Tracer kinetic studies of the low density lipoprotein metabolism in the fetal rat: an example for estimation of flux rates in the nonsteady state[1]

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Abstract To get insight into the low density lipoprotein (LDL)-apoB flux in the rat fetus near term and in the early postnatal period, homologous apoE-free ^{125}I-labeled LDL was injected into the umbilical vein of the rat fetus immediately after Caesarean section. Since the serum LDL-apoB spontaneously declined after birth, a time-dependent two-pool model was used to calculate the flux rates in the neonate from the specific activities of LDL-apoB up to 15 h post partum. An approximate value of LDL-apoB flux in the fetus at birth was obtained by extrapolation of the kinetic data to the time of injection of the tracer. The data revealed that the turnover of LDL-apoB in the fetus (18.6 μg LDL-apoB/h per g body weight) exceeded that in the adult rat (0.4 μg/h per g body weight) by at least one order of magnitude. Even 15 h after delivery, the LDL-apoB influx amounted to 2.5 μg/h per g body weight. The fractional catabolic rate of LDL-apoB in the fetus at term (0.39, h^{-1}) slightly exceeded that in the adult animal (0.15, h^{-1}) and reached the adult level within the first 3 h after birth and remained constant thereafter. In the rat fetus, LDL-apoB flux greatly exceeds that of VLDL-apoB. The data support the view of a direct synthesis and secretion of LDL, most probably by the fetal membranes.—**Plonné, D., B. Schlag, L. Winkler, and R. Dargel.** Tracer kinetic studies of the low density lipoprotein metabolism in the fetal rat: an example for estimation of flux rates in the nonsteady state. *J. Lipid Res.* 1990. **31:** 747–752.

Supplementary key words tracer kinetic • nonsteady state • LDL-turnover • fetal rat

In the late gestational period the plasma lipoprotein pattern of the rat fetus exhibits some peculiarities, the most important of which are the high LDL concentration, the extremely low concentration of VLDL, and the lack of apoE in the LDL and of apoC in the VLDL fraction (1–3). In the light of LDL as a carrier for cholesterol, it seemed to be of interest to know the LDL turnover in the fetal rat. Due to technical reasons it is not possible to study metabolic pathways of the fetus in utero by tracer kinetics without disrupting the feto-placental unit. Therefore, we tried to extrapolate from kinetic studies carried out immediately after delivery by section to the end of the prenatal phase. The perinatal phase, however, is characterized by numerous transient states due either to the alterations of pool sizes by the onset of adaptative processes or the acute interruption of the placental and yolk sac circulation at birth. For example, immediately after delivery there is a striking but transient decrease of the LDL concentration while the HDL concentration increases and the VLDL concentration remains nearly constant for several days post partum (D. Plonné et al., unpublished data). This means that studies of LDL kinetics immediately after birth have to be done in the nonsteady state.

Various mathematical solutions have been proposed for special metabolic nonsteady state problems (4–8). Usually this approach necessitates information about structure and parameters of the mathematical model in the steady state system of the metabolite in question. Obviously, the information required for the calculation of flux parameters cannot be obtained in the present case. Therefore, the aim of the present study was to determine the flux rates of LDL in the fetal and neonatal rat immediately be-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins, apo, apolipoprotein; SDS, sodium dodecyl sulfate; EIA, electroimmunoassay; BSA, bovine serum albumin; FCR, fractional catabolic rate; B.W., body weight; SD, standard deviation; SEM, standard error of the mean.

[1]This manuscript, which attempts to model a nonsteady state situation, is being published because of the Editor's belief that this work will significantly stimulate further efforts in this line of experimentation. However, an expert kinetic reviewer of this manuscript believes that several important assumptions used to develop the model may not necessarily be valid. Specifically, the Steele equations which are used in the modeling are based on glucose kinetics, but the model shown in Fig. 1 is not necessarily true for the glucose system. Conditions to be filled include *1*) that K_{12} and K_{21} must be constant and known, *2*) that the initial value $M_2(0)$ must be known, and *3*) that the initial value, $Y(0)$, must be known and that these values must be supported by experimental data. In the present situation, such data are lacking. In the glucose system, it is known that K_{12} and K_{21} vary with time. The basic assumption that these values to not vary between fetal and adult rats, therefore, may not necessarily be correct.

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fore and after birth in spite of the difficulties one encounters in the perinatal state.

MATERIALS AND METHODS

Animals

Female Wistar rats from our colony (Uje-Wist) receiving a standard diet ad lib were used. The day of pregnancy was determined by vaginal smears. The fetuses were delivered without disruption of the umbilical circulation by Caesarean section at day 22 of gestation within a 2-min period after killing the dam by dislocation of the neck.

Tracer experiments

Preparation of homologous LDL was performed as described elsewhere (3). Labeling of LDL with ^{125}I was as described by McFarlane (9). The labeled LDL were filtered through a 45-μm Millipore membrane immediately prior to use.

For measurement of the tracer decay in fetal serum, 0.3 μCi of homologous ^{125}I-labeled LDL (sp act 0.03 μCi/μg LDL protein) in 10 μl was injected into the umbilical vein. Thereafter, the umbilical circulation was blocked by a ligature and the fetus was separated from the placenta. No radioactivity was found in the placenta after this procedure. Fetuses were held at 37°C in a wet chamber. No significant changes of the hematocrit and of the body weight were observed up to 15 h after delivery. Fetal blood was collected into glass capillaries after an incision into the neck region 5, 15, 30 min, and 1, 2, 3, 4, 5, 6, 8, 10, 12, and 15 h after the tracer injection.

Each sampling point represents the mean value of five or six single fetuses. The total radioactivity was measured in a 20-μl aliquot of serum in a gamma counter (Multycrystal gamma-counter, Fa. Berthold). For estimation of the radioactivity of the ^{125}I-labeled LDL-apoB, 200 μg human LDL was added to another 20-μl aliquot of serum and the apoB was precipitated by isopropanol according to Yamada et al. (10). The activity remaining in the supernatant was subtracted from the total activity.

The tracer decay in serum of adult animals was measured after injecting 0.2 ml of the ^{125}I-labeled LDL into the tail vein as described above. Aliquots of 100 μl blood were drawn 5, 10, 15, and 30 min, and 1, 2, 3, 6, and 10 h after application of the tracer.

The LDL-apoB concentration was determined by EIA technique (11). For measuring LDL-apoB in fetal rats, the assay was performed by using whole serum because the amount of VLDL-apoB was found to be negligible (<0.5 mg/dl). The concentration of LDL-apoB in serum of adult animals was determined in the infranatant after flotation of the VLDL (100,000 g; 20 h).

Protein was determined by the method of Lowry et al. (12) in the presence of SDS (40 mg/5 ml final volume) using BSA as a standard.

Fitting methods

Nonsteady state. For calculation of metabolic fluxes in the nonsteady state, it is essential to presmooth the experimental data (5, 13). A sum of two exponentials was fitted to the data of both the tracee (LDL-apoB) concentration and the tracer (^{125}I-labeled LDL-apoB) concentration in the serum normalized to a 5 g fetus. The equation

$$C(t) = c0 \times e^{-c1 \times t} + c2 \times e^{-c3 \times t} \qquad \textit{Eq. 1}$$

represents the smoothing function for the decay of the LDL-apoB concentration in the fetal serum after section, and

$$X(t) = x0 \times e^{-x1 \times t} + x2 \times e^{-x3 \times t} \qquad \textit{Eq. 2}$$

is the smoothing function for the tracer activity in the serum after injection into the umbilical vein at $t = 0$. Neither Eq. 1 nor Eq. 2 represents a solution of any linear system in this case. The smoothing functions C(t) and X(t) allow good approximations for the time courses of tracee and tracer concentrations, and that is why they were used instead of the real but unknown functions for calculating the nonsteady state fluxes.

Steady state. The function

$$P(t) = p0 \times e^{-p1 \times t} + p2 \times e^{-p3 \times t} \qquad \textit{Eq. 3}$$

was fitted to the values of the tracer decay in the serum of the adult rat which were normalized to a 250 g animal. In contrast to Eq. 1 and Eq. 2, Eq. 3 is a real model function, i.e., the solution of a linear time-invariant two-pool system (14, 15).

In general, fitting was done by using a computer program for nonlinear regression analysis which includes fitting of the data by the method of weighted least squares whereby the sum of the residuals squared was minimized by a regularized Gauss-Newton method (16).

Mathematical modelling

Nonsteady state. The smoothing functions C(t) and X(t) and their derivatives C'(t) and X'(t) were used to calculate the flux rates for the time-dependent two-pool model (**Fig. 1**).

The differential equations for the LDL-apoB masses are

$$M1'(t) = -k12 \times M1(t) + k21 \times M2(t) + (fi(t) - fe(t)) \qquad \textit{Eq. 4}$$

$$M2'(t) = k12 \times M1(t) - k21 \times M2(t) \qquad \textit{Eq. 5}$$

The tracer decay is described by the differential equations

$$X'(t) = -k12 \times X(t) + k21 \times Y(t) - \frac{fe(t) \times X(t)}{M1(t)} \qquad \textit{Eq. 6}$$

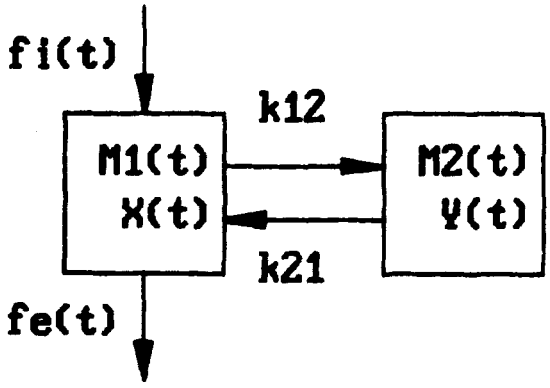

Fig. 1. Structure of the two-compartment system used for calculation of the LDL flux rates in the adult and fetal rat. M1(t): absolute LDL-apoB mass of pool 1 (μg) (intravascular pool); M2(t): absolute LDL-apoB mass of pool 2 (μg) (extravascular pool); X(t): tracer activity in pool 1; Y(t): tracer activity in pool 2; k12, k21: fractional transfer rate constants; fi(t): influx (μg/h); fe(t): efflux (μg/h) = k10(t) × M1(t); k10(t) = FCR(t) = fractional catabolic rate.

$$Y'(t) = k12 \times X(t) - k21 \times Y(t) \qquad \textit{Eq. 7}$$

The system (Eq. 4–Eq. 7) can be solved if the following conditions are fulfilled: 1) k12 and k21 must be constant and must be known. 2) The initial value M2(0) must be known. 3) The initial value Y(0) must be known.

1) The parameters k12 and k21 were assumed to be constant and to correspond to those in the steady state of adults (for details see Discussion). The latter were determined in separate experiments using the model structure of Fig. 1 for the steady state.

2) According to Radziuk, Norwich, and Vranic (5) we assumed that the volume of distribution remains constant during the nonsteady state. In this case the absolute size of pool 1 M1(t) is given by

$$M1(t) = V \times C(t) \qquad \textit{Eq. 8}$$

The volume of distribution, V, of pool 1 is the ratio of the injected total activity and the concentration of the tracer activity in the serum extrapolated to t = 0. The sizes of pool 1 and pool 2 in the prenatal steady state M1 and M2 correspond to those in the postnatal nonsteady state at t = 0 M1(0) and M2(0), respectively. That means:

$$M1(0) = M1 \qquad \textit{Eq. 9}$$

$$M2(0) = M2 \qquad \textit{Eq. 10}$$

Because in the prenatal steady state

$$k12 \times M1 = k21 \times M2$$

the initial value M2(0) is given by

$$M2(0) = M2 = \frac{k12 \times M1(0)}{k21} \qquad \textit{Eq. 11}$$

3) The activity of the tracer within the pool 2 at t = 0 is equal to zero:

$$Y(0) = 0 \qquad \textit{Eq. 12}$$

The solution of Eq. 5 with the initial value Eq. 11 is given by

$$M2(t) = V \times \left(\frac{k12(c0+c2)}{k21} \times e^{-k21\times t} + \frac{k12\times c0}{k21-c1} \times (e^{-c1\times t} - e^{-k21\times t}) + \frac{k12\times c2}{k21-c3} \times (e^{-c3\times t} - e^{-k21\times t})\right) \qquad \textit{Eq. 13}$$

The difference of the fluxes can be computer by combining Eq. 13 and Eq. 4:

$$fi(t) - fe(t) = M1'(t) + k12 \times M1(t) - k21 \times M2(t) \qquad \textit{Eq. 14}$$

The solution of Eq. 7 for Y(0) = 0 is given by

$$Y(t) = \frac{k12\times x0}{k21-x1} \times (e^{-x1\times t} - e^{-k21\times t}) + \frac{k12\times x2}{k21-x3} \times (e^{-x3\times t} - e^{-k21\times t}) \qquad \textit{Eq. 15}$$

The efflux results from substitution of Eq. 15 into Eq. 6:

$$fe(t) = M1(t) \times \left(-k12 + \frac{k21\times Y(t) - X'(t)}{X(t)}\right) \qquad \textit{Eq. 16}$$

Finally, the FCR is obtained by

$$FCR(t) = k10(t) = \frac{fe(t)}{M1(t)} \qquad \textit{Eq. 17}$$

RESULTS

The elimination of homologous ^{125}I-labeled LDL-apoB from serum in the adult rat is shown in **Fig. 2.** The values of the parameters of the normalized model function P(t) were found to equal p0 = 0.217 ± 0.035; p1 = 2.802 ± 1.102; p2 = 0.738 ± 0.029; and p3 = 0.122 ± 0.009. The

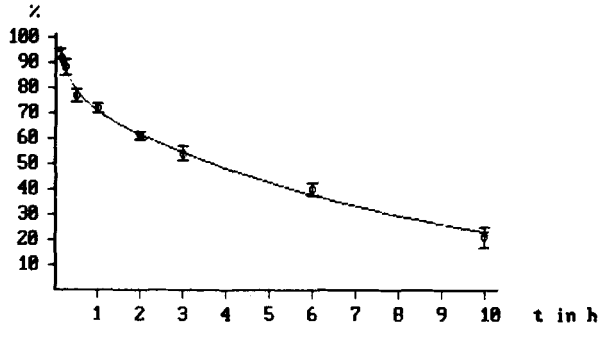

Fig. 2. Time course of tracer elimination from the serum of adult rats after injection of 200 μl (6 μCi) of homologous ^{125}I-labeled LDL (specific activity 0.03 μCi/μg LDL protein) into the tail vein (values in %). Each point represents the mean ± SEM of values from six animals.

concentration of the serum LDL-apoB in adult animals was 7.3 ± 1.7 mg/dl (mean ± SD, n = 8). The flux rates and the FCR of LDL-apoB calculated for the adult rat were 86.4 ± 10.4 μg/h per 200 g body weight and 0.154 ± 0.014 1/h, respectively. The calculated fractional transfer rate constants were k12 = 0.55 ± 0.25 and k21 = 2.22 ± 0.87 · h^{-1}.

Fig. 3 shows the time course of the LDL-apoB concentration and the ^{125}I-labeled LDL-apoB radioactivity in the fetal serum within a time period of 15 h starting at the point of tracer injection (t = 0). The LDL-apoB pool of the fetus decreased from 46 mg/dl serum at t = 0 to 20 mg/dl within this period.

The calculated flux rates and the FCR of LDL-apoB in the fetal/neonatal rat are given in **Table 1.** The values reveal that at t = 0 the influx of LDL into the compartment 1 is 27 μg/h per 5 g body weight while the efflux amounts to 93 μg/h per 5 g body weight. Thus, when related to 1 g body weight, the influx and the efflux of LDL-apoB in the fetus at t = 0 are 5.4 μg/h and 18.6 μg/h, respectively, while the LDL-apoB turnover in the adult animals is 0.43 μg/h per g body weight. That means that the LDL turnover in the fetus exceeds that in the adult animal by at least one order of magnitude. The FCR in the fetus at t = 0 was calculated as 0.39 · h^{-1} and thus was somewhat higher than in the adult animal (0.15 · h^{-1}). The FCR decreases after delivery and reaches the adult level after about 3 h. Thereafter, the FCR remains constant within the sampling period (Table 1).

In the newborn, the LDL efflux exceeds the influx at any time during the sampling period. Furthermore, both influx and efflux decrease by about 50% between 3 and 15 h after tracer injection (Table 1).

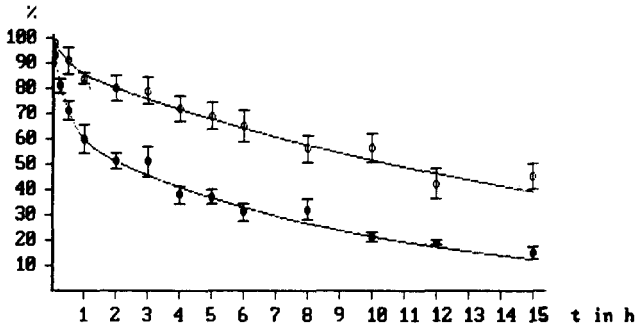

Fig. 3. Time course of the radioactivity (●; parameters of the smoothing function X(t): x0 = 37.0 ± 6.3, x1 = 2.363 ± 0.920, x2 = 63.0 ± 4.6, x3 = 0.108 ± 0.01) in the fetal serum after injection of 10 μl (0.3 μCi) of homologous ^{125}I-labeled LDL (sp act 0.03 μCi/μg LDL protein) into the umbilical vein (values in %), and time course of the LDL-apoB concentration (○; parameters of the smoothing function C(t)): c0 = 50 ± 21.5, c1 = 2.106 ± 2.09, c2 = 411 ± 13, c3 = 0.055 ± 0.0047) in the fetal serum after delivery in %; time of delivery at t = 0; 100% = 46.1 mg/dl. Each point represents the mean ± SEM of values from five to six fetuses.

TABLE 1. Influx, efflux, and fractional catabolic rate (FCR) of LDL-apoB in the newborn at designated times after delivery by Caesarean section

Time	Influx	Efflux	Influx	Efflux	FCR
h	*μg/h/fetus*		*μg/h/g body weight*		h^{-1}
0	27.1	93.0	5.42	18.6	0.390
1	26.0	49.5	5.20	9.90	0.240
2	15.1	29.7	3.02	5.94	0.155
3	12.4	25.0	2.48	5.00	0.139
5	10.8	21.9	2.16	4.38	0.136
7	9.6	19.6	1.92	3.92	0.136
9	8.6	17.5	1.72	3.50	0.136
11	7.7	15.7	1.54	3.14	0.136
13	6.9	14.1	1.38	2.81	0.136
15	6.2	12.6	1.24	2.52	0.136

DISCUSSION

Since, for technical reasons, the study of the LDL flux was not possible in the intact rat fetus under in vivo conditions, we tried to get the flux parameters by a mathematical approach that should allow us to extrapolate from the early postnatal phase to the end point of the gestational phase. A serious problem, however, was that the steady state existing immediately prior to birth abruptly shifted to a nonsteady state characterized by a marked decrease of the LDL pool when the fetus was removed from the feto-placental unit. The transition from the prenatal steady state to the nonsteady state starting with birth was caused by a suddenly appearing difference between influx and efflux, the latter having been obtained from extrapolation of the experimental data to t = 0. This difference could theoretically originate from the following alterations. *1*) Decrease of the influx with the efflux unchanged or only slightly decreased. *2*) Increase in the efflux with a constant or only slightly altered influx. *3*) Decrease of the influx accompanied by an incraese in the efflux. A sudden and marked increase in efflux during the short period of transition from the steady state to the nonsteady state (items 2 and 3) seems unlikely, since the nonspecific pathway does not change abruptly and the up-regulation of the LDL receptors needs several hours (17, 18). Furthermore, the fall of the concentration of insulin in the immediate postnatal period (19) which more rapidly modifies the LDL-receptor activity (20) would act in the opposite direction. Therefore, the rapid loss of a specific site for LDL synthesis and supply in consequence of the delivery is much more plausible (item 1). From recent studies of others (21–23) as well as from our own experiments (D. Plonné et al., unpublished data), there is an increasing body of evidence that the fetal membranes that are removed during the delivery might represent such a source.

If the postnatal nonsteady state is caused exclusively by a diminution of the influx, the efflux within the postnatal nonsteady state at t = 0 would be equal to the efflux within the prenatal steady state. Because under steady state conditions the influx rate equals that of the efflux, the LDL turnover could be estimated from the latter at t = 0. However, a simultaneous sudden decrease of both influx and efflux (e.g., by removal of the fetal membranes) cannot be completely excluded. In this case the approach described would lead to an underestimation of the fetal LDL turnover. Therefore, the calculated value represents a minimal rate of the fetal LDL turnover.

The calculation of the flux parameters in the postnatal nonsteady state as well as in the steady state (adult rat) were performed based on the two-pool model (Fig. 1) which was first applied to LDL metabolism by Langer, Strober, and Levy (24). In this model the irreversible loss occurs from an intravascular pool which is in exchange with a second extravascular pool. Using this model in the steady state we found a FCR of LDL in the adult rat that is in good agreement with findings of others (25–27).

The principal approach of Steele et al. (4) was adapted for the calculation of the fetal LDL flux rates. In this case the following suppositions must be fulfilled. *1*) Values k12 and k21 must be constant during the whole experimental period. This would be the fact if the exchange of LDL between the two pools is realized by a free diffusion process thus leading to equal concentrations in both pools. Spady, Meddings, and Dietschy (18) have shown that the resistance to diffusion of LDL in the organs important for LDL catabolism, such as liver, adrenals, ovaries, kidney, and small intestine, is negligible. Thus, free diffusion of LDL between the two pools can also be assumed for the fetal rat. *2*) The values of k12 and k21 must be known. According to Steele et al. (4) they can be obtained only from studies in the steady state. In our study, however, it was impossible for technical reasons to perform such experiments at the feto-placental unit prior to birth or at the fetus immediately after its delivery. Therefore, the values of k12 and k21 determined in the adult animals were applied to the fetus. There are no compelling reasons which, a priori, would contradict this approach. Admittedly, the identity of the constants in the fetal and the adult rat cannot be experimentally proved. It is possible, however, to illustrate the consequences brought about by different values of k12 and k21 for the kinetic parameters calculated for the fetal LDL flux. At t = 0 influx, efflux, and FCR depend only on the value of k12. Plausible results are obtained only with values of k12 ranging between 0 and 0.7. Beyond this range the results are not plausible due to negative influx rates. For the extreme cases k12 = 0 and k12 = 0.7 the following values were obtained for the rates of influx, efflux, and for FCR: 32 μg LDL-apoB/h per g, 45 μg LDL-apoB/h per g, and 0.9 $\cdot h^{-1}$ or 0, 12, and 0.24, respectively. This means that the LDL turnover in the fetus at term is at least one order of magnitude higher than in the adult animals irrespective of the value of k12 taken for the calculation of the parameters at t = 0. In the range of k12 used, the FCR calculated for the fetus exceeds the value obtained for the adult rats by 8-fold (k12 = 0) or 1.5-fold (k12 = 0.7), thus giving evidence that in the fetus the FCR for LDL is more or less elevated.

In the interval $0 < t < 15$ h, the k21/k12 ratio is also of importance. Within the plausible range of k12 given above, for every value a critical k21/k12 ratio can be calculated below which implausible results are obtained, e.g., negative efflux and/or influx rates. Above the critical ratio, however, the results are nearly independent of its absolute value. Thus, in a broad range of plausible k21/k12 ratios, the FCR declines between the 3rd and 5th hour postpartum to values measured in adults thus supporting the conclusion drawn from the experimental studies.

It is of interest that studies of cholesterol metabolism in the fetal and neonatal rat performed under in vivo conditions (28) revealed a plasma cholesterol turnover rate that is in good agreement with that calculated from our tracer kinetic data (29). At the end of gestation the rapidly growing fetus has a remarkably great need of cholesterol calculated to 1.5–2 mg per g increase in fetal body weight (23). The high flux rates determined for the LDL in the rat fetus at term allow the conclusion that this lipoprotein species essentially contributes to the need for cholesterol of the growing organism.

According to Spady et al. (18) the FCR obtained from in vivo experiments will be altered markedly in response to changes in the number of LDL receptors, a change in the production rate, a change in the K_m of the ligand to the receptor, or where other competing lipoproteins might be present. The authors inferred that it is impossible to draw any conclusion from the FCR obtained in in vivo experiments about the receptor-dependent transport activity. In fetal rats LDL are the predominant lipoprotein species in the plasma (1). Thus, provided the K_m value of the LDL-receptor and the rate constant of the receptor-independent LDL uptake are constant, one may speculate that the decrease of FCR in the early postnatal period could be the response to a hormonal signal (e.g., insulin; see also ref. 20) that triggers a diminution of the number of receptors for LDL. From the physiological point of view one might expect an increase in the number of LDL receptors in the neonate that needs cholesterol for growth. Since there is a marked reduction of the LDL efflux in the early postnatal period (Table 1) we suggest that up-regulation of the receptor takes place after the time period studied in our experiments.

In previous experiments we could show a small but constant VLDL secretion in the fetal rat at term after injecting Triton WR 1339 into the umbilical vein (2). The influx of VLDL triglycerides into the fetal serum was calculated to be 3.2 μg/h per g which corresponds to the

secretory rates obtained from tracer experiments (30) as well as from studies with isolated fetal hepatocytes (author: names and initials?, unpublished data). From these data an influx rate of about 0.3 μg VLDL-apoB was calculated which could account only for 1/60 of the LDL-apoB influx in the fetus at term and for 1/8 of that in the unsuckled newborn 15 h post partum. Therefore, it is unlikely that VLDL are the sole precursors of plasma LDL in the rat fetus. Thus, the results presented here favor the view of a direct production and secretion of LDL by a source within the feto-placental unit.

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