0031-3998/10/6801-0010
PEDIATRIC RESEARCH

Vol. 68, No. 1, 2010
Printed in U.S.A.

ARTICLES

The Effects of Intrauterine Malnutrition on Maternal-Fetal Cholesterol Transport and Fetal Lipid Synthesis in Mice

HESTER VAN MEER, ESTHER M. E. VAN STRATEN, JULIUS F. W. BALLER, THEO H. VAN DIJK, TORSTEN PLÖSCH, FOLKERT KUIPERS, AND HENKJAN J. VERKADE

Departments of Pediatrics [H.M., E.M.E.S., J.F.W.B., T.H.D., T.P., F.K., H.J.V.] and Laboratory Medicine [F.K.], University Medical Center Groningen, University of Groningen, 9713 BN Groningen, The Netherlands

ABSTRACT: Intrauterine malnutrition is associated with increased susceptibility to chronic diseases in adulthood. Growth-restricted infants display a less favorable lipid profile already shortly postnatal. Maternal low protein diet (LPD) during gestation is a well-defined model of fetal programming in rodents and affects lipid metabolism of the offspring. Effects of LPD throughout gestation on physiologic relevant parameters of lipid metabolism are unclear. We aimed to determine effects of LPD on maternal-fetal cholesterol fluxes and fetal lipid synthesis in mice. Pregnant mice (dams) were fed with a control (18% casein) or an LPD (9% casein) from E0.5 onward. We quantified maternal-fetal cholesterol transport and maternal cholesterol absorption at E19.5 using stable isotopes. We determined fetal lipid biosynthesis at E19.5, after administration of (1-^{13}C)-acetate from E17.5 onward. LPD did not change fetal and maternal plasma and hepatic concentrations of cholesterol and triglycerides. LPD affected neither the magnitudes of maternal-fetal cholesterol flux, maternal cholesterol absorption, nor fetal synthesis of cholesterol and palmitate (both groups, ~14% and ~13%, respectively). We conclude that LPD throughout gestation in mice does not affect maternal-fetal cholesterol transport, fetal cholesterol or fatty acid synthesis, indicating that programming effects of LPD are not mediated by short-term changes in maternal-fetal lipid metabolism. **(*Pediatr Res* 68: 10–15, 2010)**

Epidemiologic studies indicate that inadequate intrauterine nutrition is associated with increased susceptibility to develop chronic diseases in adulthood (*e.g.*, diabetes, hypertension, and cardiovascular disease; "metabolic programming") (1). Comparably, associations between small body size at birth and a less favorable lipid profile in adulthood have been described (2–4). Already in the first postnatal days, growth restricted human infants display a more atherogenic lipid profile (increased plasma concentrations of triglycerides, total cholesterol, and LDL (C)) compared with infants with a birth weight appropriate for gestational age (5).

Elevated plasma cholesterol is an established risk factor for the development of cardiovascular diseases later in life, however, insufficient cholesterol supply to the fetus impairs its development (6). It was demonstrated that the fetus is capable of synthesizing a large fraction of the required cholesterol *de novo* (7–9). As an independent source of cholesterol, maternal cholesterol can be transported to the fetal circulation (10). Recently, it was shown that maternal-fetal cholesterol transport is influenced by maternal plasma cholesterol levels (11). Interestingly, high maternal cholesterol levels increase the development of fetal aortic fatty streaks, as shown in human and animal studies (12,13). Hence, the metabolic condition of the mother during pregnancy can affect lipid metabolism in the offspring.

In humans, exposure to the Dutch Hunger Winter during late gestation is associated with increased adult obesity, glucose intolerance, and hypertension (14). Several animal models were developed to mimic the caloric restriction during gestation. An established animal model to investigate metabolic consequences of gestational under nutrition in the offspring is a low protein diet (LPD) during gestation (15).

Studies in rats have indicated that maternal protein restriction during gestation leads to features of the metabolic syndrome in adulthood, *e.g.*, increased blood pressure (16), decreased insulin sensitivity, and alterations in lipid profile (17,18). In mice, maternal protein restriction during pregnancy impaired glucose clearance in the adult offspring (19). Furthermore, experiments in mice showed that an LPD affected the renin-angiotensin system (important in the regulation of blood pressure) in the fetus already (20).

Recently, we demonstrated in mice that maternal gestational protein reduced the expression of genes involved in lipid synthesis and cholesterol metabolism in the fetal liver (21). Because the liver is a key player in regulating cholesterol and fatty acid homeostasis, we hypothesized that changes in fetal hepatic gene expression could translate into short-term ("acute") effects on cholesterol and fatty acid metabolism (such as maternal-fetal cholesterol flux and/or fetal cholesterol and lipid synthesis). If true, short-term effects on fetal lipid metabolism could be involved in the mechanism by which metabolic programming takes place. We aimed to determine

Received October 15, 2009; accepted March 22, 2010.

Correspondence: Henkjan J. Verkade, M.D., Ph.D., Department of Pediatrics, Beatrix Children's Hospital, University Medical Center Groningen, Hanzeplein 1, 9713 BN Groningen; e-mail: h.j.verkade@med.umcg.nl

Supported by grant 2004T048 from a Dr. Dekker fellowship of the Dutch Heart Foundation (T.P.).

H.M. and E.M.E.S. contributed equally to this work.

Abbreviations: E, embryonic day; **MIDA,** mass isotopomer distribution analysis

the acute effects of maternal protein restriction during gestation on maternal-fetal cholesterol flux, on *de novo* fetal cholesterol synthesis, and on fatty acid synthesis in mice.

MATERIALS AND METHODS

Animals. C57BL/6J mice (age 20 ± 1 wk) were obtained from Harlan (Horst, The Netherlands). Animals were housed in temperature-controlled rooms (23°C) with 12-h light cycling and free access to standard RMH-B mouse chow before the experiments (Arie Blok BV, Woerden, The Netherlands) and water *ad libitum*. Experimental procedures were approved by the local Ethical Committee for Animal Experiments of the University of Groningen.

Experimental procedures. All female rats received the control diet containing 180 g casein/kg (purified diet, 4400.18, Arie Blok BV, Woerden, The Netherlands) 2 weeks before mating. Virgin females were time mated using vaginal smears to assess their stage of estrous before introducing the male. After confirmation of mating by detection of a vaginal plug, the females were allocated to either the control diet or the isocaloric LPD (90 g casein/kg, purified diet 4400.17, Arie Blok BV, Woerden, The Netherlands) during the gestational period. The experimental diets are identical to the diets used by Langley-Evans *et al.* (22) and are described in detail in Table 1. We originally planned an independent intervention study with a fourth experimental group ($n = 5$) and own controls ($n = 5$). However, the intervention was not executed, in contrast to the control treatment in the second group of five (control) mice. Because the design on the control group was identical, we considered it scientifically appropriate to include the control mice into this study, although it caused unequal distribution ($n = 10$ for controls and $n = 5$ for treated dams).

Animals were weighed every 2 d. On embryonic day 14.5 (E14.5), pregnant mice received an i.v. dose of 0.52 mg (1.265 μmol) cholesterol-D_7 dissolved in Intralipid (20%, Fresenius Kabi, Den Bosch, The Netherlands) and an oral dose of 0.97 mg (2.30 μmol) cholesterol-D_5 dissolved in medium-chain triglyceride oil. Weights of the dams ranged from 27.3 to 33.1 g at E14.5 of gestation and were not significantly different between the two dietary groups. From E17.5 onward both diets were supplemented with 10 mg/g [1-^{13}C]-acetate (Isotec, Miamisburg, OH). Blood spots were collected from the tail on filter paper before administration of labeled cholesterol and acetate and daily from E14.5 onward to E19.5 of gestation. At E19.5, females were anesthetized with isoflurane and terminated by cardiac puncture. Figure 1 shows a schematic overview of the experimental setup.

Blood was collected in EDTA tubes. Liver and brain were snap frozen in liquid nitrogen. Fetuses were removed from *uteri*, weight and length were measured, and fetuses were killed and dissected. Blood samples were taken by exsanguination. Liver and brain of fetuses were collected, immediately snap frozen in liquid nitrogen and stored at −80°C. Sex of the fetuses was determined by PCR as previously described (23,24).

Analytical procedures. Cholesterol was extracted from blood spots and plasma according to Neese *et al.* (25). Hepatic and brain lipids from mothers and pups and lipids from whole (complete) pups were extracted according to Bligh and Dyer (26). Unesterified cholesterol from blood spots, plasma, and tissues were analyzed by gas chromatography quadrupole mass spectrometry (27). Commercially available kits were used for the determination of total cholesterol in brain, liver, and plasma samples (Roche, Mannheim, Germany). Hepatic fatty acids from livers of mothers and fetuses were analyzed by gas chromatography quadrupole mass spectrometry according to Oosterveer *et al.* (28). In short, lipids were extracted from livers and were hydrolyzed using HCL and acetonitrile. Fatty acids were extracted with hexane, after which the samples were derivatized using pentafluorobenzyl:triethanolamine:acetonitril solution. Enrichments of the PFB-derivatives of palmitate were measured by gas chromatography-mass spectrometry. The monitored ion were m/z 255–259 corresponding to the m0–m4 mass isotopomers for C16:0.

Fractional cholesterol absorption measurement. Fractional cholesterol absorption was measured using an adapted plasma dual isotope ratio method (29) using blood spots obtained at 120 h after i.v. and oral administration of stable-isotopically labeled cholesterol. For calculation of the fractional cholesterol absorption, the ratio of D_5-cholesterol and D_7-cholesterol in blood spots was divided by the ratio of D_5-cholesterol and D_7-cholesterol in the administered dose. The resulting value represents the fractional cholesterol absorption (27).

Mass isotopomer distribution analysis (MIDA). To determine *de novo* cholesterol and palmitate synthesis, the MIDA approach was used (25,30). MIDA allows determination of the enrichment of the pool of acetyl-CoA precursor units that has entered newly synthesized cholesterol or palmitate molecules during the course of (1-^{13}C)-acetate administration. Analysis of the isotopomer pattern of these molecules allows for determination of the fraction (f) of newly synthesized cholesterol or palmitate, respectively, in plasma or tissue. All normalized mass isotopomer distributions measured by GC-MS (m_0–$m_{0\times}$) were corrected for the natural abundance of ^{13}C by multiple linear regression as described by Lee *et al.* (31) to obtain the excess fractional distribution of mass isotopomers (m_0–$m_{0\times}$) because of incorporation of the infused (1-^{13}C)-acetate as described in Ref. 28. For determination of the absolute amount of newly synthesized hepatic palmitate, we multiplied *f* by the total amount of hepatic palmitate at the end of the experiment.

Statistics. We regarded the whole litter as one experimental unit, according to Festing (32). All data are presented as means ± SD. Statistical analyses were performed using SPSS 14.0 for Windows software (SPSS Inc., Chicago). All data analyzed using the Mann-Whitney U-test with a $p < 0.05$ were considered significant.

Table 1. *Composition of the diets provided*

Component (g per 100 g diet)	Control diet (18% casein)	Low protein diet (9% casein)
Casein	18.00	9.00
Sucrose	21.30	24.30
Corn Starch	42.5	48.50
Cellulose Fibre	5.00	5.00
Vitamin mix AIN-76	0.12	0.12
Mineral mix AIN-76	0.12	0.12
Corn oil	10.00	10.00
Choline choride	0.20	0.20
Methionine	0.50	0.50

Figure 1. Experimental setup.

RESULTS

Parameters in the control group. As stated above, the group size of the control mice was twice that of the experimental (low protein) intervention, based on the original design of two control groups of each $n = 5$, undergoing identical (control) treatment. We analyzed whether results between the two $n = 5$ control mice differed, but the parameters measured were virtually identical. Based on this observation, we pooled the data of the $n = 10$ control mice.

LPD affects body weight in dams but not in fetuses. At E0.5, there was no difference in body weight of dams between both groups. At E19.5, dams on a LPD had lower body weights than control dams (Table 2). Food intake during gestation was not different until E17.5 (data not shown) but

Table 2. *Parameters of dams and fetuses at E19.5 of gestation*

Parameter	Control diet (18% casein)	Low protein diet (9% casein)
Weight dam (g)	35.6 ± 2.0	32.1 ± 1.0*
Food intake dam (g/d)	6.3 ± 1.2	4.2 ± 0.7*
Liver weight to body weight dam (%)	4.5 ± 0.4	5.0 ± 0.2
Litter size (n)	7.3 ± 1.7	6.2 ± 2.1
Weight fetus (g)	1.07 ± 0.11	0.99 ± 0.15
Length fetus (mm)	20.4 ± 1.4	20.5 ± 1.5
Liver weight to body weight fetus (%)	2.8 ± 0.8	2.9 ± 1.1

Parameters of C57BL/6J dams and fetuses at E19.5 of gestation receiving control diet or low protein diet during gestation. Data are mean ± SD. N = 5 (low protein) and 10 (control) dams per group.

* $p < 0.05$ low protein diet *vs* control diet.

was lower in dams on a LPD between E17.5 and E19.5. There was no significant difference in the other parameters analyzed, including liver weight to body weight ratio of dams, litter size, body weight, body length, or liver weight to body weight ratio in fetuses (Table 2).

LPD does not affect total cholesterol concentrations in tissues. To investigate whether protein restriction diet during gestation influenced total cholesterol concentrations in dams and fetuses, we measured total cholesterol in tissues on E19.5. Figure 2*A* shows that total cholesterol levels in liver or brain in dams did not significantly differ between the two groups. In fetuses, protein restriction did not significantly affect total cholesterol levels in liver, brain, or whole body (the complete fetus; Fig. 2*B*).

LPD does not affect maternal fractional absorption of cholesterol. We investigated whether the LPD quantitatively affected cholesterol absorption in the dam and cholesterol transport to the fetus during the last stage of gestation. Cholesterol absorption in dams was found to be 64 ± 8% in control dams and 67 ± 8% in low protein (NS).

LPD does not quantitatively affect maternal-fetal cholesterol transport. Maternal-fetal transport of cholesterol was determined by measurement of the fraction of labeled cholesterol in several tissues of dams and fetuses at E19.5. To determine whether the administration route to the dam influenced on cholesterol transport to the fetus, fractions of both D_5 and D_7-labeled cholesterol in tissue and plasma were calculated. The rate of elimination of D_5 and D_7 cholesterol was measured every 24 h after administration (E14.5) until termination (E19.5) and did not significantly differ between dams on LPD or on control diet (data not shown). The fraction of orally administered D_5 cholesterol constituted ~0.5% of total cholesterol in livers and plasma of both groups. The fraction of orally administered D_5 cholesterol in brain was near detection level in dams of both groups (Fig. 3*A*). In fetuses at E19.5, the fraction of D_5 cholesterol was measurable in liver, plasma, brain, and whole body albeit very low in both groups (Fig. 3*B*).

The fraction of i.v.-administered D_7 cholesterol in plasma was higher in low protein dams compared with control dams (0.16 ± 0.04% in control dams and 0.36 ± 0.06% in low protein dams; $p = 0.028$). In livers, this fraction was 2.2 ± 0.2% in control dams and 2.8 ± 0.2% in low protein dams. In brains of dams, the fraction of i.v.-administrated D_7 was below detection at E19.5 of gestation for both groups (Fig. 3*C*). In fetuses at E19.5, the fraction of D_7 cholesterol was 1.0 ± 0.1% in liver of control fetuses and 1.1 ± 0.1% in liver of low protein fetuses. Fractions of D_7 cholesterol in plasma brain and whole body of fetuses were detectable, but below 0.5% in both groups (Fig. 3*D*). No significant differences were found in the fractional enrichments of D_5 or D_7 cholesterol between control or low-protein dams or fetuses.

LPD does not quantitatively affect maternal or fetal lipid synthesis. All dams received (1-^{13}C)-acetate in the diet from E17.5 onward to investigate whether receiving an LPD during gestation would influence cholesterol or palmitate synthesis in dams and fetuses in the last stage of gestation. Precursor pool enrichments were ~9% for control dams and low protein dams at 24 and 48 h (Fig. 4*A*), indicating steady-state and comparable levels of precursor pool enrichment in both groups. Fractional contribution of newly synthesized cholesterol was 11.9 ± 0.4% for control dams and 9.2 ± 2.7% for low protein dams at 24 h (NS) and 17.0 ± 0.6% for control dams and 14.9 ± 3.2% for low protein dams at 48 h (NS, Fig. 4*B*).

We determined the incorporation of newly synthesized cholesterol in the fetus by calculating fractional newly syn-

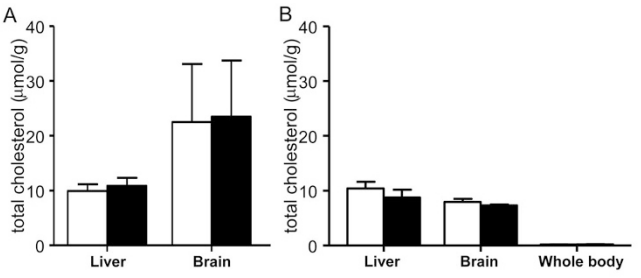

Figure 2. Total cholesterol levels in tissues of (*A*) dams and (*B*) fetuses. Whole body cholesterol in fetuses represents the amount of total cholesterol in the complete fetal body. □: control mice; ■: mice receiving an LPD during gestation. Values represent the mean ± SD $n = 5$ for low-protein dams and 10 for control dams.

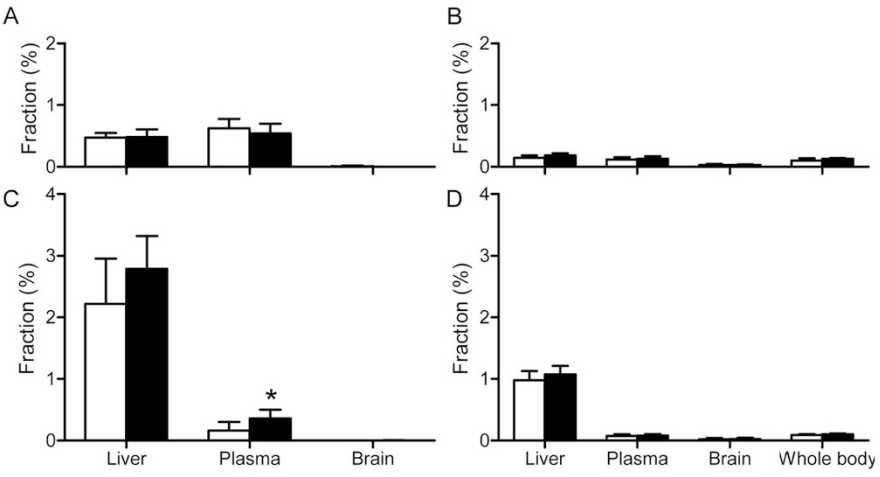

Figure 3. Fractional enrichments of orally administrated D_5–cholesterol in tissues of (*A*) dams and (*B*) fetuses and of i.v.-administrated D_7-cholesterol in tissues of (*C*) dams and (*D*) fetuses at day 19.5 of gestation. □: control mice; ■: mice receiving an LPD during gestation. Values represent the mean ± SD $n = 5$ for low-protein dams and 10 for control dams.

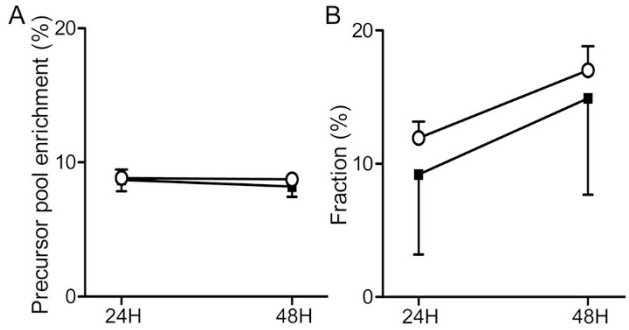

Figure 4. Fractional precursor pool enrichment (*A*) and fractional contribution of newly synthesized cholesterol (*B*) in dams on a control diet (○) or an LPD (■) during gestation. Fractions were measured making use of blood spots collected on filter paper ($n = 5$ for low-protein dams and 10 for control dams). Dams received ^{13}C-labeled acetate in the diet from day 17.5 of gestation onward. Blood spots obtained 24 and 48 h after administration were used for the calculations of the precursor pool enrichment and fractional contribution of newly synthesized cholesterol. Values represent the mean ± SD.

thesized cholesterol at E19.5 in different tissues of dams and fetuses receiving the control diet or the LPD using MIDA. Fractional synthesis in dams was 17.4 ± 0.6% in liver of control dams and 15.0 ± 3.0% in livers of low protein dams, 16.7 ± 1.5% in plasma of control dams and 14.4 ± 6.9% in plasma of low protein dams, 1.4 ± 0.4% in brain of control dams and 1.5 ± 0.4% in brain of low protein dams (all NS, Fig. 5*A*).

Fractional synthesis in fetuses was 12.6 ± 0.5% in liver of control fetuses and 14.8 ± 2.1% in liver of low protein fetuses, 13.1 ± 2.1% in plasma of controls and 15.4 ± 5.3% in plasma of low protein fetuses, 3.3 ± 0.02% in brain of control fetuses and 4.0 ± 0.4% in brain of low protein fetuses, 9.7 ± 1.4% in whole body of control fetuses, and 10.7 ± 1.2% in whole body of low protein fetuses (all NS, Fig. 5*B*). No significant differences were found in fractional cholesterol synthesis between dams and fetuses on a control diet compared with dams and fetuses on an LPD.

Fractional and absolute *de novo* synthesis of palmitate (C16:0) in livers of dams and fetuses was calculated using MIDA. Fractional *de novo* synthesized palmitate was 23 ± 2% in control dams and 23 ± 5% in low protein dams. Fractional *de novo* synthesized palmitate was 12.3 ± 0.4% in control fetuses and 14.8 ± 1.0% in low protein fetuses (Fig. 6*A*). Absolute *de novo* synthesis of palmitate was 7.3 ± 1.4 μmol/g in liver of control dams and 4.7 ± 0.9 μmol/g in liver of low protein dams (p 0.2). Absolute *de novo* synthesis of palmitate was 18.7 ± 1.4 μmol/g in liver of control fetuses and 25.0 ± 2.8 μ/g in liver of low protein fetuses (Fig. 6*B*). No significant differences considering fractional or absolute *de novo* synthesis of palmitate between the two groups of dams or fetuses were found.

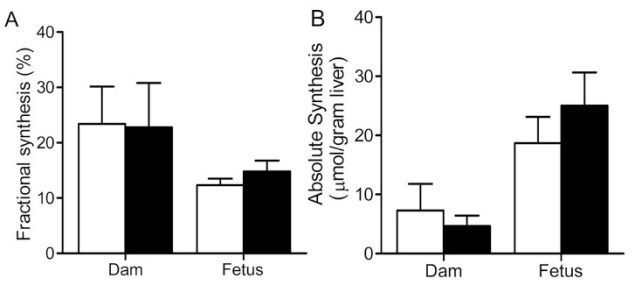

Figure 6. *De novo* (*A*) fractional and (*B*) absolute palmitate (C16:0) synthesis in livers of dams and fetuses. □: control mice; ■: mice receiving an LPD during gestation. ^{13}C-acetate was administrated in the diet and fractional and absolute synthesis were calculated using MIDA. $n = 5$ for low-protein dams and 10 for control dams. Values represent the mean ± SD.

DISCUSSION

A maternal LPD during the gestational period is a widely used animal model for intrauterine malnutrition to study the mechanisms underlying "metabolic programming." The purpose of using this diet during gestation is to mimic intrauterine malnutrition and its possible metabolic consequences. Most frequently, the LPD has been applied in rats in which gestational protein restriction resulted in impaired nephrogenesis and hypertension (33), disturbed glucose tolerance, obesity, and alterations in lipid metabolism (17) in the adult offspring. Several studies in mice demonstrate that maternal low protein during gestation leads to features of the metabolic syndrome in offspring. Male offspring from protein-restricted dams developed increased adiposity and glucose intolerance at adult age (34). When fed with a high-fat diet, male offspring from protein-restricted dams show a higher increase in body weight, relative fat mass, hyperglycemia, hypercholesterolemia, and hyperleptinemia, compared with high-fed diet offspring from control dams (35). Studying the metabolic effects of protein restriction during gestation in mice allows for extrapolation to studies on programming effects in different knock out mice. In the ApoE*3-Leiden mouse, a model for development of atherosclerosis, maternal protein restriction "aggravated" the dyslipidaemia and induced more severe atherosclerotic lesions in female adult offspring fed an atherogenic diet (36).

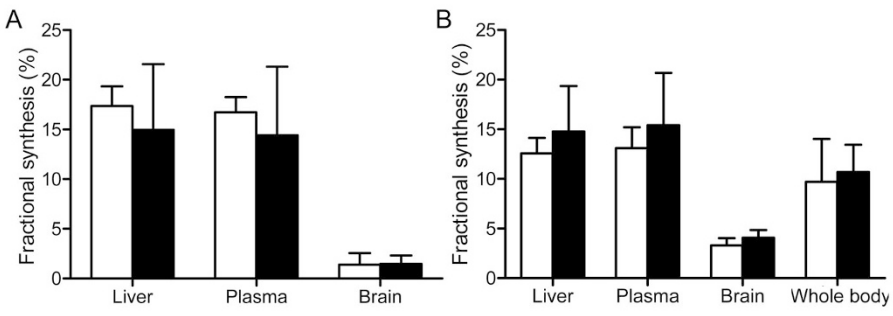

Figure 5. *De novo* fractional cholesterol synthesis in tissues of (*A*) dams and (*B*) fetuses. □: control mice; ■: mice receiving an LPD during gestation. ^{13}C-acetate was administrated in the diet and fractional synthesis was calculated using MIDA. $n = 5$ for low-protein dams and 10 for control dams. Values represent the mean ± SD.

Some human and animal studies showed that metabolic programming has differential effects in adult male and female offspring, albeit inconsistent. In this study, no differences between male and female pups in the studied parameters were observed.

We used a unique stable isotope method to determine maternal-fetal cholesterol fluxes and fetal lipid synthesis in mice. Administration of D_5 and D_7-labeled cholesterol to the dam enabled us to distinguish between cholesterol of different maternal sources transported from dam to fetus. The simultaneous administration of ^{13}C-acetate to the dam allows determination of cholesterol and fatty acid synthesis in maternal and fetal organs.

In this study, the LPD slightly decreased maternal food intake and maternal weight, when compared with control diet. However, no differences in fetal weight or length were seen in protein-restricted fetuses compared with controls. The observation on fetal weight corresponds with several other studies using the same diet in rats (37,38).

In previous experiments in mice, we showed that protein restriction during gestation decreased mRNA expression of genes important in cholesterol transport and fatty acid synthesis in fetal livers (21). These data supported the hypothesis that acute effects on lipid homeostasis could be related to the induction of programming. However, this study unequivocally demonstrates that maternal protein restriction during gestation does not induce acute, major quantitative changes in maternal-fetal cholesterol transport, cholesterol biosynthesis, or fatty acid biosynthesis in the last stage of pregnancy in mice. Discrepancy between mRNA data and metabolic fluxes has been reported before, underscoring the notion that the "proof of the pudding" of reporting changes in mRNA and even protein activities is in measuring the relevant metabolic fluxes *in vivo*.

The fraction of i.v. administered cholesterol was significantly higher in plasma of the low protein dams compared with controls. However, this effect was not reflected in the total plasma cholesterol levels of dams or in the fractions of D_7-labeled cholesterol in plasma or other tissues of pups. Therefore, we assume that the effect on i.v. fractions is of limited physiologic relevance.

We do realize that a theoretical limitation of this is the rapidly changing metabolism of dam and fetus during gestation, which constitutes metabolically a "nonsteady state" condition per definition. However, we feel that this limitation most likely applies equally for both the control and the low protein group and does not invalidate our main conclusions. Another realization involves the timing of our experiment to the late stage of gestation. Theoretically, the protein restriction diet could have influenced cholesterol flux to the fetus in the first stage of gestation. We do not have indications for this possibility as we did not find any differences in total cholesterol content in fetal organs in the last stage of gestation. Based on the results, it seems reasonable to assume that the effects of the maternal diet on cholesterol fluxes and/or on fetal hepatic lipid synthesis during the first stage of gestation are negligible. The presently used model does allow determination of effects of the maternal environment on maternal-fetal transport and on fetal cholesterol and fatty acid synthesis. The method could be applicable to studies investigating the influence of various maternal conditions such as maternal overnutrition or maternal diabetes on fetal lipid metabolism. In conclusion, programming effects of maternal protein restriction during gestation are not accompanied by immediate changes in fetal-maternal cholesterol transport or lipid synthesis in mice in the last stage of pregnancy. Apparently, programming effects of maternal LPD on lipid metabolism are mediated by more complex pathways than these acute effects.

REFERENCES

1. Osmond C, Barker DJ 2000 Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. Environ Health Perspect 108:545–553
2. Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH 1993 Growth in utero and serum cholesterol concentrations in adult life. BMJ 307:1524–1527
3. Fall CH, Osmond C, Barker DJ, Clark PM, Hales CN, Stirling Y, Meade TW 1995 Fetal and infant growth and cardiovascular risk factors in women. BMJ 310:428–432
4. Kajantie E, Barker DJ, Osmond C, Forsen T, Eriksson JG 2008 Growth before 2 years of age and serum lipids 60 years later: the Helsinki Birth Cohort study. Int J Epidemiol 37:280–289
5. Wang X, Cui Y, Tong X, Ye H, Li S 2007 Glucose and lipid metabolism in small-for-gestational-age infants at 72 hours of age. J Clin Endocrinol Metab 92:681–684
6. Herman GE 2003 Disorders of cholesterol biosynthesis: prototypic metabolic malformation syndromes. Hum Mol Genet 12:R75–R88
7. Belknap WM, Dietschy JM 1988 Sterol synthesis and low density lipoprotein clearance in vivo in the pregnant rat, placenta, and fetus. Sources for tissue cholesterol during fetal development. J Clin Invest 82:2077–2085
8. Woollett LA 1996 Origin of cholesterol in the fetal golden Syrian hamster: contribution of de novo sterol synthesis and maternal-derived lipoprotein cholesterol. J Lipid Res 37:1246–1257
9. Haave NC, Innis SM 2001 Cholesterol synthesis and accretion within various tissues of the fetal and neonatal rat. Metabolism 50:12–18
10. Yoshida S, Wada Y 2005 Transfer of maternal cholesterol to embryo and fetus in pregnant mice. J Lipid Res 46:2168–2174
11. Burke KT, Colvin PL, Myatt L, Graf GA, Schroeder F, Woollett LA 2009 Transport of maternal cholesterol to the fetus is affected by maternal plasma cholesterol concentrations in the golden Syrian hamster. J Lipid Res 50:1146–1155
12. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, Palinski W 1997 Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. J Clin Invest 100:2680–2690
13. Napoli C, Witztum JL, Calara F, de Nigris F, Palinski W 2000 Maternal hypercholesterolemia enhances atherogenesis in normocholesterolemic rabbits, which is inhibited by antioxidant or lipid-lowering intervention during pregnancy: an experimental model of atherogenic mechanisms in human fetuses. Circ Res 87:946–952
14. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP 2001 Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Mol Cell Endocrinol 185:93–98
15. Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L 2004 Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J Physiol 561:355–377
16. Langley-Evans SC, Phillips GJ, Jackson AA 1994 In utero exposure to maternal low protein diets induces hypertension in weanling rats, independently of maternal blood pressure changes. Clin Nutr 13:319–324
17. Erhuma A, Salter AM, Sculley DV, Langley-Evans SC, Bennett AJ 2007 Prenatal exposure to a low-protein diet programs disordered regulation of lipid metabolism in the aging rat. Am J Physiol Endocrinol Metab 292:E1702–E1714
18. Lucas A, Baker BA, Desai M, Hales CN 1996 Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. Br J Nutr 76:605–612
19. Roghair RD, Segar JL, Kilpatrick RA, Segar EM, Scholz TD, Lamb FS 2007 Murine aortic reactivity is programmed equally by maternal low protein diet or late gestation dexamethasone. J Matern Fetal Neonatal Med 20:833–841
20. Goyal R, Galffy A, Field SA, Gheorghe CP, Mittal A, Longo LD 2009 Maternal protein deprivation: changes in systemic renin-angiotensin system of the mouse fetus. Reprod Sci 16:894–904
21. van Straten EM, Bloks VW, Huijkman NC, Baller JF, Meer H, Lutjohann D, Kuipers F, Plosch T 2010 The liver X-receptor gene promoter is hypermethylated in a mouse model of prenatal protein restriction. Am J Physiol Regul Integr Comp Physiol 298:R275–R282
22. Langley-Evans SC, Gardner DS, Jackson AA 1996 Association of disproportionate growth of fetal rats in late gestation with raised systolic blood pressure in later life. J Reprod Fertil 106:307–312
23. van Straten EM, Huijkman NC, Baller JF, Kuipers F, Plosch T 2008 Pharmacological activation of LXR in utero directly influences ABC transporter expression and

function in mice but does not affect adult cholesterol metabolism. Am J Physiol Endocrinol Metab 295:E1341–E1348
24. Kunieda T, Xian M, Kobayashi E, Imamichi T, Moriwaki K, Toyoda Y 1992 Sexing of mouse preimplantation embryos by detection of Y chromosome-specific sequences using polymerase chain reaction. Biol Reprod 46:692–697
25. Neese RA, Faix D, Kletke C, Wu K, Wang AC, Shackleton CH, Hellerstein MK 1993 Measurement of endogenous synthesis of plasma cholesterol in rats and humans using MIDA. Am J Physiol 264:E136–E147
26. Bligh EG, Dyer WJ 1959 A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917
27. van der Veen JN, van Dijk TH, Vrins CL, van Meer H, Havinga R, Bijsterveld K, Tietge UJ, Groen AK, Kuipers F 2009 Activation of the liver X receptor stimulates trans-intestinal excretion of plasma cholesterol. J Biol Chem 284:19211–19219
28. Oosterveer MH, van Dijk TH, Tietge UJ, Boer T, Havinga R, Stellaard F, Groen AK, Kuipers F, Reijngoud DJ 2009 High fat feeding induces hepatic fatty acid elongation in mice. PLoS One 4:e6066
29. Turley SD, Herndon MW, Dietschy JM 1994 Reevaluation and application of the dual-isotope plasma ratio method for the measurement of intestinal cholesterol absorption in the hamster. J Lipid Res 35:328–339
30. Hellerstein MK, Neese RA 1992 Mass isotopomer distribution analysis: a technique for measuring biosynthesis and turnover of polymers. Am J Physiol 263:E988–E1001
31. Lee WN, Byerley LO, Bergner EA, Edmond J 1991 Mass isotopomer analysis: theoretical and practical considerations. Biol Mass Spectrom 20:451–458
32. Festing MF 2006 Design and statistical methods in studies using animal models of development. ILAR J 47:5–14
33. Langley-Evans SC, Welham SJ, Jackson AA 1999 Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. Life Sci 64:965–974
34. Bhasin KK, van Nas A, Martin LJ, Davis RC, Devaskar SU, Lusis AJ 2009 Maternal low-protein diet or hypercholesterolemia reduces circulating essential amino acids and leads to intrauterine growth restriction. Diabetes 58:559–566
35. Bol VV, Delattre AI, Reusens B, Raes M, Remacle C 2009 Forced catch-up growth after fetal protein restriction alters the adipose tissue gene expression program leading to obesity in adult mice. Am J Physiol Regul Integr Comp Physiol 297:R291–R299
36. Yates Z, Tarling EJ, Langley-Evans SC, Salter AM 2009 Maternal undernutrition programmes atherosclerosis in the ApoE*3-Leiden mouse. Br J Nutr 101:1185–1194
37. Langley SC, Browne RF, Jackson AA 1994 Altered glucose tolerance in rats exposed to maternal low protein diets in utero. Comp Biochem Physiol Physiol 109:223–229
38. Langley-Evans SC, Gardner DS, Jackson AA 1996 Maternal protein restriction influences the programming of the rat hypothalamic-pituitary-adrenal axis. J Nutr 126:1578–1585