Protein Turnover in Tissues of the Fetal Rat after Prolonged Maternal Malnutrition

JOHN D. JOHNSON AND TRACY DUNHAM

Department of Pediatrics, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

ABSTRACT. Protein turnover in fetal diaphragm, heart, liver, and brain was determined at 21 days gestation in fetal rats whose mothers had received a protein-restricted diet (6% protein) throughout gestation. This diet resulted in severe combined protein-calorie malnutrition. Fetal body weight was significantly decreased at days 19–22 gestation *versus* controls (27% protein diet) when pregnant animals were protein-restricted (*e.g.* 40% decrease in body weight at day 22 gestation *versus* controls). Protein synthesis was determined by intravenous injection of “massive” amounts of [³H]phenylalanine to pregnant animals and measuring free and protein-bound specific radioactivities in fetal tissues. Rate constants for protein degradation were calculated by subtracting fractional growth rate from protein synthesis. Fractional protein synthesis was reduced in diaphragm (0.26 *versus* 0.41 days⁻¹), heart (0.41 *versus* 0.52 days⁻¹), and liver (0.35 *versus* 0.89 days⁻¹) in fetuses from malnourished mothers relative to controls. Similarly, fractional protein degradation was decreased in these fetal tissues – diaphragm (0.03 *versus* 0.06 days⁻¹), heart (0.14 *versus* 0.18 days⁻¹), and liver (0.25 *versus* 0.80 days⁻¹). Reduced protein accretion during maternal malnutrition in these fetal tissues is secondary to decreased protein synthesis out of proportion to the concurrent reduction in protein degradation. Protein synthesis and degradation in fetal brain from malnourished mothers were not altered *versus* controls. These effects of malnutrition on protein turnover in fetal tissues throughout pregnancy in the rat correspond closely with the effect of protein restriction in young adult rats and the effect of protein-calorie malnutrition on whole body protein turnover in human infants. (*Pediatr Res* 23: 534–538, 1988)

Abbreviations

Ks, fractional rate of protein synthesis
Kd, fractional rate of protein degradation
Kg, fractional rate of protein accretion
LP, low-protein diet (6% protein)

During normal fetal growth, protein accretion in heart and diaphragm at the time of maximal fetal growth (day 19 gestation) results from higher rates of synthesis and lower rates of degradation when compared to a time of slower rate of growth (1). When pregnant rats are fasted beginning on day 18 gestation, the decreased rate of protein accretion observed at day 21 gesta-

tion in fetal heart, diaphragm, and brain results from enhanced protein degradation without major changes in the Ks (2).

A diet restricted in protein results in reduced protein synthesis and reduced protein degradation in striated muscles of the young adult rat (3–5). We have now studied the effects of dietary restriction of protein and calories throughout gestation in pregnant rats on protein turnover in tissues of fetal rats. The results obtained contrast sharply with those previously reported during short-term maternal starvation.

METHODS

Animals. Virgin Sprague-Dawley rats were mated at approximately 100 days of age. Day zero of gestation was defined as the day on which a pan plug was found (6). Throughout these experiments, animals were kept in a room controlled at 22 ± 1° C with a 12-h-on-off light cycle. Control animals were fed a pelleted diet containing 27% protein (ICN Biochemicals, Cleveland, OH) *ad libitum* throughout gestation. Protein-restricted pregnant animals were fed a pelleted diet containing 6% protein (ICN Biochemicals) *ad libitum* throughout gestation. The diets were isocaloric. Their compositions are shown in Table 1. Minerals and vitamins were equivalent in the two diets.

Pregnant animals were weighed periodically during gestation and food intake was estimated at discrete intervals by differences in pelleted food weight in the feeding containers of the cages over various time intervals.

Determination of protein synthesis. The method of Garlick *et al.* (7) was used to measure fractional rates of protein synthesis in fetal tissues. This technique involves the rapid infusion of very large amounts of [³H]phenylalanine to rapidly achieve constant specific activity of the precursor pool for protein synthesis in tissues. Flooding amounts of [³H]phenylalanine were injected intravenously into pregnant animals at 21 days gestation (150 μmol phenylalanine containing 65 μCi L-4-[³H]phenylalanine [25 μCi/mmol] per 100 g body weight) with the animals under light ether anesthesia. Pregnant rats were killed with an overdose of diethyl ether 20 min after injection of phenylalanine. Previous studies have shown that calculated values for Ks (expressed as fraction/day) in fetal tissues remain constant between 10–30 min after maternal injection of [³H]phenylalanine (2). The applica-

Table 1. Composition of diets

	Control* (%)	LP† (%)
Casein	27	6
Corn starch	19	26
Sucrose	40	54
Vegetable oil	10	10
Salt mixture (USP XIV)	4	4
Vitamin fortification mixture	+	+

* ICN Biochemicals no. 902487.

† ICN Biochemicals, special ordered.

Received September 21, 1987; accepted January 20, 1988.
Correspondence and reprints John D. Johnson, M.D., Department of Pediatrics, University of New Mexico School of Medicine, Albuquerque, NM 87131.
Supported by National Institute of Child Health and Human Development Grant HD-15243

bility of this technique to measurement of fetal protein synthesis has been established previously (2, 8, 9).

Samples of maternal and fetal blood were obtained and fetuses were submerged immediately in ice-cold 0.9% NaCl. Fetal tissues were dissected rapidly and frozen in liquid N₂. Processing of blood and tissue samples to determine the specific radioactivities of free and protein-bound [³H]phenylalanine was conducted according to the method of Garlick *et al.* (7). Ks values in fetal tissues were calculated from the equation $K_s = S_b / (S_a \times t)$ where S_b = specific radioactivity (dpm/nmol) of protein-bound phenylalanine, S_a = specific radioactivity of free phenylalanine in tissues, t = time in days.

Determination of protein degradation. K_d was calculated by subtraction of the fractional growth rate, K_g (at day 21 gestation) from K_s. K_g was determined by constructing protein accretion curves using a separate group of fetuses. Data for protein accretion from 19 through 22 days gestation obtained at 24-h intervals were analyzed by polynomial regression and the quadratic equations for these analyses are shown in "Results." Growth rates (dP/dt) were obtained from derivatives of these equations and fractional growth rates (K_g) from the relation $K_g = dP/dt \div P(t)$, where P(t) represents the protein content at 21 days gestation.

Biochemical determinations. Protein concentration was measured in tissue homogenates by the method of Lowry *et al.* (10) using bovine albumin as the standard. RNA and DNA concentrations were determined as previously described (1, 2).

Statistical analysis. The two-tailed Student's *t* test for independent samples was used to compare various results in control versus malnourished animals. Polynomial regression was used to analyze protein accretion curves of fetal tissues as a function of gestational age.

RESULTS

Fetal growth curves. The effect of maternal protein-calorie restriction on fetal body weight is shown in Figure 1. Significantly decreased fetal body weight was apparent by day 19 gestation

Fig. 1. Body weight of fetuses from control and protein-calorie-restricted mothers. Each point represents the mean body weight ($\pm$ SD) of fetuses from six litters. There were no significant differences in litter size between control and protein-calorie-restricted mothers at any gestational age. Fetal body weight was reduced significantly in the malnourished animals versus controls at all gestational ages studied ($p < 0.003$).

and became accentuated through the remainder of the gestation. Maternal weight gain was significantly reduced for the entire gestational period in the malnourished group (Fig. 2). Cumulative maternal weight gain during pregnancy averaged 126 ± 23 g (mean $\pm$ SD) in controls and -7 ± 24 g in malnourished animals. Total caloric intake was depressed in the protein-restricted group from days 6–22 gestation (Fig. 3). There was a progressive dramatic decrease in total food intake in mothers fed the LP.

The effects of prolonged maternal malnutrition on fetal tissue protein content are shown in Figure 4. Protein accretion is dramatically reduced in fetal liver and diaphragm with maternal protein-calorie restriction. Heart and brain protein contents are less dramatically, but significantly, affected. Quadratic equations for changes in fetal organ protein contents from both control and malnourished groups are expressed in Table 2.

Organ contents of RNA and DNA are shown in Table 3 and Figures 5 and 6. RNA and DNA contents of liver are reduced in fetuses from mothers consuming the LP at all gestational ages. RNA is decreased in the heart at days 19, 20, and 22 and at days 19–22 in diaphragm, but is not reduced significantly in brain of LP fetuses. DNA is significantly lower in heart (days 19 and 22) and diaphragm (day 22) from LP fetuses, but not in brain at any age. Ratios of protein/RNA and protein/DNA are not significantly different in any tissue at any gestational age (data shown only for day 21). RNA/DNA ratio is decreased in diaphragm at days 21 and 22 in LP fetuses but not in other tissues. All three

Fig. 2. Maternal weight gain throughout pregnancy in control and protein-calorie-restricted mothers. Each bar represents the mean $\pm$ SD of daily weight gain for the time intervals indicated ($n = 14$ –37). Open bars signify control animals and cross-hatched bars signify protein-calorie-restricted animals (LP). Maternal weight gain was significantly less ($p < 0.001$) in LP animals versus controls at all time intervals.

Fig. 3. Dietary intake of control and protein-calorie-restricted pregnant rats. Each bar represents the mean $\pm$ SD of food intake in g/day for the time intervals indicated. Open bars represent control animals, cross-hatched bars protein-calorie-restricted pregnant animals (LP). * $p < 0.004$ versus controls.

ratios were constant throughout gestation in heart and brain in control and LP animals. Protein/DNA and protein/RNA increased from days 19 to 22 in diaphragm and liver both in control and LP fetuses.

Protein turnover. Ks values for fetal organs of control and malnourished fetuses at 21-days gestation are shown in Table 4. Ks is depressed significantly in fetal heart, diaphragm, and liver from fetuses of malnourished mothers *versus* controls, but is not affected in brain. Kg values are lower in the fetuses of protein-calorie-deficient mothers at day 21 gestation in heart and diaphragm. Kd values are also lower in all fetal tissues in the malnourished *versus* the control group.

Table 5 illustrates that total protein synthesis per unit RNA and DNA in fetal organs is reduced by maternal protein-calorie restriction.

DISCUSSION

Restriction of protein (6%) throughout gestation results in significant fetal growth retardation that is evident by day 19 in body weight as well as tissue weights of liver, diaphragm, and heart. Protein, RNA, and DNA are most significantly reduced in liver, followed by diaphragm and heart. As in many models of fetal growth retardation in the rat, brain composition is least affected; protein is decreased on day 22, but neither RNA nor DNA contents are significantly reduced at any gestational age. Whole body and tissue weights at term gestation are reduced to a greater extent in this model of fetal growth retardation than with total maternal fasting beginning on day 18 gestation (2). Our results of body and tissue weights and tissue protein, RNA, and DNA are similar to those of Zeman (11, 12) who first described the effect on fetal cellular development of a 6% protein diet throughout pregnancy in the rat, *viz*, lesser reductions of these measurements in brain than in liver and heart. We chose a 6% protein diet because diets containing more protein may require maintenance of the protein-deficient diet before mating, as well as throughout gestation, to obtain similar inhibition of fetal growth (13).

Fig. 4. Organ protein content in fetuses from control and protein-calorie-restricted mothers. Each point represents the mean ($\pm$ SD) of six litters (total of 50–90 fetuses/time point). * $p < 0.05$ versus controls.

Table 2. Fetal organ protein accretion (quadratic equations)*

Organ	Equation	R ² value
Heart		
C	$P = 22.00 - 2.62A + 0.080A^2$	0.88
LP	$P = -10.85 + 0.702A - 0.0046A^2$	0.85
Diaphragm		
C	$P = 11.46 - 1.58A + 0.054A^2$	0.88
LP	$P = -11.31 + 0.913A - 0.015A^2$	0.81
Liver		
C	$P = -644.3 + 61.40A - 1.397A^2$	0.68
LP	$P = -230.0 + 21.87A - 0.481A^2$	0.57
Brain		
C	$P = 85.79 - 9.58A + 0.292A^2$	0.75
LP	$P = 154.7 - 15.69A + 0.4242A^2$	0.68

* P, protein content (mg/organ); A, gestational age (days); C, control.

As shown in Figure 3, maternal food intake on the LP diet is markedly less than in controls from day 6 gestation until its completion, with accelerated decline in intake from days 16–22. Thus, this model is not one of “pure” protein deficiency, but rather severe combined protein-calorie deficiency. A similar pattern of maternal food intake was observed by Zeman (11) with a 6% protein diet. However, when she pair-fed controls receiving a 24% casein diet with those receiving a 6% protein diet, newborns of the pair-fed controls *versus ad libitum*-fed controls were indistinguishable in body weight and chemical composition, suggesting that the effects on fetuses of the protein-restricted diet were due to protein restriction per se, and not of combined protein-calorie deficiency. However, Zeman’s protein-restricted rats were not as anorectic as ours. Her protein-restricted pregnant animals gained 33 g and were still consuming 7 g food/day on day 21 gestation; ours actually lost weight and were consuming only 4 g food/day late in gestation. We know that total starvation

Table 3. Fetal tissue composition, day 21 gestation*

Tissue	Wet wt (mg/tissue)	Protein (mg/tissue)	RNA (μ g/tissue)	DNA (μ g/tissue)	Protein/RNA	Protein/DNA	RNA/DNA
Heart							
C	23.6 $\pm$ 4.4	2.09 $\pm$ 0.44	105 $\pm$ 26	78 $\pm$ 25	20.1 $\pm$ 2.1	28.1 $\pm$ 5.9	1.42 $\pm$ 0.34
LP	20.7 $\pm$ 3.2	1.93 $\pm$ 0.33	94 $\pm$ 27	85 $\pm$ 34	21.1 $\pm$ 3.3	24.4 $\pm$ 6.1	1.17 $\pm$ 0.25
Diaphragm							
C	18.4 $\pm$ 4.1	1.95 $\pm$ 0.40	86.4 $\pm$ 30.0	61.3 $\pm$ 17.4	23.5 $\pm$ 4.3	33.4 $\pm$ 7.9	1.46 $\pm$ 0.43
LP	12.1 $\pm$ 2.9†	1.26 $\pm$ 0.19†	50.4 $\pm$ 11.8†	52.8 $\pm$ 20.2	25.3 $\pm$ 4.3	25.7 $\pm$ 6.8	1.01 $\pm$ 0.23‡
Liver							
C	274 $\pm$ 45	28.6 $\pm$ 4.8	2348 $\pm$ 348	1446 $\pm$ 296	12.2 $\pm$ 1.1	20.1 $\pm$ 2.8	1.64 $\pm$ 0.17
LP	157 $\pm$ 30§	11.5 $\pm$ 1.5§	1034 $\pm$ 178§	763 $\pm$ 161§	13.4 $\pm$ 1.3	23.3 $\pm$ 6.7	1.72 $\pm$ 0.35
Brain							
C	204 $\pm$ 22	13.40 $\pm$ 1.59	657 $\pm$ 161	967 $\pm$ 187	21.0 $\pm$ 3.7	14.2 $\pm$ 2.4	0.71 $\pm$ 0.23
LP	173 $\pm$ 18‡	11.81 $\pm$ 1.57	558 $\pm$ 46	853 $\pm$ 185	22.5 $\pm$ 4.4	14.5 $\pm$ 2.3	0.68 $\pm$ 0.15

* Values represent mean $\pm$ SD of pooled tissue from six litters.† $p < 0.02$.‡ $p < 0.05$.§ $p < 0.002$. Fig. 5. Organ RNA content. Each point represents mean $\pm$ SD of fetuses from six litters. * $p < 0.05$ versus controls. Fig. 6. Organ DNA content. Each point represents mean $\pm$ SD of fetuses from six litters. * $p < 0.05$ versus controls

over the last 4 days of gestation results in significantly reduced fetal body weight and tissue weights (2). We cannot be certain that the effects of the protein-deficient diet on fetuses were due to protein deficiency per se or combined protein-calorie deficiency in our experiments.

Fetuses from LP mothers exhibited reduced Ks for protein in heart, diaphragm, and liver and diminished Kd in all four tissues studied (Table 4). Thus, reduced protein accretion during protein-restriction in these tissues is secondary to decreased protein synthesis out of proportion to the concomitant reduction in protein degradation. These results contrast sharply with those obtained after 72 h of maternal fasting (2). In the latter studies, reduced protein accretion in these organs occurred as a result of elevated protein degradation with relatively constant fractional rates of protein synthesis. A number of studies in young adult

rats demonstrate reduced protein synthesis in skeletal muscle occurring during protein-free or protein-restricted diets, with simultaneous decrements in rates of protein degradation (3–5, 14). In this regard, the rat fetus whose mother is subjected to protein-deficiency throughout pregnancy responds in a fashion similar to the young adult rat. Similarly, human infants suffering from severe protein-calorie undernutrition exhibit reductions in total body protein synthesis and breakdown and a reduced rate of skeletal muscle contractile protein breakdown (15, 16).

The mechanism by which reduced Ks occurs in tissues of fetuses from LP mothers involves reduced rate of protein synthesis per unit RNA (Table 5). This could be due to a diminished rate of translation of mRNA or a selective decrease in mRNA for rapidly turning over proteins. The total amount of RNA per

tissue was also decreased in these tissues, thus further reducing the protein synthetic capacity. This differs from the effect of 72 h of maternal starvation late in gestation in the rat. In the latter circumstance, the rate of protein synthesis per unit RNA is maintained at a level comparable to controls (2). Protein-free diets and starvation both result in a decreased rate of protein synthesis per unit RNA in skeletal muscle of young adult rats (14). The mechanisms for diminished protein breakdown under these conditions are totally unknown.

In conclusion, reduced protein accretion in various tissues of the fetal rat resulting from maternal protein-calorie restriction throughout gestation occurs as a result of decreased fractional rates of protein synthesis out of proportion to accompanying decreased fractional rates of protein breakdown. In view of previous results obtained in tissues of fetuses from "acutely" starved mothers, we speculate that the rat fetus is able to maintain normal Ks in response to short-term protein-calorie deprivation, but that prolonged deprivation results in inhibited protein synthesis. Similarly, short-term deprivation accelerates protein

breakdown, but unexplained adaptive mechanisms result in decreased protein breakdown during long-term protein-calorie deprivation. Additional studies are necessary to dissect the cellular mechanisms of these responses to nutritional alterations.

Acknowledgments. The authors thank Betty J. Skipper for statistical and mathematical advice, Robert E. Greenberg for helpful comments and critique, and Elizabeth Kerl for preparation of the manuscript.

REFERENCES

1. Johnson JD, Wetmore DL 1984 Protein turnover in heart and diaphragm of rat fetus. *Am J Physiol* 247:E781-E785
2. Johnson JD, Dunham T, Skipper BJ, Loftfield RB 1986 Protein turnover in tissues of the rat fetus following maternal starvation. *Pediatr Res* 20:1252-1257
3. Garlick PJ, Millward DJ, James WPT, Waterlow JC 1975 The effect of protein deprivation and starvation on the rate of protein synthesis in tissues of the rat. *Biochim Biophys Acta* 414:71-84
4. Millward DJ, Waterlow JC 1978 Effect of nutrition on protein turnover in skeletal muscle. *Fed Proc* 37:2283-2290
5. Laurent BC, Moldawer LL, Young VR, Bistrian BR, Blackburn GL 1984 Whole-body leucine and muscle protein kinetics in rats fed varying protein intakes. *Am J Physiol* 246:E444-E451
6. Szabo KT, Free SM, Birkhead HA, Gay PE 1969 Predictability of pregnancy from various signs of mating in mice and rats. *Lab Anim* 19:822-825
7. Garlick PJ, McNurlan MA, Preedy VR 1980 A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of ^3H -phenylalanine. *Biochem J* 192:719-723
8. Goldspink DF, Kelly FJ 1984 Protein turnover and growth in the whole body, liver and kidney of the rat from the foetus to senility. *Biochem J* 217:507-516
9. Lewis SEM, Kelly FJ, Goldspink DF 1984 Pre- and post-natal growth and protein turnover in smooth muscle, heart and slow- and fast-twitch skeletal muscles of the rat. *Biochem J* 217:517-526
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265-275
11. Zeman FJ 1967 Effect on the young rat of maternal protein restriction. *J Nutr* 93:167-173
12. Zeman FJ, Stanbrough EC 1969 Effect of maternal protein deficiency on cellular development in the fetal rat. *J Nutr* 99:274-282
13. Zamenhof S, Van Marthens E, Margolis FL 1968 DNA (cell number) and protein in neonatal brain: alteration by maternal dietary protein restriction. *Science* 160:322-323
14. Millward DJ, Garlick PJ, Nnanyelugo DO, Waterlow JC 1976 The relative importance of muscle protein synthesis and breakdown in the regulation of muscle mass. *Biochem J* 156:185-188
15. Golden MHN, Waterlow JC, Picou D 1977 Protein turnover, synthesis and breakdown before and after recovery from protein-energy malnutrition. *Clin Sci Mol Med* 53:473-477
16. Nagabushan VS, Narasinga Rao BS 1978 Studies on 3-methyl histidine metabolism in children with protein-energy malnutrition. *Am J Clin Nutr* 31:1322-1327

Table 4. Rate constants for protein synthesis, accretion, and degradation in fetal tissues*

Tissue	Ks (days ⁻¹)	Kg (days ⁻¹)	Kd (days ⁻¹)
Heart			
C†	0.52 (0.06)	0.34	0.18
LP	0.41 (0.05)‡	0.27	0.14
Diaphragm			
C	0.41 (0.13)	0.35	0.06
LP	0.26 (0.03)‡	0.23	0.03
Liver			
C	0.89 (0.05)	0.09	0.80
LP	0.35 (0.18)‡	0.10	0.25
Brain			
C	0.39 (0.06)	0.20	0.19
LP	0.34 (0.02)	0.17	0.17

* Values for Ks represent means $\pm$ SD of five experiments. Tissues from all individual fetuses were pooled for each experiment. All values were determined at 21 days gestation.

† Control (27% protein).

‡ $p < 0.05$ versus C using two-tailed t test.

Table 5. Relationship of protein synthesis to RNA and DNA content of fetal rat tissues at day 21 gestation

Tissue	Ks (days ⁻¹)	Protein synthesis/unit RNA (g protein/g RNA/day)	Protein synthesis/unit DNA (g protein/g DNA/day)
Heart			
C*	0.52	10.5	14.7
LP	0.41	8.6	9.9
Diaphragm			
C	0.41	9.7	13.8
LP	0.26	6.5	6.6
Liver			
C	0.89	10.8	17.9
LP	0.35	4.7	8.2
Brain			
C	0.39	8.3	5.6
LP	0.34	7.6	4.9

* Control (27% protein).