Hepatic Protein Synthesis in Suckling Rats: Effects of Stage of Development and Fasting

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ABSTRACT. We studied the developmental changes in hepatic protein synthesis in suckling rats between postpartum d 1 and 28 and investigated the effect of fasting for 10 or 18 h on hepatic protein turnover at postpartum d 5, 10, 16, and 28. Fractional protein synthesis rates (K_s , %/d) were measured *in vivo* using a flooding dose of L-[4-³H] phenylalanine. Although hepatic K_s and translation efficiency (protein synthesis/unit RNA) were significantly higher at postpartum d 28 than d 1, the pattern of change was biphasic: K_s and translational efficiency were higher at d 10 and 28 than at d 5 and 16. The largest increase in K_s and translational efficiency occurred during the period normally associated with weaning (between postpartum d 16 and 28). At all stages of development, the K_s and translational efficiency in fasted rats were significantly lower than those in control (fed) rats, although the relative decline in both measurements was largest at postpartum d 10. The absolute rates of hepatic protein synthesis declined to similar levels on d 5, 10, and 16 after 10 h of fasting and changed little after 18 h of fasting; this level was significantly higher at postpartum d 28. Our results suggest that postnatal development in suckling rats was marked by a biphasic pattern in the rates of hepatic protein synthesis, which increased during the neonatal and weaning periods. The relative changes in the synthesis and loss of hepatic protein in response to fasting were greater during the neonatal than during the late suckling and weaning periods. (*Pediatr Res* 31: 247–252, 1992)

Abbreviations

K_s , fractional synthesis rate
ASR, absolute synthesis rate
rASR, relative absolute synthesis rate
PCA, perchloric acid
EBW, empty body weight

enzyme expression, which occur in two clusters during the neonatal and late suckling periods (3). The neonatal period between birth and 10 d of age is marked by an increase in the expression of hepatic enzymes involved in gluconeogenesis and fatty acid oxidation in response to the high fat, low carbohydrate composition of milk. During the transition from suckling to weaning, however, an increase occurs in the expression of lipogenic and ureagenic enzymes as the proportion of dietary carbohydrates increases.

In addition to dietary changes during the late suckling period, circulating thyroxine, corticosterone, and insulin concentrations increase, all of which play a regulatory role in the expression of hepatic enzyme activities (4). The changes in hepatic enzyme expression necessarily involve changes in the relative rates of mRNA and protein synthesis or turnover. Studies in growing weaned rats have shown that thyroxine and corticosterone can increase the expression of specific hepatic enzyme mRNA (5, 6) and increase hepatic protein synthesis (7). Despite documented changes in metabolic and endocrine status between birth and weaning, the ontogeny of hepatic protein synthesis is poorly understood.

During periods of acute starvation or fasting, however, conservation of body protein mass is crucial. A short-term fast (24 h) in young growing and adult rats reduces the hepatic protein synthesis rate and protein mass by approximately 30 and 20%, respectively (8–10). Alternatively, in skeletal muscle, reduced protein synthesis in response to a short-term fast is greater in young rats than in older weaned rats (11, 12). Given the inherent metabolic fragility of the neonate, the conservation and maintenance of hepatic protein mass and perhaps hepatic function may be crucial during periods of acute nutrient deprivation.

On the basis of these observations, our study was designed to answer two questions: 1) Do changes in hepatic protein synthesis accompany the ontogenic changes in the composition of the diet and the concentrations of circulating hormones, and 2) is the effect of fasting on hepatic protein mass and synthesis influenced by stage of development?

MATERIALS AND METHODS

Experimental Design. Experiment 1. A more complete description of the animals and techniques has been reported (13). Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) with timed pregnancies were obtained at 14 d of gestation. Each dam was housed individually in a solid-bottom cage with wood-chip bedding and maintained in a temperature-controlled room at 23°C with a 12-h on (0400–1600 h)-off (1600–0400 h) light cycle. Animals had free access to deionized water and food (22% protein, 5% fat; Wayne Research Animal Diets, Chicago, IL). At 1 d of age, litters were standardized to 10 pups and weighed every 3 d thereafter. In the period from 1 to 20 d of age, pups and dams were housed such that the food was available to both. At 21 d of age, pups were physically separated from their dams and housed together as a litter in cages with free access to

The liver undergoes significant changes in growth and functional development from birth through weaning. Hepatic growth in suckling rats has been characterized as hyperplastic shortly after birth and as hypertrophic at weaning (1, 2). Postnatal changes in functional development include those in hepatic

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Fig. 1. Developmental changes in RNA protein ratio, K_s , and translational efficiency in hepatic tissue of suckling rats. RNA protein ratios were different ($p < 0.01$) d 1 vs 5, d 5 vs 18, and d 21 vs 28; K_s were different ($p < 0.05$) d 1 vs 10, d 10 vs 18, and d 18 vs 28 (postpartum day effect, cubic $p < 0.10$); translational efficiencies were different ($p < 0.01$) d 1 vs 18 and d 16 vs 28 (postpartum day effect, quadratic $p < 0.05$). Values are means $\pm$ SEM ($n = 8$). Data were analyzed by t test and analysis of variance.

water and food as described above for dams. Pups were randomly selected from two litters used for each day and were studied between 0900 and 1200 h on d 1, 5, 10, 16, 18, 21, and 28 postpartum.

Experiment 2. A second set of litters was used to study the effect of fasting and was housed in the same manner as that in experiment 1. Animals in experiment 2 were fed Purina Rodent Chow (Ralston-Purina Co., St. Louis, MO) (22.5% protein, 4% fat) and water *ad libitum*. All litters were standardized to 10 pups within 1 d of birth. At postpartum d 5, 10, 16, and 28, pups from three litters were randomly assigned to one of three treatment groups and were studied during both normal feeding and after a period of 10 or 18 h of fasting. On postpartum d 4, 9, and 15, the normally fed control pups were studied at 2000 h. The remaining dams were separated from their respective pups at 2200 h and the dams' mammary ducts were surgically ligated under ether anesthesia; dams were then returned to their pups for the remainder of the experiment. The elapsed time between removal of the dam for surgery and return to her pups was approximately 30 min. The following day, the remaining pups in each of the three litters were studied after 10 h (0800 h) or 18 h (1600 h) of fasting. Rats studied at 28 d were separated from their respective dams at postpartum d 21 and fed Rodent Chow *ad libitum*. These rats were fasted by removing the food from their cages and were studied in the same manner as those on postpartum d 5, 10, and 16. To verify that each pup had been fasted, stomach contents were measured after the animals were killed. Fasted pups whose stomach contents amounted to more than 25% of the average contents found in control (fed) pups

were excluded from further analysis. The protocol was approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Measurement of In Vivo Protein Synthesis. Rats were decapitated, and trunk blood was obtained 2 and 10 min after a flooding dose of L-[4- 3 H]phenylalanine (50–100 μ Ci/rat, 150 μ mol/mL/100 g body wt) was administered i.v. The peritoneal cavity was immediately flushed with ice-cold PBS (CaCl₂, 0.90 mM; KCl, 2.68 mM; KH₂PO₄, 1.47 mM; MgCl₂·H₂O, 0.88 mM; NaCl, 137 mM; Na₂HPO₄·7H₂O, 15.22 mM), pH 7.4. The liver was removed and weighed and a subsample was frozen in liquid nitrogen.

Tissue Preparation and Analysis. Liver samples were homogenized in 0.2 M PCA using a Duall glass grinder and centrifuged at 3000 $\times$ g for 20 min at 4°C. The PCA-soluble homogenate supernatant was separated from the PCA-insoluble pellet, neutralized with 4 M KOH and centrifuged at 3000 $\times$ g for 20 min to remove insoluble KClO₄ salts. The resulting neutralized supernatant was the PCA-soluble fraction that contained the free amino acid pool; it was further analyzed to determine the specific radioactivity of phenylalanine. The PCA-insoluble pellet precipitated from the tissue homogenate contained protein and predominantly ribosomal RNA; it was washed twice with 0.2 M PCA and solubilized in 0.3 M NaOH at 37°C for 1 h. An aliquot was removed for determination of total protein as described by Smith *et al.* (14). The remaining solution was acidified with 2 M PCA and centrifuged at 3000 $\times$ g for 20 min, and the RNA concentration of the acid-soluble supernatant was determined as described by Munro and Fleck (15). The remaining PCA-insoluble protein pellet was washed again twice with 0.2 M PCA and hydrolyzed to free amino acids in 6 N HCl at 110°C for 24 h under an atmosphere of nitrogen gas. The PCA-soluble (free amino acid pool) and PCA-insoluble (amino acid incorporated into protein) fractions of the liver tissue homogenate were dried under vacuum (Speedvac concentrator-SVC200H; Savant Instruments Inc., Farmingdale, NY), washed three times with water, and resuspended in H₂O before determination of phenylalanine sp act.

HPLC. Phenylalanine was separated from other amino acids in the samples using anion-exchange chromatography (AS8 column; Dionex Corp., Sunnyvale, CA) as described by Davis *et al.* (13). Fractions were collected, and the radioactivity associated with the phenylalanine peak was measured in a liquid scintillation counter (TM Analytic, Elk Grove Village, IL).

Calculations. The K_s (%/d) was calculated for rats killed 10 min after the injection using the equation (16)

$$K_s = (S_B/S_A) \times (1440/t) \times 100$$

where t is the time of labeling in min, S_A represents the specific radioactivity of phenylalanine in the PCA-soluble tissue free pool, and S_B represents that incorporated into protein. The labeling times used in the calculations are the actual times at which tissues were chilled with ice-cold saline. Because most of the RNA in tissues is ribosomal, the RNA to protein ratio (μ g RNA/mg protein) is an estimation of the protein synthetic capacity of the tissue. Translational efficiency was estimated by multiplying the $K_s \cdot d^{-1}$ by the ratio of protein to RNA to yield g protein synthesized per g RNA per d. The hepatic protein ASR (mg protein/d) was calculated by multiplying the $K_s \cdot d^{-1}$ by the protein content (mg protein/liver). The hepatic protein ASR was also calculated relative to EBW because of the significant changes in body weight during development. It was expressed as mg protein $\cdot d^{-1} \cdot EBW^{-1}$ (rASR). In our calculations, EBW represented an animal's live body weight minus the weight of stomach contents. We used EBW rather than live body weight because we found significant differences in stomach contents between fed and fasted rats.

In experiment 2, at each stage of development, the fractional

Table 1. Effect of fasting and stage of development on EBW and relative liver weight, protein, and RNA content*

	Day postpartum			
	5	10	16	28
EBW (g)				
Control (fed)	10.0 ± 0.4	20.0 ± 1.1	34.5 ± 1.0	78.3 ± 5.9
10-h fasted	9.8 ± 0.9	19.8 ± 0.8	33.4 ± 1.2	75.9 ± 5.0
% change	(-3)	(-1)	(-3)	(-3)
18-h fasted	9.6 ± 0.1	19.5 ± 1.5	31.3 ± 1.5	73.9 ± 10.8
% change	(-4)	(-3)	(-9)	(-6)
Liver wt (mg/g EBW)				
Control (fed)	36.2 ± 1.5	31.5 ± 2.5	31.3 ± 2.4	44.0 ± 2.1
10-h fasted	31.4 ± 0.9†	26.9 ± 0.8†	26.7 ± 0.8†	35.1 ± 1.7†
% change	(-13)	(-14)	(-15)	(-20)
18-h fasted	28.4 ± 0.4†‡	24.3 ± 0.3†‡	27.3 ± 0.7	34.0 ± 1.5†
% change	(-21)	(-23)	(-12)	(-23)
Liver protein content (mg/g EBW)				
Control (fed)	4.51 ± 0.14	4.46 ± 0.50	4.29 ± 0.25	6.84 ± 0.37
10-h fasted	4.10 ± 0.19†	3.98 ± 0.17	4.10 ± 0.07	5.94 ± 0.31†
% change	(-9)	(-10)	(-4)	(-13)
18-h fasted	3.53 ± 0.10†‡	3.48 ± 0.17†‡	4.15 ± 0.15	5.87 ± 0.31†
% change§	(-22)	(-21)	(-3)	(-14)
Liver RNA content (mg/g EBW)				
Control (fed)	0.334 ± 0.014	0.272 ± 0.034	0.270 ± 0.019	0.385 ± 0.017
10-h fasted	0.307 ± 0.006†	0.244 ± 0.024†	0.249 ± 0.014†	0.355 ± 0.021
% change	(-8)	(-10)	(-8)	(-8)
18-h fasted	0.274 ± 0.007†‡	0.177 ± 0.016†‡	0.232 ± 0.015†	0.354 ± 0.035
% change	(-18)	(-35)	(-14)	(-8)

* Values are means ± SD. Values in parentheses represent % change from control fed group.

† $p < 0.05$ vs controls.

‡ $p < 0.05$ vs 10 h.

§ Day postpartum effect ($p < 0.05$).

|| Day postpartum effect (quadratic, $p < 0.01$).

rate of change in hepatic protein content was determined in the first 10 h and between 10 and 18 h of fasting. We used the relative hepatic protein content per unit of EBW rather than the absolute hepatic protein content. The fractional rate of change in hepatic protein content between 0 and 10 h and 10 and 18 h of fasting was assumed to be linear and was therefore calculated by dividing the difference in hepatic protein content between the beginning (HP_i) and end (HP_f) of each interval by the average protein content during each interval:

$$[(HP_f - HP_i)/(\bar{X}_{HP_i,HP_f})] \times [100/\Delta t]$$

The rates were corrected for the differences in interval duration (i.e. 10 or 8 h) and expressed as %/h. A negative fractional rate of change indicated hepatic protein loss, and a positive fractional rate of change indicated hepatic protein gain.

Statistics. In experiment 1, differences associated with days postpartum were determined by analysis of variance; comparisons between days postpartum were also determined using t test with a Bonferroni correction for comparison of four means. Polynomial regression was used to analyze changes associated with days postpartum. In experiment 2, means represents data from three litters; each of the three litter means was calculated using data from two to three rats. Differences associated with day postpartum and fasting time were first analyzed by analysis of variance. In those variables in which there was a significant interaction between days postpartum and fasting time, statistical comparisons between fed, 10-h fasted, and 18-h fasted groups were made using a modified paired t test; the three litters were paired across each treatment, within a given day postpartum. Comparisons between days postpartum were evaluated using Student's t test with a Bonferroni correction for comparison of three means. Polynomial regression was also used to analyze changes associated with days postpartum.

RESULTS

Experiment 1. Hepatic RNA/protein ratios decreased linearly ($p < 0.05$) by approximately 47% between postpartum d 1 and 28, although most of the decrease occurred during the first 5 d (Fig. 1). The pattern of change in hepatic protein K_s during the first 28 d postpartum was different from that of the RNA/protein ratio (Fig. 1). Although hepatic tissue K_s was nearly 40% lower at birth than at 28 d postpartum, the pattern of change appeared to be biphasic; K_s increased (21%) between postpartum d 1 and 10, decreased (30%) between d 10 and 18, and rose sharply (67%) between d 18 and 28 to its highest level (124%/d). The biphasic pattern of change in K_s seen in control (fed) rats was also indicated by the cubic response which approached significance ($p < 0.10$) between postpartum d 5 and 28. During the first 28 d postpartum, the pattern of change observed in hepatic translational efficiency of protein synthesis was quadratic ($p < 0.01$); translational efficiency was greater at d 28 than at d 16. The proportional changes in hepatic translational efficiency, however, were much greater than those found in K_s . Translational efficiency was nearly 3-fold lower at birth than at 28 d postpartum; the largest difference was observed during the period normally associated with weaning in rats (16–28 d postpartum).

Experiment 2. EBW did not decrease after either 10 or 18 h of fasting at any stage of development (Table 1). Liver weights were lower in experimental than in control (fed) rats at postpartum d 5, 10, and 28 when measured at 10 and 18 h of fasting; the only decrease in liver weight among experimental rats occurred on d 16 at 10 h (Table 1). In addition, liver weight in experimental rats declined significantly at postpartum d 5 and 10, but not at d 16 and 28, between 10 and 18 h of fasting. Liver protein and RNA contents decreased in response to fasting (Table 1). The proportional changes in liver protein and RNA contents at 10 and 18 h of fasting were similar to those seen in liver weight, except that no changes were found on postpartum d 16.

Fig. 2. Changes in hepatic RNA protein ratio, K_s , and translational efficiency in control (fed), 10-h fasted, and 18-h fasted rats during different stages of development. *, Different from control (fed) rats ($p < 0.01$); **, different from 10-h fasted rats ($p < 0.01$). RNA protein ratio (postpartum day effect) in control (fed) rats was linear ($p < 0.01$); K_s (postpartum day effect) in control (fed) rats was cubic ($p < 0.05$) and in 10-h and 18-h fasted rats was quadratic ($p < 0.05$); and translational efficiency (postpartum day effect) in control (fed) rats was quadratic ($p < 0.01$) and in 10-h and 18-h fasted rats was linear ($p < 0.01$). Bars represent means ($n = 3$ litters) $\pm$ SD. Effects of fasting were analyzed by a paired t test. Postpartum day effects were determined by analysis of variance and polynomial regression.

Fig. 3. Percentages of change in K_s after either 10 (solid bars) or 18 (hatched bars) h of fasting. Postpartum day effects were determined by analysis of variance for 10 and 18 h of fasting. Polynomial regression analysis indicated that changes with days postpartum were quadratic ($p < 0.05$).

At postpartum d 5 and 10, but not at d 16 or 28, there was a significant decline in liver protein and RNA between 10 and 18 h of fasting. The percentage of change in liver protein and RNA content after 18 h of fasting was greater at postpartum d 5 and 10 than at d 16 and 28.

In experiment 2, the developmental decline in RNA/protein ratios measured in control (fed) rats was similar to that observed in experiment 1 (Fig. 2). At postpartum d 5 and 28, fasting for either 10 or 18 h did not influence RNA/protein ratios. On d 10 and 16, however, the RNA/protein ratio in rats fasted for 18 h was lower than that in control (fed) rats. As in experiment 1, hepatic K_s exhibited a biphasic pattern (cubic $p < 0.05$) of change between postpartum d 5 and 28; K_s increased between d 5 and 10, decreased between d 10 and 16, and increased between d 16 and 28 (Fig. 2). At all stages of development, hepatic K_s in fasted rats was significantly lower than that in control (fed) rats. Most of the decrease in hepatic K_s occurred in the first 10 h of fasting; only small decreases were seen with continued fasting for 18 h. The percentage of decline in hepatic K_s after either 10 or 18 h of fasting was greatest at 10 d postpartum, as indicated by the quadratic effect ($p < 0.05$) with day postpartum (Fig. 3).

Estimates of protein ASR and rASR were calculated to illustrate the changes in whole liver protein synthesis (Table 2). The ASR in control (fed) rats was significantly ($p < 0.01$) higher at postpartum d 28 than at d 5. The ASR in control (fed) rats was 3-fold higher at postpartum d 10 than at d 5 and 4.8-fold higher at d 28 than at d 16. The ASR, however, was only 24% lower at d 16 than at d 10. ASR and rASR were significantly lower after 10 or 18 h of fasting than estimates in control (fed) rats, regardless of stage of development. The rASR increased quadratically ($p < 0.01$) between postpartum d 5 and 28 in rats fasted 10 and 18 h; the largest increases (2.8-fold) occurred between d 16 and 28. The relative decreases in both ASR and rASR after 18 h of fasting were greatest at d 10 and significantly different from those at postpartum d 28.

The fractional rates of change in hepatic protein content tended to be lowest at postpartum d 16 (Table 3). At d 5 and 10, the rate of hepatic protein loss seemed to be greater between 10 and 18 h than in the first 10 h, whereas at d 28, the rate of loss was greater in the first 10 h of fasting. The fractional rate of hepatic protein loss between 10 and 18 h of fasting was greater ($p < 0.05$) at postpartum d 5 and 10 than at either d 16 or d 28.

DISCUSSION

Developmental changes. Our initial objective was to examine the ontogeny of hepatic protein synthesis during the suckling period. We were particularly interested in the relationship between changes in hepatic protein synthesis and the well-established temporal changes in gene expression, circulating hormone concentrations, and dietary composition. The most striking result that we observed in fed rats from both studies was the biphasic nature of changes in hepatic protein synthesis between postpartum d 1 and 28, i.e. the protein K_s increased between d 1 and 10, decreased slightly between d 10 and 16, and increased between d 16 and 28.

The increase in hepatic K_s by d 10 coincided with the reported increase in gluconeogenic and ketogenic enzyme expression (3, 4). The reason for the decline in hepatic K_s between d 10 and 16 is less clear; it occurred, however, in both experiments 1 and 2. We have found (17), as have others (18), that the energy and protein intakes of suckling rats decline between d 10 and 16 and are associated with a decrease in the fractional rate of weight gain. Thus, the transient decline in hepatic K_s between d 10 and 16 may be a consequence of a progressively limited nutrient intake.

The increase in all estimates of protein synthesis between postpartum d 16 and 28 is concurrent with the transition from milk to solid food. During this time, the liver also makes its maximum contribution to body protein mass (19). In our study,

Table 2. *Effect of fasting and stage of development on liver protein ASR**

	Day postpartum			
	5	10	16	28
ASR (mg protein/d)				
Control (fed)	37.11 ± 2.0	111.9 ± 5.3	138.7 ± 27.1	667.6 ± 48.8
10-h fasted	24.26 ± 4.0†	52.0 ± 12.8†	81.6 ± 1.8†	422.6 ± 26.2†
% change	(-35)	(-54)	(-41)	(-37)
18-h fasted	19.12 ± 4.5†	42.1 ± 15.7†	77.5 ± 16.1†	397.4 ± 99.1†
% change‡	(-49)	(-62)	(-44)	(-40)
rASR (mg protein · d ⁻¹ · EBW ⁻¹)				
Control (fed)§	3.71 ± 0.17	5.60 ± 0.27	4.02 ± 0.44	8.53 ± 0.29
10-h fasted	2.48 ± 0.20¶	2.63 ± 0.32†	2.44 ± 0.06†	5.56 ± 0.31¶
% change	(-34)	(-53)	(-40)	(-36)
18-h fasted	1.99 ± 0.27†	2.16 ± 0.37†	2.47 ± 0.24†	5.38 ± 0.34¶
% change‡	(-47)	(-62)	(-40)	(-37)

* Values are means ± SD. Numbers in parentheses represent % change from control fed group.

† $p < 0.05$ vs controls.

‡ Day postpartum effect ($p < 0.05$).

§ Day postpartum effect (cubic, $p < 0.05$).

|| Day postpartum effect (quadratic, $p < 0.05$).

¶ $p < 0.01$ vs controls.

Table 3. *Effect of stage of development on fractional rate of change in hepatic protein content between 10- and 18-h intervals of fasting**

	Day postpartum			
	5	10	16	28
Fasting interval (h)				
0-10	-0.96 ± 0.15	-1.09 ± 0.70	-0.43 ± 0.30	-1.42 ± 0.35
10-18†	-1.85 ± 0.49	-1.67 ± 0.29	+0.15 ± 0.23	-0.14 ± 0.14

* Values (%/h) are means ± SD. Positive (+) = gain; negative (-) = loss. Rates are calculated as described in Materials and Methods.

† Day postpartum effect ($p < 0.01$).

the rASR at d 28 (8.53) was significantly higher than at any earlier stage of development (3.71 to 5.60). Although the energy intakes (kcal/body wt) of suckling rats at postpartum d 16 and weaned rats at d 28 are similar (17, 20), between d 16 and 28 the composition of dietary energy changes from predominantly fat to predominantly carbohydrate. As the intake of dietary carbohydrate increases during weaning, both plasma corticosterone concentrations and tissue responsiveness to corticosterone also increase (4). Studies in young, growing, weaned rats suggest that corticosterone can stimulate hepatic protein synthesis (7). A direct link between the surge in plasma corticosterone concentrations and the increase in hepatic protein synthesis has not been established. Nevertheless, the increase in hepatic protein synthesis is consistent with a surge of new enzyme expression (3) that reflects the change in hepatic metabolic function in response to increased carbohydrate intake.

Effects of fasting. The neonate may be particularly vulnerable to the effects of acute nutritional deprivation because of its inherent metabolic fragility. Because hepatic metabolic function depends on the relative expression of enzymes, the conservation of hepatic protein is crucial to sustained metabolic function during acute nutritional deprivation. Our results indicate that during acute fasting, hepatic protein was reduced as a result of decreased protein synthesis. The relative magnitude of these changes seemed to be greater during the neonatal (especially postpartum d 10) than during the late suckling or weaning periods.

Regardless of the stage of development, hepatic protein K_s and ASR decreased in response to fasting. Most of the decrease occurred within the first 10 h and was accompanied by significantly reduced liver weight and protein and RNA content. Despite these changes, there was no significant decrease in EBW. With few exceptions, hepatic RNA/protein ratios were unaffected by fasting. Thus, decreased hepatic K_s was largely a result

of decreased translational efficiency rather than a decrease in ribosomal number.

The effects of fasting on hepatic protein synthesis in the suckling rats in our study were qualitatively similar to those reported in adult and young weaned rats (8-10). We found quantitative differences, however, depending on the stage of development. Regression analysis of the percentage of change in K_s after either 10 or 18 h of fasting indicated that the change at postpartum d 10 was larger than that at either d 16 or 28. In addition, the relative decreases in hepatic K_s in suckling rats fasted for 18 h ranged from 25 to 52% compared with 17 to 28% reported in young, growing animals fasted for 24 to 48 h (9, 10). We were concerned about a potential effect of differences in the stomach emptying rate in fasted rats at different stages of development. At d 5, 10, and 16, the rates of stomach emptying were similar (approximately 20%/h); at d 28, however, they were significantly higher (40%/h) (21). Nevertheless, on all days measured, the stomachs were approximately 90% empty after 4 h of fasting. Taken together, these findings suggest that the relative decrease in hepatic protein synthesis in response to fasting was greater in 10-d-old neonatal rats than in older suckling or weaned rats.

Alternatively, one could hypothesize that the rate of hepatic protein synthesis during fasting was the "basal rate" and that the differences in hepatic protein synthesis rate associated with stage of development resulted from changes in the sensitivity and/or responsiveness of the liver to nutrient intake. Two findings support this speculation: first, we found that the values for hepatic K_s and rASR on d 5, 10, and 16 after either 10 or 18 h of fasting were remarkably similar, approximately 60%/d and 2.40 mg · d⁻¹ · g EBW⁻¹. These values support the premise that a basal rate of hepatic protein synthesis attained in suckling rats after 10 h of fasting may have been related to the maintenance of vital metabolic functions; the basal rate of protein synthesis,

however, was higher after weaning than during the suckling period. Second, we found that food intake and stage of development may have affected the degree to which hepatic protein synthesis was increased above the basal rate. Hepatic protein synthesis decreased in the fed state between d 10 and 16, as the nutrient intake of the suckling pups was reduced. As a result, the relative decrease in hepatic protein synthesis in response to fasting at postpartum d 16 was smaller than that at d 10. The relative differences in the fed and fasted states at postpartum d 16 and 28 were similar, despite significant increases in the hepatic protein ASR in both the fed and fasted rats during this period. Thus, the relative differences in hepatic protein synthesis between fed and fasted rats reflected relative differences in nutrient stimulation in the fed state, as well as the basal level of protein synthesis at a given stage of development.

Changes in protein synthesis, however, could not account for the total reduction in hepatic protein content in response to fasting. The changes in hepatic protein content at either 10 or 18 h of fasting were influenced by stage of development. At postpartum d 5 and 10, the absolute decrease in hepatic protein content was linear between 0 and 18 h of fasting. On the other hand, at postpartum d 16 and 28, most of the decrease in protein content occurred in the first 10 h, with no further significant decrease between 10 and 18 h. Although the fractional rate of hepatic protein loss seemed to increase with the duration of fasting in neonatal rats at postpartum d 5 and 10, it declined in 28-d-old weaned rats. These data, combined with the fact that the decline in protein synthesis was essentially completed by 10 h of fasting, suggest that protein content may have been regulated by degradation rather than synthesis after 10 h.

We conclude that developmental changes in hepatic protein synthesis in the rat are biphasic. We documented increases in hepatic protein synthesis between birth and 10 d and again after weaning. The increases are associated with temporal changes in enzyme expression, diet composition, and circulating hormone concentrations that have been reported to occur during the neonatal and late suckling or weaning periods. The differences in the response to fasting with stage of development appeared to reflect differences in the responsiveness of hepatic protein synthesis to nutrient intake above a basal rate. This basal rate was relatively constant during the suckling period and increased after weaning. Nevertheless, reduced protein synthesis, combined with a greater fractional rate of hepatic protein loss, contributed to the greater proportional decrease in protein mass in neonatal rats than in older weaned rats. Thus, if hepatic function is linked to protein mass, our results suggest that metabolic function may be more compromised in response to fasting in the neonatal rats than in older weaned animals.

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