Effects of maternal dietary protein content on cerebral ketone body-metabolizing enzymes in the progeny of rats

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The effect of maternal dietary protein content on blood glucose and ketone bodies and on cerebral ketone body-metabolizing enzymes in the rat progeny was investigated. Four different diets having either 15.5% (adequate) or 5.4% (low) calories from protein with 12.2% or 47.1% calories from fat (AP-HC, adequate protein-high carbohydrate diet; AP-HF, adequate protein-high fat diet; LP-HC, low protein-high carbohydrate diet; and LP-HF, low protein-high fat diet) were fed to females starting at 2 weeks prior to mating and continued throughout pregnancy and lactation. Compared to the pups of mothers on the diets with adequate levels of protein, the pups of mothers on the low protein diets had stunted growth and also had significant reductions in brain weight gains in the postnatal period. Undernutrition due to maternal low protein diets (LP-HC and LP-HF) resulted in significantly higher activities of brain D-3-hydroxybutarate dehydrogenase and 3-oxoacid CoA-transferase in the pups on day 18 than the corresponding values for pups in adequate protein (AP-HC and AP-HF) groups. The results show that the reduction in protein content in the maternal diet increases the activity levels of cerebral ketone body-metabolizing enzymes in suckling rats.

Keywords: maternal diet; dietary protein; blood 3-hydroxybutyrate; brain weight; D-3-hydroxybutryate dehydrogenase; 3-oxoacid CoA-transferase

Introduction

The importance of ketone bodies in the developing rat brain has been well documented.[1-3] Ketone bodies, D-3-hydroxybutyrate and acetoacetate, are used both as fuels for energy production and as lipogenic precursors.[2,3] Compared to adult rats, suckling rats demonstrate higher rates of both cerebral uptake and oxidation of ketone bodies.[4-6] These observations are consistent with findings that activities of ketone body-metabolizing enzymes are elevated 3-fold to 5-fold in brains of suckling rats compared to adult animals.[7-9] In contrast to the adult, ketone body-metabolizing enzymes in fetal and suckling rat brains respond in varying degrees to maternal dietary manipulations.[10-13] Maternal ketonemia induced by feeding pregnant rats a high-fat diet or by fasting for 5 days resulted in a significant increase in the activities of succinyl-CoA: 3-oxoacid CoA-transferase (CoA-transferase) (EC 2.8.3.5) and D-3-hydroxybutyrate dehydrogenase (BDH) (EC 1.1.1.30) in the brains of term fetuses or newborns.[10,11,13,14] Similarly, the postweaning decline in cerebral BDH activity observed in rats weaned on laboratory chow is delayed either by weaning rats onto a high-fat diet or by fasting animals for 48 hr.[12,15]

Undernutrition is associated with variable changes in blood ketone body concentrations and in the activity levels of ketone body-metabolizing enzymes in brains of suckling rats.[16-19] In these studies, undernutrition was instituted by either increasing the litter size

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or restricting maternal food intake. The present study was initiated to investigate the possible effects of early undernutrition due to a low level of protein in the maternal diet on cerebral ketone body-metabolizing enzymes in the progeny.

Materials and methods

Animals, diets and experimental design

Rats (the Charles-Foster strain) inbred in our animal house and maintained at 30° C were used in this study. Adult animals were caged individually and were kept in normal 12-hr day-light and night-dark cycles. Female rats weighing 150-200 g were divided at random into four groups and were fed one of the four diets ad libitum:

- Group AP-HC: adequate protein (15.5%)-high carbohydrate diet
- Group AP-HF: adequate protein (15.5%)-high fat diet
- Group LP-HC: low protein (5.4%)-high carbohydrate diet
- Group LP-HF: low protein (5.4%)-high fat diet

The composition of these four diets and caloric contributions of fat, carbohydrate, and protein together with the American Institute of Nutrition (AIN) vitamin and mineral mixtures[20] are presented in *Table 1*. Female rats were maintained on one of these four dietary regimens starting at 2 weeks prior to breeding and continued during pregnancy and lactation. Pregnant rats were housed individually and 24-hr food intake was recorded for 6 consecutive days in each stage prior to mating as well as during pregnancy and lactation. To investigate possible intrauterine effects of maternal nutritional status, newborn pups were killed within 12 hr after birth. For preweaning studies, pups were weighed and pooled within the same dietary regimen at the time of delivery and litter size was adjusted to seven in each litter. Females were continued on the same dietary regimen until pups were killed on postnatal day 10 or 18. These pups were not separated from their mothers until the time of decapitation. For the postweaning study, pups were weighed and separated from their mothers on postnatal day 21 and were housed in groups of four in plastic cages. Weaned rats were continued on the same dietary regimen as their mothers, with free access to food and water until they were killed on postnatal day 30. All animals were killed between 10 AM and noon. Pups from all four groups were decapitated on the same day, and the preparation of samples and determination of each parameter were performed simultaneously in the four groups. For each time period, only two or three pups from a given litter were used, and hence a mean value represents pups from several mothers on the same dietary regimen. By redistributing pups within the same dietary treatment the litter size (seven pups/mother) was maintained until postnatal day 21.

Table 1 Composition of diets

Ingredients	Diets[a] AP-HC (g)	AP-HF (g)	LP-HC (g)	LP-HF (g)
Casein	200	200	70	70
DL-methionine	3	3	1	1
Sucrose	200	200	200	200
Sago flour (Metroxylon)	730	280	860	410
Peanut oil	70	70	70	70
Hydrogenated vegetable fat (Dalda)	—	200	—	200
Choline chloride	2	2	2	2
Vitamin mixture (20)	10	10	10	10
Mineral mixture (20)	35	35	35	35
% Calories from fat	12.2	47.1	12.2	47.1
% Calories from protein	15.5	15.5	5.4	5.4
% Calories from carbohydrate	72.3	37.4	82.4	47.5

[a] AP-HC: adequate protein-high carbohydrate diet; AP-HF: adequate protein-high fat diet; LP-HC: low protein-high carbohydrate diet; LP-HF: low protein-high fat diet.

Analytical procedures

Pups were killed by decapitation and blood samples were collected in preweighed glass tubes containing 0.2 mL of 12% cold perchloric acid. The deproteinized blood was centrifuged at 3,000 rpm for 10 min and the supernatant was collected. Precipitates were washed twice each with 0.1 mL of 6% perchloric acid. The three supernatants were combined and neutralized with 10% KOH, and the supernatants were stored frozen for subsequent determination of glucose[21] and D-3-hydroxybutyrate[22] concentrations.

The brain minus olfactory bulb was quickly removed and weighed. Brain homogenates were prepared in 0.1 M Tris-HCl buffer, pH 8.5 using a Potter-Elvehjem homogenizer. A portion of homogenate was sonicated using four 15-sec pulses at 10 kHz with an interval of 15 sec after each pulse. Brain BDH activity was determined by measurement of acetoacetate formed[23] during incubation of the sonicated homogenate with 3-hydroxybutyrate in the presence of NAD^+.[24] For the assay of CoA-transferase, an aliquot of the homogenate was stored in liquid nitrogen. Preliminary studies indicated no appreciable loss of enzyme activity during storage. CoA-transferase activity was assayed by measuring changes in the absorbance of acetoacetyl-CoA before and after the addition of sodium succinate.[24] Protein and DNA were determined by the methods of Lowry et al.[25] and Burton,[26] respectively.

Statistical analysis

All data are expressed as mean ± SEM. Data presented in *Table 2* were analyzed by Student's *t* test. All other data were analyzed by ANOVA (one factor) and compared by the Fisher's protected least significant difference (Stat View, Brain Power, Inc., Calabasas, CA). In all analyses differences were considered significant at $P < 0.05$.

Table 2 Caloric intake of females fed high fat with adequate or low levels of protein in the diet

Animals	Dietary treatments			
	AP-HC	AP-HF	LP-HC	LP-HF
	Caloric intake (Kcal/day)			
Non-pregnant	45 ± 0.8	50 ± 1.1[a]	44 ± 1.4	54 ± 2.3[a]
Pregnant	87 ± 2.6	98 ± 1.4[a]	62 ± 1.0	67 ± 1.7[a]
Lactating	107 ± 2.0	103 ± 2.1	74 ± 3.1	70 ± 1.4

[a] $P < 0.05$.
Mean ± SEM (n = 8-10); at each stage (non-pregnant, pregnant, and lactation) eight to ten females were randomly selected in week 2 and the food intake was recorded for 6 consecutive days.

Results

Female rats maintained on low protein diets (groups LP-HC and LP-HF) consumed fewer calories per day during pregnancy and lactation than did females maintained on high protein diets (groups AP-HC and AP-HF) (*Table 2*). The daily caloric intake was significantly higher in both nonpregnant and pregnant rats consuming diets high in fat (AP-HF and LP-HF) compared to the corresponding groups consuming diets high in carbohydrate (AP-HC and LP-HC). Similarly, daily calories derived from protein were also moderately higher between two groups of rats consuming the same level of protein in the diet under a similar experimental condition (LP-HC versus LP-HF) (data not shown). These findings on daily caloric intake on two diets, namely AP-HC and LP-HC (*Table 2*) are consistent with earlier studies reported from this laboratory.[27,28] The consumption of a high fat diet containing either adequate or low levels of protein did not significantly alter any of the reproductive parameters including average litter size, stillborn, or resorption (data not shown). Maternal weight loss due to pregnancy and lactation stress was marginally reduced in females fed high fat diets (AP-HC versus AP-HF) (data not shown). The effect of maternal low protein diet (LP-HC) on low birth weights as well as retarded postnatal growth of pups was consistent with earlier studies from this laboratory.[28]

The high fat diet (AP-HF), as compared with the AP-HC, had a significant effect only on the body weight on day 18 (*Figure 1*) and brain weight and DNA content on day 0 (*Table 3*). Similarly, the substitution of low protein-high carbohydrate (LP-HC) diet with low protein-high fat (LP-HF) diet did not improve the body weight (*Figure 1*) and brain weight of newborns at birth or of pups during the first 30 postnatal days (*Table 3*). Compared to both the AP-HC and AP-HF groups progeny of the LP-HC group were progressively growth retarded and addition of fat did not improve the growth status in the LP-HF group.

Blood glucose levels were not significantly different among the four groups of growing rats (compare AP-HC versus AP-HF and LP-HC versus LP-HF) on any given day (*Figure 1*). Blood glucose levels, however, increased progressively with age and reached adult levels by postnatal day 18. As expected, blood levels of D-3-hydroxybutyrate in the pups of the AP-HF group compared with the AP-HC group were significantly higher on postnatal day 10. Blood D-3-hydroxybutyrate levels in the pups of the LP-HC and LP-HF groups were also higher than age-matched pups of the AP-HC group on day 10. On day 18 there was a significant difference in the blood ketone body concentration between LP-HC and LP-HF groups (Figure 1).

Consistent with the previous reports,[7,8] the ontogenetic pattern of cerebral BDH activity in the AP-HC group indicated a progressive rise in the activity until postnatal day 18 and a decline thereafter (*Figure 2*). A significantly higher activity expressed as either units/g of brain (results not shown) or specific activity (milliunits/mg protein) (*Figure 2*) was, however, observed on days 10, 18, and 30 in the AP-HF group compared with the age-matched AP-HC group. Compared with the LP-HC group, the LP-HF group had significantly higher BDH activity only on postnatal days 10 and 18, and both of these groups had higher activity than that observed in the age-matched AP-HC group on day 18.

The developmental pattern of cerebral CoA-transferase in the AP-HC group was similar to those

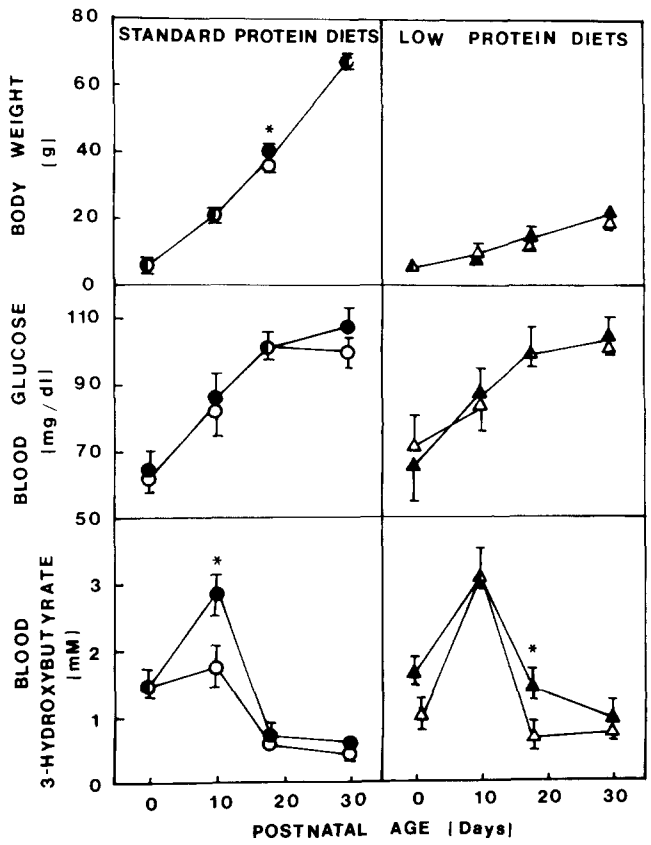

Figure 1 Effects of maternal diet [AP-HC (○), AP-HF (●), LP-HC (△) and LP-HF (▲)] on body weights and blood levels of D-3-hydroxybutyrate and glucose at different ages of the progeny. The results are the means ± SEM for 8-10 pups. Standard protein diets = Adequate protein (AP) diets. *$P < 0.05$.

Table 3 Effects of maternal diet on brain weights and brain DNA and protein content at different ages of the progeny

Parameters	Dietary treatments			
	AP-HC	AP-HF	LP-HC	LP-HF
Brain weights (mg)				
Day 0	276 ± 6	249 ± 7[a]	246 ± 17	228 ± 4
10	945 ± 24	901 ± 17	747 ± 20	719 ± 22
18	1283 ± 29	1282 ± 15	1026 ± 24	1046 ± 18
30	1393 ± 22	1425 ± 34	1121 ± 20	1141 ± 27
Brain DNA (mg/g tissue)				
Day 0	1.78 ± 0.02	1.95 ± 0.03[a]	1.96 ± 0.06	1.84 ± 0.05[a]
10	2.17 ± 0.02	2.20 ± 0.02	2.16 ± 0.01	2.18 ± 0.01
18	0.78 ± 0.05	0.76 ± 0.03	0.70 ± 0.06	0.66 ± 0.04
30	0.79 ± 0.04	0.78 ± 0.04	0.72 ± 0.03	0.71 ± 0.05
Brain protein (mg/g tissue)				
Day 0	48.1 ± 1.7	48.4 ± 1.6	49.3 ± 2.4	46.2 ± 3.3
10	63.7 ± 1.4	63.1 ± 1.6	59.8 ± 1.1	59.7 ± 1.1
18	65.9 ± 1.5	68.2 ± 1.7	55.9 ± 0.6	56.7 ± 1.4
30	94.0 ± 1.2	96.5 ± 2.5	92.0 ± 2.5	88.7 ± 2.1

[a] $P < 0.05$.
Means ± SEM, n = 7-11 pups except for n = 4 for LP-HC and LP-HF pups on day 0.

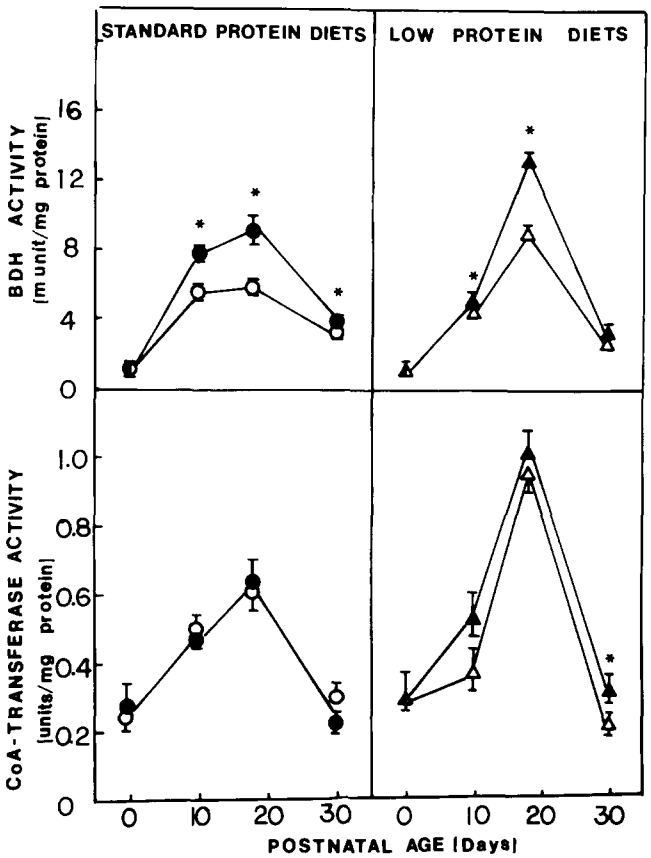

Figure 2 Effects of maternal diet [AP-HC (○), AP-HF (●), LP-HC (△), and LP-HF (▲)] on cerebral BDH and CoA-transferase activities at different ages of the progeny. The results are the means ± SEM for 8-10 pups except in the LP-HC group at birth only 4 pups were used. Standard protein diets = Adequate protein (AP) diets. *$P < 0.05$.

reported previously.[8,9,13] In contrast to BDH, CoA-transferase activity was not responsive to maternal dietary fat content except between LP-HC and LP-HF groups on day 30 (*Figure 2*). Interestingly, on day 18 CoA-transferase activity in both the LP-HC and LP-HF groups was significantly higher than that observed in the age-matched AP-HC and AP-HF groups (*Figure 2*). Thus, maternal dietary manipulation, especially its protein content, influenced the activity levels of cerebral ketone body-metabolizing enzymes in suckling rats.

Discussion

In the present study we investigated a possible interrelationship between protein and fat content in the maternal diet and its effect on postnatal development of cerebral ketone body-metabolizing enzymes in the progeny. An increase in cerebral BDH activity, but not in CoA-transferase activity, was observed in 10, 18, and 30 day-old pups whose mothers consumed a diet high in fat with adequate level of protein (AP-HF) prior to the initiation of pregnancy compared with the AP-HC group (*Figure 2*). However, other investigators observed no significant effect of the high fat content in maternal diet during lactation on the postnatal development of cerebral BDH activity in suckling pups.[14] This discrepancy may be attributed to the differences in the experimental variations, such as the content of fat in the diet, the time of initiation of the diet, and possible strain difference.

Several lines of investigation have suggested that maternal dietary manipulation, especially with respect to fat, can have significant effects on cerebral ketone body-metabolizing enzymes. Such studies have been carried out by giving pregnant females a high fat diet starting from the first week of pregnancy with the percentage of calories derived from fat ranging from 60% to 80%,[11,13,14] resulting in considerable elevation in the maternal circulating levels of ketone bodies. The resultant increases in cerebral BDH or CoA-transferase activity in term fetuses or newborns have been attributed to either in utero exposure to elevated levels of circulating ketone bodies or altered metabolic milieu.[11,13,14] A significant reduction in the activities of BDH and CoA-transferase is observed in brains of suckling rats subjected to either intrauterine growth retardation or perinatal undernutrition by food restriction to dams during pregnancy and lactation.[19,21] In contrast, no significant changes were observed in the activities of cerebral ketone body-metabolizing enzymes (expressed as units/g tissue) in undernourished pups (by increasing the litter size) compared to well nourished age-matched pups.[17] However, a reduction has been observed in the capacity to incorporate both [3-^{14}C]-D-3-hydroxybutyrate-carbon and [U-^{14}C]glucose-carbon into cerebral lipids by undernourished suckling rats.[17] It should be emphasized that no attempt was made to reduce protein content of the maternal diet in these studies.

The most significant observation of this study is

the effect of maternal low protein diets on the attainment of maximum activities of both ketone body-metabolizing enzymes in 18 day-old pups (*Figure 2*: compare AP-HC versus LP-HC, and AP-HF versus LP-HF). Significantly higher activities of BDH and CoA-transferase in the LP-HC group are consistent with the suggestion of higher proportion of ketone body utilization in undernourished rats.[29] Although the mechanism(s) responsible for such a response to the maternal low protein diet is not known, it is evident that the content of fat in the maternal diet plays a minor role, if any, in this response. The significance of this increment in ketone body-metabolizing enzymes in brains of pups of mothers on low protein diets (both LP-HC and LP-HF groups) on the contribution of ketone bodies to the energy metabolism remains to be investigated.

Abbreviations

AP-HC	adequate protein-high carbohydrate diet
AP-HF	Adequate protein-high fat diet
LP-HC	low protein-high carbohydrate diet
LP-HF	low protein-high fat diet
BDH	D-3-hydroxybutyrate dehydrogenase
CoA-transferase	succinyl-CoA:3-oxoacid CoA-transferase

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