity binding, with minimal effects on low-affinity sites; manganese ions enhance agonist binding by increasing the binding capacity of high-affinity opiate receptor sites. Low concentrations of protein-modifying reagents, such as *N*-ethylmaleimide and iodoacetamide, also lower agonist binding by destroying predominantly high-affinity agonist binding sites (*3*, *4*).

Irreversibly acting drugs can often be used as probes for characterizing the functional correlates of receptors. The opiate antagonist chlornaltrexamine and the agonist chloroxymorphamine appear to act irreversibly (*5*). Naloxazone is a monosubstituted C-6 hydrazone derivative of naloxone (Fig. 1) (*6*). In the present study, we show that naloxazone blocks high-affinity binding of ^{3}H-labeled opiates selectively and irreversibly in vivo. Since naloxazone markedly attenuates morphine analgesia with little effect on the lethality of narcotic overdose, it may prove possible to identify highly analgesic opiates, acting at high-affinity sites, that have a lesser potential for lethal effects and are mediated via low-affinity receptors.

Naloxazone was synthesized by adding naloxone to a tenfold excess of hydrazine in ethanol. After 2 hours, sodium borate solution was added and the product extracted into chloroform. After recrystallization from chloroform and petroleum ether, thin white crystals of free base with a melting point of 163°C were obtained; mass spectroscopy showed a single parent-ion with a mass-to-charge ratio (m/e) of 341 (M^+) corresponding to the molecular weight of 341; elemental analysis of C (66.61); H (6.90); O (14.36); N (12.13) compared favorably to the theoretical value for $C_{19}H_{23}O_3N_3$ (C, 66.84; H, 6.79; O, 14.06; N, 12.31). Nuclear magnetic resonance showed a downshift of 0.17 part per million for the hydrogen at C-5. Infrared spectroscopy showed disappearance of the ketone absorbance at 1700 cm^{-1} of naloxone, and a positive test with trinitrobenzylsulfonic acid indicated the presence of free $^-NH_2$. Thin-layer chromatography of naloxazone yielded a single spot with an R_F of 0.74 on silica gel (chloroform, methanol, and concentrated ammonium hydroxide at 132:12:0.9) and an R_F of 0.44 on alumina (chloroform and methanol at 9:1). The R_F of naloxone was 0.86 in the silica gel system and 0.74 in the alumina system.

Table 1. Morphine analgesia and death in naloxone- and naloxazone-treated mice. Mice treated with naloxone or naloxazone were given intraperitoneal injections of morphine sulfate 16 to 20 hours later; survivors were tested for analgesia with the tail-flick assay. Each animal received only one injection of morphine. A mouse was considered insensible to pain if the tail-flick latency was twice as long after morphine administration as before. Control latencies ranged from 3 to 5 seconds.

Morphine dose (mg/kg)	Preliminary treatment			
	Naloxone (200 mg/kg)		Naloxazone (200 mg/kg)	
	Analgesia	Death	Analgesia	Death
2.5	1/10			
5	4/10			
10	14/19		0/10	
25			1/10	
30			0/8	
60			3/9	
90			7/9	
100			8/10	
400		3/8		3/7
500		13/16		10/16

Solutions of naloxone or naloxazone in 0.9 percent NaCl were made with glacial acetic acid (final pH, 7.0) and injected subcutaneously into the back of the neck. Binding assays were performed as previously described, using extensive washing techniques that have been demonstrated to remove reversible ligands (*4*). The effects of naloxazone on morphine analgesia were examined by treating mice with naloxone, naloxazone, or saline, giving them various doses of morphine sulfate, and testing them with the tail-flick assay (*7*); morphine lethality was determined through intraperitoneal injection of high doses of morphine sulfate in groups of mice treated in the same manner.

Opiate receptor binding assays in vitro show that naloxazone competes with both the narcotic agonist [^{3}H]dihydromorphine and the antagonist [^{3}H]naloxone. The inhibition constant of naloxazone for both the [^{3}H]naloxone and [^{3}H]dihydromorphine binding sites is 2 to 4 nM (four replications with 10 to 20 percent variation) and is unaffected by either sodium or manganese ions. In the experiments, single concentrations of the ^{3}H-labeled ligands were used, with no separation of high- and low-affinity sites. Since sodium ions decrease agonist inhibition of [^{3}H]naloxone binding and manganese ions increase it (*3*), the absence of any effect by these ions supports naloxazone's antagonistic character.

One day after the subcutaneous injection of naloxazone (200 mg/kg), [^{3}H]-naloxone binding in whole brain membranes was reduced by 40 percent. Binding remained reduced despite several washings, resuspension, and incubation at 37°C to remove any drug only

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