A Low-Protein Isocaloric Diet During Gestation Affects Brain Development and Alters Permanently Cerebral Cortex Blood Vessels in Rat Offspring

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ABSTRACT In humans, low birth weight is associated with nonfatal stroke, cardiovascular disease and diabetes at adulthood. The aim of this study was to investigate in rats the effect of early protein restriction, inducing low birth weight, on brain and endocrine pancreas vascularization at birth and to study if such alterations lasted until adulthood. Pregnant rats were fed either 20 or 8% protein isocaloric diets. Control newborns were nursed by their dams fed the 20% protein diet and low protein (LP) pups by dams fed either the 8 or 20% protein diet. The diets given during lactation were maintained until adulthood. The blood vessel density of cerebral cortex analyzed by morphometry in 3-d-old pups from dams fed the 8% protein diet was lower than in control (C). It remained lower at adulthood whether a LP or a C diet was given postnatally. Reduction of vascularization at adulthood induced by the LP diet limited to fetal life seems characteristic for the brain since vascularization of islets of Langerhans was reduced in neonates but normalized at adulthood by a C diet postnatally. Body and brain weights were lower in LP pups and adults. DNA concentration was lower in forebrain and higher in cerebellum in LP pups. In brain of LP adults, DNA, protein, cholesterol and phospholipid concentrations were lower and were restored at adulthood by a normal diet after birth. In conclusion, cerebral cortex of offspring exposed to a LP isocaloric diet during fetal development showed reduced vascularization which remained throughout life. J. Nutr. 129: 1613–1619, 1999.

KEY WORDS: • *protein-malnutrition* • *vascularization* • *cerebral cortex* • *brain composition* • *rats*

Cardiovascular disease is more frequent in men who were small for gestational age and below normal weight at 1 yr of age (Barker et al. 1993). Nonfatal stroke is also more frequent in adult women who were small for gestational age (Rich-Edwards et al. 1997). Diabetes and also hypertension are more likely to develop at adulthood in people who had a low birth weight (Hales and Barker 1992). Thus, chronic degenerative diseases such as vascular disease, diabetes and hypertension might originate in derangements affecting fetal development (Goldberg and Prentice 1994). In rats, a low birth weight may be induced in pups when dams are fed by an isocaloric low protein (LP) diet during pregnancy (Snoeck et al. 1990). The offspring may retain lasting anatomical and functional defects at adulthood in different organs. The islets of Langerhans of pups born to LP diet-fed dams are affected in their morphology and function (Snoeck et al. 1990) with consequences during adulthood (Dahri et al. 1991, Dahri et al. 1995). Moreover, these alterations at birth are associated with a deficient vascularization of the islets (Snoeck et al. 1990). Likewise, other studies showed that the number of renal glomeruli is reduced in these pups (Merlet-Benichou et al. 1994) and remains low when a normal diet is given after birth. Anatomical and physiological variables in the brain of this offspring are also altered when a LP diet is given to the dams before and during pregnancy. The number of dendrites is reduced. The sensory cortico-cortical- and thalamo-cortical-evoked potentials are lower, and the brain tissue has elevated biogenic amines with modification in tryptophan metabolism (Resnick et al. 1979). Vascular bed density of the brain is influenced by metabolic requirements, at least in normal adult rats (Bar 1980). Hence, the LP diet modifies the metabolic environment of the fetus and leads to alterations of cerebral variables. The effect of the LP diet on brain vascularization of the newborn and 30-d-old pups has already been investigated (Conradi et al. 1979), but its putative lasting consequences at adulthood were not investigated and require further exploration.

The aim of this study was therefore to explore the vascularization and composition of the neonatal brain when an isocaloric LP diet is given to the dams during pregnancy as well as to evaluate the capacity of recovery when a normal diet is given postnatally. To that end, morphological measurements of blood vessel density were performed on neonatal and adult brain sections from animals submitted to LP diet during fetal life only or until adulthood. To further assess the specificity of sensitivity of the brain vascularization to the LP intake, a comparative study was designed on the endocrine pancreas and duodenum vascularization.

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0022-3166/99 $3.00
Manuscript received 12 May 1998. Initial review completed 30 July 1998. Revision accepted 18 May 1999.

TABLE 1

Composition of the diets

	Control	Low protein
	g/kg diet	
Standard Vitamin Mix[1]	10	10
Standard Trace Element Mix[1]	10	10
KH_2PO_4	7	7
KCl	7	7
Dicalcium phosphate	6.5	11.5
Calcium carbonate	15	11
$MgSO_4.7H_2O$	4	4
MgO	2	2
NaCl (iodized)	3	3
Choline chloride (50%)	4	4
Casein (88% protein)	220	90
Dl-methionine	2	0.8
Corn starch	80	80
Cellulose	50	50
Soybean oil/safflower oil (1:1)	43	43
Glucose	536.5	666.7

[1] Hope Farms (Woerden, The Netherlands).

MATERIALS AND METHODS

Animals

Virgin female Wistar rats, 110 d old weighing 200 g belonging to a local stock bred at the Animal Center of the University of Louvain, Belgium, were caged with males overnight, and pregnancy was determined by inspection of vaginal smears. Pregnant rats were kept in individual cages, at 24°C and 60% humidity, under controlled dark-light cycle (10 and 14 h). The rats were given free access to their respective diets and to water. They were divided randomly into two groups from d 1 of gestation until parturition: one control (C)[2] group was provided with a 20% protein diet, and the second LP group with an 8% protein diet. Both diets were purchased from Hope Farms (Woerden, The Netherlands). The diets were similar in fat content and were rendered isocaloric by the addition of carbohydrates to the LP diet (**Table 1**). The food intake was similar during gestation in both groups of rats as in our previous study (Snoeck et al. 1990) and was slightly lower in the LP adults as also reported previously (Dahri et al. 1995).

At birth, all litters in both diet groups were culled to 8 pups with a similar number of males and females per treatment and a third recuperation (R) group was formed with pups born to dams fed an 8% protein diet which were cross-fostered to lactating dams that consumed the 20% protein diet throughout the pregnancy and lactation. Pups from these three groups were weaned at 27 d and then fed either the 8% protein diet (LP) or the 20% protein diet (C and R) until the age of 110 d, when the measurements were made.

At either 3 or 110 d of age, rats were weighed and killed by decapitation. The whole brain was quickly removed and dissected into forebrain and cerebellum. The weights were recorded separately. The tissues were then immediately homogenized in 5 mL of phosphate-buffered saline with Ultra-Turrax for 2–3 min and stored as 3 aliquots at −20°C before analysis.

The institution's guide for the care and use of laboratory animals was followed, and all procedures were performed with approval of the animal ethics committees of the Université Catholique de Louvain, Belgium.

[2] Abbreviations used: C, control; DAPI, 4′,6-diamidino-2-phenylindole-2HCl; IGF, insulin-like growth factor; LP, low protein; PEG, polyethylene glycol; R, recuperation.

Analytical methods

Proteins in the samples were determined by the method of Bradford. The DNA assay of Kapuscinski and Skoczylas (1977) was slightly modified. The 500 μL of homogenized samples were transferred into tubes and adjusted to 2.5 mL with buffer to which 500 μL of a DAPI (4′, 6-diamidino-2-phenylindole-2-HCl) solution (125 μg/L; Serva Feinbiochemica, Heidelberg, Germany) were added. Fluorescence was measured in a Kontron fluorimeter (Zürich, Switzerland), using calf thymus DNA (Boehringer, Mannheim, Germany) up to 150 μg/L as standard. The excitation and emission wave lengths were 370 nm and 460 nm, respectively. Cholesterol and phospholipids were measured enzymatically (Boehringer), using, respectively, the methods of Kattermann et al. (1984) and Takayama et al. (1977).

Morphometrical analysis

Cerebral cortex. The cerebral cortex vascularization from 3-d-old pups or adult female rats (90-d-old) was studied on thick sections. Animals were killed under ether anesthesia and perfused intra-aortically with a nuclear track emulsion (L-4 Ilford) according to the method of de Paermentier et al. (1986). Three-day-old rats were perfused first with 3 mL of a washing solution (8 g/L of NaCl, 1 g/L of procaine hydrochloride and 1 g/L of glucose) and 5 mL of the diluted (1:4) emulsion at 37°C, at the rate of 30 mL/h. Adult female rats were perfused with 30 mL of a washing solution and 50 mL of the diluted emulsion. The forebrain which included telencephalon, diencephalon and mesencephalon was dissected, fixed overnight in 40 g/L paraformaldehyde in 0.1 mol/L of phosphate buffer, at pH 7.4. After dehydration, the forebrain was cut into three blocks (anterior, median and posterior) and embedded in polyethylene glycol 1000 (PEG 1000; Acros Organics, Geel, Belgium). A 30-μm thick section was cut transversally in each block. The sections were photographically developed for 5 min at room temperature in D19 developer (Kodak, Chalon-sur-Saone, France). After a rapid rinse in distilled water, the sections were fixed for 5 min in sodium thiosulfate (300 g/L) (Janssen Chemica, Beerse, Belgium), then rinsed again in distilled water, stained with light green (1 g/L in distilled water), dehydrated, mounted and studied microscopically.

To validate the technique of emulsion perfusion, the vascularization of adult female rats was also studied on thin sections. Anesthetized rats were killed by an intra-aortic perfusion of fixative mixture (paraformaldehyde 40 g/L, lysine 75 mmol/L, and periodate 20 mmol/L. The fixation of the brain was continued for 4 h in the same mixture, followed by dehydration and embedding in PEG 1000. Section of 6 μm was stained with a solution of ammonium oxalate 20 g/L, Arsenazo III 10 g/L (Sigma, Bornem, Belgium).

The length and perimeter of the blood vessels in the cerebral cortex were measured, respectively, on thick and thin sections using an electronic planimeter (MOP Digiplan, Kontron, Germany) and reported to the cortex area measured, giving a blood vessel density. Four fields per section (two fields closed to the plane of symmetry and two other fields lateral) were selected on the three sections. Each field contained 160 mm^2. All vessels, including terminal and lateral branches, were measured in each field and values were summed.

Duodenum. Since the analysis of the perfused of emulsion gave inconsistent results in the neonates, measurements were only calculated in adult animals. Three 20-μm sections and four fields per section were analyzed per animal using the method described above for brain.

Endocrine pancreas. Pancreas of the neonates and adult rats were removed. Pieces of pancreas were fixed for 2 h in glutaraldehyde (25 g/L) in 0.1 mol/L of phosphate buffer pH 7.2, rinsed and fixed in osmium tetroxide (10 g/L) in phosphate buffer for 1 h. The samples were washed in phosphate buffer, dehydrated in ethanol and embedded in epon. Three sections (1 μm) were taken at random from each sample of pancreas. After staining with toluidine blue, blood vessel volume density in the islets was calculated as the ratio of intrainsular blood vessel area to islet tissue and reported as percentage.

TABLE 2

Body and brain weight in 3-d-old rats and adult rats in relation to the protein concentration of maternal and postnatal diets[1,2]

	3-d-old rats		Adult rats		
	Control	Low protein	Control	Low protein	Recuperation
n	11	9	55	55	46
Body, *g*	9.36 ± 0.15	7.11 ± 0.20*	274 ± 8	202 ± 5*	271 ± 8
Brain, *mg*	364 ± 12	316 ± 16*	1611 ± 20	1511 ± 17**	1602 ± 20
mg/g BW[3]	39 ± 1.5	45 ± 2.3*	6.06 ± 0.14	7.65 ± 0.17**	6.19 ± 0.19
Forebrain, *mg*	271 ± 13	265 ± 17	1295 ± 14	1230 ± 12*	1270 ± 13
mg/g BW	29.1 ± 1.5	37.3 ± 2.2**	4.9 ± 0.1	6.3 ± 0.1**	4.9 ± 0.1
Cerebellum, *mg*	94 ± 0.4	45 ± 1.7**	319 ± 13	283 ± 11*	340 ± 14
mg/g BW	10 ± 0.4	6.3 ± 0.8**	1.2 ± 0.1	1.4 ± 0.1*	1.3 ± 0.1

[1] Values represent means ± SEM.
[2] *$P < 0.05$, **$P < 0.01$ indicate difference from control values.
[3] BW, body weight.

Statistical analysis

Data were analyzed by one-way ANOVA including the Bartlett test for homogeneity of variance, followed by Scheffé's tests. Differences were considered significant when $P < 0.05$. Values presented are means ± SEM.

RESULTS

Body and brain weight. LP offspring had lower body weight (−23%, $P < 0.01$) at 3 d of age compared to C (**Table 2**). The reduction persisted until adulthood when malnutrition was prolonged postnatally. In R adult rats, body weight was not different from that of C rats.

In LP neonates, the total brain weight was lower ($P < 0.05$) than in C. However, it was significantly higher when expressed as a proportion to the body weight ($P < 0.05$). Interestingly, the weight of the cerebellum was half that of the C, whereas the forebrain weight was not affected. When expressed as a proportion of body weight, the weight of the forebrain was higher (28%), and that of the cerebellum remained lower (37%) then the C. In adult LP rats, total brain weight was significantly lower than in C rats, but expressed as a proportion of the body weight it was significantly higher ($P < 0.01$). Absolute weight of forebrain and cerebellum was slightly but significantly diminished, by 5 and 11% respectively, compared to C rats. When expressed per g body weight they were 28% and 16% higher, respectively. No significant differences were observed between C and R rats.

DNA, protein and lipid concentration

In 3-d-old LP pups, DNA concentration was 43% lower in the forebrain ($P < 0.05$) and 50% higher in the cerebellum, compared to C pups (**Table 3**). Protein concentration was not different from C in either part of the brain. In adult LP rats, DNA, protein and cholesterol concentrations were significantly lower in the forebrain and the cerebellum, but phospholipids were lower only in the cerebellum. The normal diet given after birth in the R group restored to normal the DNA, protein, cholesterol and phospholipid concentrations in both parts of the brain.

TABLE 3

DNA, protein and lipid concentrations in 3-d-old rats and adult rats in relation to the protein concentration of maternal and postnatal diets[1,2]

	3-d-old rats		Adult rats		
	Control	Low protein	Control	Low protein	Recuperation
n	11	9	55	55	46
Forebrain					
DNA, *mg/g*	2.70 ± 0.37	1.53 ± 0.33*	3.01 ± 0.11	2.77 ± 0.07*	3.04 ± 0.1
Protein, *mg/g*	120 ± 17	92 ± 17	289 ± 19	210 ± 6.4***	299 ± 16
Cholesterol, *μmol/g*	ND	ND	58 ± 2.2	50 ± 1.9**	58 ± 2.7
Phospholipid, *μmol/g*	ND	ND	2.87 ± 0.11	2.64 ± 0.11	2.99 ± 0.11
Cerebellum					
DNA, *mg/g*	2.19 ± 0.26	3.29 ± 0.24*	13.9 ± 0.84	11.74 ± 0.38*	12.36 ± 0.86
Protein, *mg/g*	99 ± 15	112 ± 15	494 ± 17	380 ± 18***	443 ± 20
Cholesterol, *μmol/g*	ND	ND	105 ± 3.2	84 ± 2.5***	102 ± 1.4
Phospholipid, *μmol/g*	ND	ND	5.46 ± 0.15	4.63 ± 0.34***	5.52 ± 0.16

[1] Values represent means ± SEM for *n* rats per group.
[2] *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ indicate difference from control values, ND, not determined.

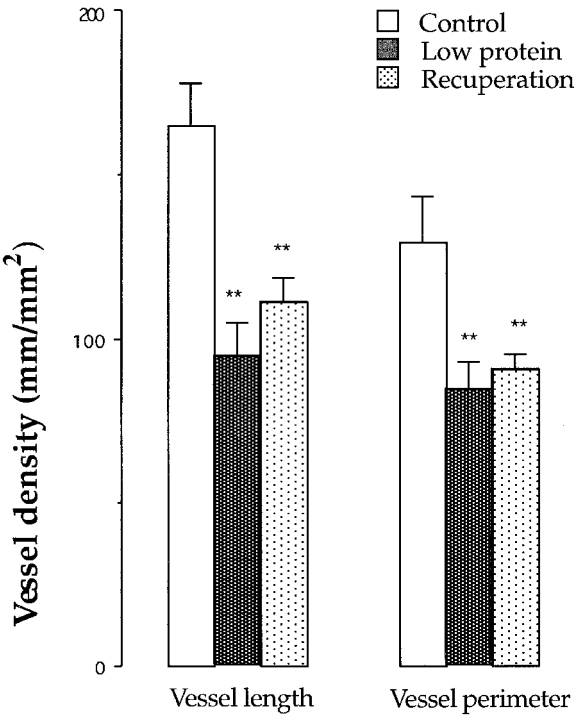

FIGURE 1 Morphometrical quantification of the vascularization in cerebral cortex of adult female rats in the control, low protein and recuperation groups. The vessel lengths were determined on thick sections after a perfusion of a photographic emulsion; the perimeters of the vessel profile were traced on thin sections and measured using an electronic planimeter in four selected fields containing 160 mm^2 per section on three different sections. The values are means ± SEM, $n = 7$. **: indicates significant difference ($P < 0.001$) from respective control values.

Vascularization. The vascularization of the cerebral cortex in 3-d-old pups born from dams fed a LP diet was analyzed by measuring the length of blood vessel profiles on thick sections. The density reached 54.8 ± 5.4 mm/mm^2 in C pups but 72% lower, 15.2 ± 2.6 mm/mm^2, in LP pups ($n = 7$/group, $P < 0.01$). In adult rats, the density of the blood vessel profiles estimated by either length or perimeter (see the Materials and Methods section) was respectively reduced relative to C by about 35% ($P < 0.05$) and 43% ($P < 0.001$) in the LP group and by 30% (<0.05) and 33% ($P < 0.05$) in the R group (Figs. 1 and 2).

In both the head and tail of the endocrine pancreas from LP newborns, the blood vessel volume density in the islets of Langerhans was significantly ($P < 0.001$) lower in LP than in C pups (**Table 4**). Blood vessel density was significantly lower in the islets of LP adult rats ($P < 0.01$) than C and R rat islets, which did not differ from one another.

In the duodenum from adult LP rats, blood vessel density of the duodenum was significantly lower ($P < 0.05$) than that of the C and the R groups. No differences were found between C and R rats (Fig. 3).

TABLE 4

Blood vessel volume density in the endocrine pancreas of newborn rats and adult rats in relation to the protein concentration of maternal and postnatal diets[1,2]

	Control	Low protein	Recuperation
		%	
Newborn			
Pancreatic head	7.24 ± 1.12 (7)	2.93 ± 0.98** (9)	—
Pancreatic tail	7.47 ± 0.47 (6)	2.79 ± 0.34** (9)	—
Adult	3.34 ± 0.28 (4)	1.96 ± 0.11* (4)	3.41 ± 0.37 (4)

[1] The values represent means ± SEM (n).
[2] *$P < 0.01$, **$P < 0.001$ indicates significant difference from control values.

DISCUSSION

The aim of this study was to quantify the impact of LP exposure during fetal life on brain vascularization at birth and

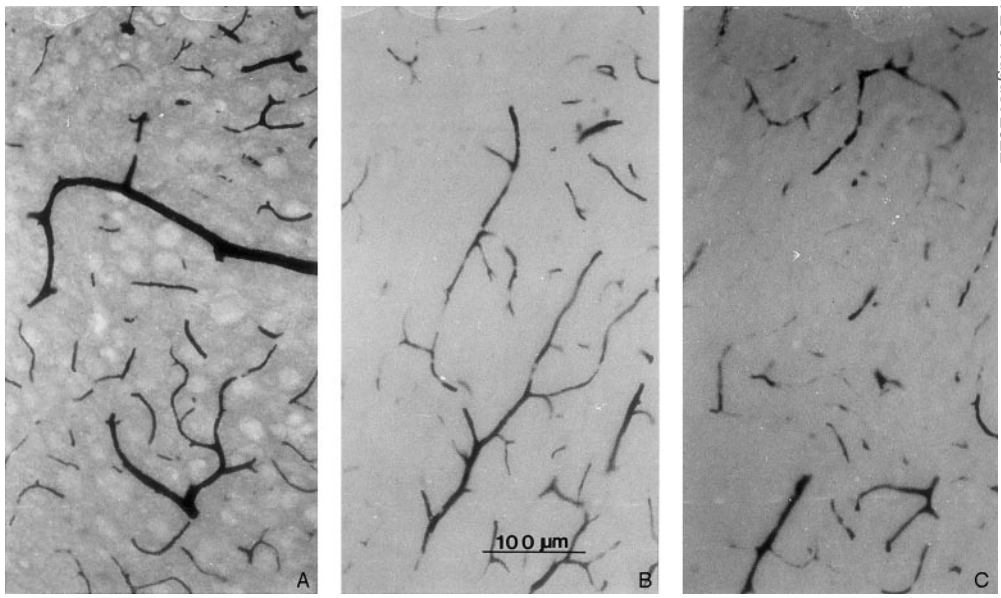

FIGURE 2 Photomicrographs showing the vascularization in frontal thick section (30 μm) of cerebral cortex of 90-d-old rats (see the Materials and Methods section). Control (A), recuperation (B) and low protein (C).

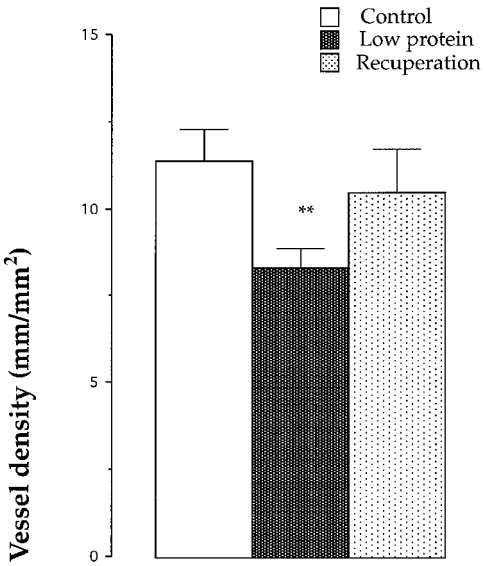

FIGURE 3 Morphometrical quantification of the vascularization in duodenum of adult female rats in the control, low protein and recuperation groups. The vessel lengths were calculated on thick sections after a perfusion of a photographic emulsion, measured using an electronic planimeter in four selected fields amounting to 160 mm^2 per section. The values are means ± SEM, $n = 7$ indicates significant difference ($P < 0.001$) from control value.

its consequences at adulthood. The results show that the development of the fetal brain vascularization is hindered by a poor intake of proteins by dams during pregnancy although the diets were isocaloric. At adulthood, the brain vascularization remains less dense when the LP diet was maintained, but the impaired brain vascularization persists also even when a normal protein diet was given from birth. Therefore, the brain vessels that were disturbed early in development do not recover in the offspring born from dams fed a poor protein diet during pregnancy. In line with this finding, a LP diet given 2 wk before mating, during gestation and lactation was reported to reduce the length of thin neocortical blood vessels in the offspring at 30 d (Conradi et al. 1979). In another experiment in which pups were underfed after birth, fewer capillary profiles were also found per unit area section in the cortex of 30-d-old pups; however, the fraction of section area occupied by capillary lumen was normal due to a compensatory increase in mean cross-sectional area (Peeling and Smart 1994b). In our experiments, we found also a decrease in length density of the vessels on section area which would reflect a reduced number of vessel profiles. Possibly the thinnest capillaries may be weakly perfused by the emulsion marker, thereby hampering their identification. However, in comparing the C with experimental group, a change in vascularization due to the LP diet is observed. In addition, the vascular quantifications in rats, by use of two different techniques, revealed the same difference between the groups, validating once more the methodology used. To our knowledge, it is the first time that the recovery of brain blood vessel alterations by a normal diet given immediately after birth to LP newborns has been investigated. It is worth noting that other anatomical and physiological changes in developing brain are graded according to the quantity of protein intake (Resnick et al. 1982) and are not restored with a normal protein diet postnatally (Resnick et al. 1979, Stern et al. 1984). These perturbations are associated with behavioral changes. It is unclear if the alterations in the vascular system are the cause or the consequence of the quantitative biochemical modifications in the brain tissue, or if the observed changes in the blood vessels and the neural tissue are independent. The latter may be more likely since in our study, the forebrain weight and composition recovered with a normal diet after birth but the blood vessels did not. In the 3-d-old LP pups and the adult R rats, we observed a reduced vascular density in the presence of a normal weight of the forebrain. A normal growth of neural tissue may then occur despite such an alteration in vascularization, indicating that the density of the vascular bed might not be a critical limitation in terms of growth. Blood perfusion to the brain may be normal, notwithstanding lessened vascular density, and could account for the maintenance of normal brain weight. We have not measured the blood flow in the brain. Although, in the endocrine pancreas of our LP adult rats, where the blood vessel density was lower, we previously showed that blood flow was lower in basal state, but reached the level observed in C rats when stimulated with glucose. In the duodenum, where the blood vessel density was also lower, the basal blood flow was normal (Iglésias-Barreira et al. 1996). These observations show that a reduced vascular density does not always lead to lower tissue perfusion.

The total brain weight was lower in the LP pups and LP adults, but, relative to body weight, was significantly higher. Therefore, the brain is less affected than the body, consistent with a study in LP neonates (Resnick et al. 1982) and in underfed pups at 30 d (Peeling et Smart 1994a). This may be due to the direction of nutrients to the brain at the detriment of other organs. The newborns which have a low body weight as in our study (Snoeck et al. 1990) have a normal absolute forebrain weight. Although DNA concentration was lower in the forebrain, protein concentration was in the normal range in 3-d-old LP pups, in agreement with Prasard (1991). Both parameters as well as cholesterol and phospholipid levels remained low in the adult brain when the LP diet was maintained postnatally but normalized when a normal diet was given immediately after birth. Similar observations of recovery after LP diet during fetal life only have been described in rats at 13 d (Shambaugh et al. 1995). Cerebellum weight, in contrast to the forebrain weight, was lower in 3-d-old LP pups. The greater magnitude of change in the cerebellum is a reflection of its different growth rate rather than a selective sensitivity of this organ to malnutrition (Peeling and Smart 1991, Shambaugh et al. 1995). The normalization of the weight of the cerebellum at adulthood by a normal diet given immediately after birth confirms earlier results (Winick et al. 1968). However, if the LP diet is more severe during fetal life (6%), the brain weight remains lower at 21 d of age despite a normal diet after birth (Resnick et al. 1982) which confirms the graded effect of the LP content in the diet during gestation.

The development of blood vessels during the fetal growth phase may be affected by the hormonal changes induced by the LP diet. Insulin is reduced in the pancreas of fetuses from rat dams fed the LP diet (Snoeck et al. 1990) and is thought to be a growth factor for endothelial cells in particular microvessels (Crow et al. 1994). Plasma insulin-like growth factor (IGF) levels are lower in the LP fetus (Muaku et al. 1995, Shambaugh et al. 1995) and IGF stimulate proliferation of endothelial cells and blood vessel formation (Davies and Hagen 1993). A LP diet was also shown to reduce the activity of the placental enzyme 11β-hydroxysteroid dehydrogenase (Langley-Evans 1994), leading to an increased placental passage of glucocorticoids to the fetus. Glucocorticoids are potent inhib-

itors of angiogenesis (Crum et al. 1985). Decreases in nutrient availability, such as of amino acids, may also play a role in the reduction of vascularization. Indeed high doses of L-amino acids added or not to glutamine favor long-term growth of endothelial cells (Gorman et al. 1996). Moreover, protein deficiency was demonstrated to have an etiological role in the induction of both vascular and cardiac changes in subadult monkeys (Sandhyamani 1992). Notably essential amino acids as well as taurine and alpha aminobutyric acid plasma levels are lowered in our LP fetuses (Reusens et al. 1995). The precise role of amino acids on endothelial cell growth is not well-known, but increased homocysteine plasma levels in men prior to their cardiovascular disease suggested their implication in the disease (Nygard et al. 1997).

The most important finding of this study is the dramatic reduction of brain vascularization, which does not recover with a normal diet postnatally. In contrast, the vascularization of the endocrine pancreas, which was also affected at birth was restored at adulthood when a normal protein diet was given from birth onward. Although the vascularization of the neonatal duodenum is altered when the dams have metabolic alterations such as diabetes (Reusens-Billen et al. 1989), the duodenum showed normal vascularization when a normal diet was given until adulthood after exposure to a LP diet. The former observations indicate the possible recovery of the vascular changes, especially in organs with a higher developmental plasticity, like the endocrine pancreas and the digestive tract. In those organs, vascularization was, however, most impaired at adulthood when the rats were fed a LP diet throughout life. Moreover, this impairment of the vascularization was demonstrated to have functional consequences in these organs. Basal blood flow was shown to be significantly decreased in islets as well as in exocrine pancreas but was normal in duodenum, colon and kidney in adult rats chronically exposed to 8% protein since early fetal development. Those functional defects were corrected at adulthood when LP pups were given a normal protein diet postnatally (Iglesias-Barreira et al. 1996). Notably that blood pressure was normal in the three experimental groups (unpublished data, Iglesias-Barreira and Reusens).

To conclude, protein deficiency during gestation reduces the development of the cerebral cortex vascularization which does not recover with a normal diet after birth. The biochemical factors involved in this alteration must be specified as well as the increased vulnerability at adulthood to aggressive situations like hypoxia-, hyper- or hypoglycemia. The permanent effect of fetal protein malnutrition on blood vessels' development irrespective of postnatal nutrition seems to be specific for the brain. Therefore the vascular bed of each organ appears to have its own sensitivity to a LP diet during development. Further experimental investigations on blood vessel development are required to understand the specific consequences of chronic protein undernutrition on pathological vascular diseases later in life, especially in socio-economically unprivileged populations.

Epidemiological observations in humans who have had poor fetal growth as indicated by a low birth weight and poor infant growth have a higher incidence of stroke, cardiovascular disease and type 2 diabetes later in life (Barker et al. 1993, Hales and Barker 1992, Rich-Edwards et al. 1997). Our present experimental data on cerebral vessels support such former findings.

ACKNOWLEDGMENT

We are grateful to Bernadette Bréant for her critical advice, to Marie-Thérèse Ahn for skillful technical assistance and to Veronique Guns for expert secretarial help.

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