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Body Weight Deficit in the Absence of Reduction in Cerebrum Weight and Nucleic Acid Content in Progeny of Swine Restricted in Protein Intake during Pregnancy (42715)

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Abstract. Fifty-six castrated male progeny of crossbred (Chester White × Landrace × Large White × Yorkshire) dams fed an adequate diet (control, C), a control diet fed at one-third of C (restricted, R), or diets severely deficient in protein (PF) or restricted in nonprotein calories (RCal) were killed at age 25 weeks. Dams were fed their respective diets in the following regimens: C, 1.8 kg (6000 kcal daily) throughout pregnancy; R, 0.6 kg of C diet daily for 70 days, then 1.8 kg of C daily to parturition at about 114 days; PF, 1.8 kg of a "protein-free" diet (<0.2% protein) throughout pregnancy; RCal, 0.6 kg daily (2000 kcal) of a diet containing three times the concentration of protein, minerals, and vitamins provided by the C diet for 70 days, then 1.8 kg of C daily to parturition. All dams were fed an adequate diet *ad libitum* through a 28-day lactation. Castrated male progeny were assigned to one of two replicates based on birth date and fed a corn–soybean meal diet *ad libitum* from weaning to age 25 weeks, supplemented from age 10 to 12 weeks with 0, 110, or 220 mg/kg of thyroprotein (iodinated casein). Cerebrum weight was unaffected by maternal diet, despite a significant ($P < 0.001$) reduction in body weight of progeny of PF dams compared with other groups, resulting in a higher relative cerebrum weight in progeny of PF dams than in progeny of C, R, and RCal dams. Absolute and relative weights of RNA, DNA, and total protein in cerebrum were unaffected by maternal diet. Thyroprotein supplementation to the diet of the progeny had no effect on cerebrum weight or its protein or nucleic acid content. It is concluded that maternal protein deprivation but not restriction of feed or nonprotein calorie intake to one-third of recommended allowance during gestation results in stunting of body weight in young adult progeny but does not affect cerebrum weight, cerebrum cell number (DNA), or protein synthetic activity (RNA), or RNA-to-protein ratio.

Prenatal and perinatal dietary restriction and protein–calorie malnutrition alter cellular growth of the brain and other tissues in laboratory animals (1–7) and humans (8–11). Much of the effort in human studies has been directed toward effects of early malnutrition on cognitive development (9); the evidence suggests a causal association between early nutrition and cognition at preschool age (12) and beyond (13), presumably related to brain function at the cellular level. Such relationships are difficult to quantify in clinical studies with humans. Data obtained using the pig, whose nervous system ontogeny probably resembles that of the human more closely than that of the rat (14), provide the possibility for developing inferences on differential effects of human maternal protein malnutrition on body mass and brain mass.

The growth retardation that accompanies malnutrition is influenced by the stage of development at which the nutritional insult is imposed (15). Winick and Noble (1) extended this observation in whole animals to cellular events and showed that early malnutrition impeded cell division (as measured by DNA) and that the animal did not recover, whereas malnutrition at a later stage of growth allowed recovery. Davison and Dobbing (14) compared rates of brain growth and development in several species and reported that maximum growth of the brain of pigs occurs earlier than that in rats, suggesting species differences in the time at which vulnerability to malnutrition is greatest. The

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central nervous system of the pig grows most rapidly from about 6 weeks before to about 5 weeks after birth (16–18) as measured by protein, RNA and DNA content of cerebrum, and cerebellum.

The purpose of the work reported here was to test the hypothesis that maternal protein or energy restriction during pregnancy has permanent effects on body weight and on cerebrum protein, RNA, and DNA content of progeny during young adulthood.

Materials and Methods. Crossbred (Chester White × Landrace × Large White × Yorkshire) castrated male progeny of female swine fed an adequate diet (control, C), control diet fed at one-third of C (restricted, R), or diets essentially protein-free (PF) or restricted in nonprotein calories (RCal) were used. Composition of the diets fed to the dams during gestation has been described in detail elsewhere (19). The diet regimens were as follows: C, 1.8 kg (6000 kcal DE) daily of a corn–soybean meal-type diet throughout pregnancy; R, 0.6 kg of C diet daily for 70 days, then 1.8 kg of C daily to parturition at about 114 days; PF, 1.8 kg of a "protein-free" diet (<0.2% protein) throughout pregnancy; RCal, 0.6 kg daily (2000 kg DE) for 70 days of a diet containing three times the concentration of protein, minerals, and vitamins provided by the C diet, then 1.8 kg of C daily to parturition. All dams were fed an adequate corn–soybean meal-type diet *ad libitum* through a 28-day lactation.

Randomly selected castrated male progeny were assigned to one of two replicates based on birth date and fed *ad libitum* a corn–soybean meal-type grower diet (18% protein) supplemented with 0, 110, or 220 mg iodinated casein during weeks 10 to 16 of the postweaning period from age 5 weeks to age 25 weeks. All pigs were fed the basal diet (B) from 5 to 10 weeks and from 16 to 25 weeks of age, while 18 were fed B throughout the period 5 to 25 weeks of age, 19 were fed B supplemented with 110 mg thyroprotein (iodinated casein), and 19 were fed B supplemented with 220 mg thyroprotein/kg of diet from week 10 to 16 (20). Since supplemental thyroprotein failed to affect any of the traits reported, the data from the three postweaning dietary groups were combined to provide the means shown in Table I.

Cerebrum was removed from each pig at age 25 weeks following electrical stunning and exsanguination, weighed, frozen in liquid nitrogen, and later analyzed for protein (21), DNA (22), and RNA (23).

TABLE I. PROTEIN, RNA, AND DNA IN CEREBRUM OF PROGENY OF PIGS RESTRICTED IN PROTEIN, FEED, OR NONPROTEIN CALORIES DURING PREGNANCY

Trait	Maternal diet[a]				Overall mean ± SD	Probability
	C	PF	R	RCal		
No. of animals	18	17	9	12		
Body wt (kg)	103.8[b]	87.8[c]	101.5[b]	100.4[b]	97.5 ± 8.6	<0.0001
Cerebrum wt (g)	65.6	62.5	65.9	64.5	64.8 ± 4.4	NS
Cerebrum wt (% of body wt)	0.0635[b]	0.0718[c]	0.0652[b]	0.0648[b]	0.0672 ± 0.0065	<0.003
Protein ($mg \cdot g^{-1}$)	109.9	107.6	116.3	108.5	110.8 ± 15.2	NS
Protein, total (g)	7.24	6.69	7.69	6.99	7.19 ± 1.13	NS
RNA ($mg \cdot g^{-1}$)	2.54	2.48	2.75	2.49	2.61 ± 0.36	NS
RNA, total (mg)	167.7	154.9	181.3	160.8	169.6 ± 26.0	NS
DNA ($mg \cdot g^{-1}$)	0.538	0.529	0.537	0.504	0.531 ± 0.074	NS
DNA, total (mg)	35.39	32.88	35.28	32.49	34.42 ± 4.98	NS
RNA:DNA ($mg \cdot mg^{-1}$)	4.74	4.73	5.13	4.95	4.93 ± 0.50	NS
DNA:protein ($mg \cdot g^{-1}$)	4.92	4.96	4.66	4.64	4.83 ± 0.55	NS
RNA:protein ($mg \cdot g^{-1}$)	23.1	23.2	23.8	22.9	23.6 ± 2.5	NS

[a] C, control diet fed at 1.8 kg daily throughout gestation; PF, "protein-free" diet fed at 1.8 kg daily throughout gestation; R, conrol diet fed at 0.6 kg daily for the first 70 days of gestation, then at 1.8 kg daily to parturition; RCal, diet containing three times the concentration of protein, vitamins, and minerals of control diet and fed at 0.6 kg daily (one-third of the amount fed to C group) for the first 70 days of gestation, then the control diet at 1.8 kg daily to parturition.

[b,c] Means in the same row with different superscripts are significantly different from each other.

Data were subjected to least-squares means analysis of variance (24) with orthogonal comparisons to detect differences. Maternal diet and progeny postweaning diet effects and their interactions were tested.

Results. The effects of maternal gestation diet on pig progeny body weight and on cerebrum weight and protein, RNA, and DNA content at age 25 weeks are summarized in Table I. Although body weight at slaughter was less ($P < 0.001$) in progeny of dams fed the PF diet than in other progeny, weight of the cerebrum was not significantly affected by maternal diet. Therefore, when expressed as a percentage of body weight, cerebrum weight was greater ($P < 0.003$) in progeny of dams fed the PF diet than in other progeny. Indices of cell number (DNA concentration and total organ DNA) and of protein synthetic activity (RNA and protein concentration and total organ RNA and protein) were unaffected by maternal gestation diet. The absence of a maternal diet effect on cerebrum cell number and on protein and RNA content was substantiated further by the expression of constituents as ratios to each other, i.e., RNA-to-DNA, DNA-to-protein, and RNA-to-protein ratios, which were similar across treatments (Table I).

Supplemental thyroprotein (110 or 220 mg/kg of diet) had no effect on any of the traits measured and there were no interactions between maternal diet and progeny postweaning diet effects. Ratios of RNA to DNA, DNA to protein, and RNA to protein and of each constituent to body weight were similar for all groups. Although there were no significant main effects of maternal diet (MD) or progeny postweaning diet (PD) on any trait, there was an MD × PD interaction for DNA-to-protein ratio ($P < 0.03$) and for RNA-to-protein ratio ($P < 0.03$). The interaction for DNA-to-protein ratio resulted from a trend toward an increase in the ratio in C progeny fed 110 mg thyroprotein/kg of diet compared with progeny fed the basal diet (5.30 vs 4.78) but a decrease in PF and R progeny fed 110 mg thyroprotein/kg of diet (4.58 vs 5.25 in PF progeny and 4.00 vs 4.75 in R progeny, respectively). The interaction for RNA-to-protein ratio resulted from a trend toward a decrease in the ratio in PF and R progeny fed 110 mg thyroprotein/kg of diet (21.5 vs 24.4 and 20.6 vs 24.0 for 110 and 0 ppm thyroprotein in PF and R progeny, respectively) but a similar ratio in C progeny fed 110 mg thyroprotein/kg of diet compared with those fed no thyroprotein. There was a significant difference ($P < 0.03$) between replicates in all traits except DNA concentration and DNA ratio. The replicate effect may have been related to litter, since replicates were formed on the basis of birth data (approximately 2 weeks difference in average age of pigs in the two replicates).

Discussion. The results reject the hypothesis that maternal protein or energy restriction during pregnancy in the pig has permanent effects in cerebrum protein, RNA, and DNA content of the progeny. Although body weight of progeny was reduced by severe protein restriction of the dam throughout pregnancy, in agreement with previous observations (19, 25, 26), the data on cerebrum weight and protein and nucleic acid concentrations indicate that brain development, as measured by these biochemical parameters, was protected from adverse effects of prenatal protein restriction. The ability of the fetal pig to survive essentially complete maternal protein deprivation has been documented (25, 26). The consequences for normal subsequent development are less certain, although there is clear evidence (27) available for an association between learning behavior and early postweaning severe protein restriction of pigs.

The reduced height and weight of large numbers of children in Third World countries is well documented (9, 28, 29). The effects of early protein deficiency are clearly related to changes in the endocrine system (26, 30–38), but the relative importance of interactions among maternal diet, maternal endocrine changes, and endocrine changes associated with fetal growth and subsequent development is unclear. The persistently elevated plasma somatotropin postnatally in progeny of protein-restricted animals despite retarded weight gain suggests a deficit in growth hormone receptors or a decrease in synthesis of somatomedins in response to somatotropin stimulation (34). The results of the present experiment provide evidence that the weight of the cerebrum and its protein, RNA, and DNA contents are in some way

protected from the stunting effect produced on other tissues by maternal protein restriction in the pig. Although such quantifiable relationships are difficult to determine in humans, this information in the pig, whose nervous system ontogeny resembles that of the human more closely than that of the rat (14, 39), provides the possibility for developing inferences on the effects of protein malnutrition on human body and brain development. Dobbing and Sands (40) and Dobbing (41) emphasized the importance of critical interspecies extrapolation of data relating to the developing brain and other tissues.

The failure of exogenous thyroprotein fed to progeny of protein-deprived dams to overcome growth depression in the present experiment suggests that thyroid function is not directly involved in the observed reduction in growth rate, despite the fact that depressed serum thyroxin concentration at birth and persisting to at least 12 weeks of age has been observed in progeny of pigs deprived of protein during pregnancy (33). It has been suggested (36) that thyroid hormones may play a greater role in the regulation of somatotropin secretion than somatomedins during malnutrition. Whether the absence of a beneficial effect of dietary thyroprotein on body weight gain of progeny of protein-deprived dams was related to inappropriate timing (fed from 10 to 16 weeks of age), to reduced tissue responsiveness to thyroxin, or to a fundamental defect in development unrelated to thyroid function is unknown.

Our data confirm in pigs the suggestion by Hsueh *et al.* (42) based on rat data that maternal protein restriction is of more importance than restriction in energy, vitamins, and minerals in producing depressed weight gain of progeny. There appears to be no published data available indicating clearly the relative importance of protein restriction versus energy restriction in humans during pregnancy on long-term brain development of the progeny. The degree of feed and nonprotein calorie restriction imposed in this study, i.e., intake of one-third of National Research Council recommended levels (43) during the first two-thirds of pregnancy, however, is not directly comparable to the imposition of the essentially protein-free diet fed throughout gestation. Further effort is needed to quantify the degree of feed or energy or protein restriction required to cause reduced postweaning development in brain and other tissues of animals.

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