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Nutritional influences on pancreatic development and potential links with non-insulin-dependent diabetes

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‘Whosoever was the father of a disease, an ill diet was the mother’

(George Herbert, 1660)

‘But more so with a poor diet during pregnancy’

(J. J. H. Manchester, 1993)

Diabetes is widespread throughout the world and affects young children as well as adults, male and female alike. The prevalence of diabetes may be 3–5% in the Western hemisphere but may reach 50% in specific populations like the Nauruans or the Pima Indians and 15–20% in Hispanics and Afro-Americans. It is associated with major complications such as blindness, renal insufficiency and cardiovascular diseases including hypertension and myocardial infarction. Diabetes afflicts the less privileged and deprived in a society (King & Rewers, 1993). Immigrants and specific populations such as Ethiopian Jews immigrating to Israel also have a high prevalence (Cohen *et al.* 1988). The disease and its complications run in families, often on the mother’s side (Liao *et al.* 1993; Mitchell *et al.* 1993). Impaired glucose tolerance is strikingly more common in women than men. Between 21 and 39 years of age, 11–20% of many populations worldwide may be afflicted by impaired glucose tolerance. In some populations, such as female Muslim Asian Indians it may reach 32% (King & Rewers, 1993). Population studies have indicated that progeny weighing less than 2.5 kg at birth and 8 kg at 1 year are afflicted with diabetes and cardiovascular diseases in more than 45% of the individuals reaching the age of 64 years (Hales *et al.* 1991). Recent data indicate also that young children born in underprivileged areas where low birth weight is endemic have high cholesterol and blood sugar levels, as well as abnormal insulin levels and hypertension (King *et al.* 1990). These abnormal biological features are not an acquisition of adult age because they may be present already at 3 years of age (The Bogalusa Heart Study, 1987). The yardstick frequently used to evaluate mothers’ health and its influence on the neonate is to record birthweight and other fetal variables which have been correlated with later degenerative diseases in the progeny (Barker, 1992). The foregoing implies that the metabolic and vascular anomalies leading to chronic ailments such as diabetes, hypertension and cardiovascular diseases originate early in life and are initiated, in all likelihood, *in utero*. The issue remains why a greater prevalence of

diabetes mellitus type 2 and of impaired glucose tolerance exist in developing countries or less-privileged communities and why their transmission seems to be more prevalent in females than in males. What kind of exposure does the maternal–fetal unit need in order to programme diabetes and its complications in the offspring?

Biological events in the mother such as poor early nutrition transmit signals in the intra-uterine milieu which affect the programme of cell differentiation and cell growth and alter functions of enzymic systems. The issue of the effect of previous scarcity of food and/or protein during pregnancy in this programming should be raised. Normal nutrition or overfeeding in postnatal life may keep deleterious metabolic effects in the progeny because of faulty early development.

We have focused experimentally on this issue by feeding pregnant rats on a diet which was low in protein (80 g/kg) but adequate in energy, and verified the adaptation of the progeny throughout life. The lack of adequate protein was chosen because of its continuous or cyclic scarcity or irregular affordability in communities where type 2 diabetes is also most prevalent (King & Rewers, 1993). Chronic malnutrition and deficiency in total amount and quality of dietary protein may alter its metabolism affecting the long-term health status of the individual (Jackson, 1993). Acute and chronic infections may play a role by themselves or by altering the nutrition of the fetus.

The objective of our current analysis is to verify the development of the fetus, in particular the alterations in structure and function of the fetal endocrine pancreas, when the mother is fed on a low-protein–normal-energy diet, as well as the long-term consequences for the progeny and their own offspring. This approach may clarify the global picture of the high prevalence of diabetes as well as its associated vascular lesions which affect all ages and races, and men and women but to differing extents.

EXPERIMENTAL APPROACH

The low-protein–normal-energy diet during pregnancy: consequences for the mother and the offspring

The mother and her weight gain. When pregnant rats were fed on the low-protein–normal-energy diet (LP group, 80 g protein/kg) from the first day until the end of gestation, the daily body-weight gain remained similar to that of pregnant rats fed on the control diet (C group, 200 g protein/kg) until day 19.5 of gestation, but was lower at parturition. The final total body-weight gain was significantly decreased ($P < 0.001$) in the LP pregnant rats (Table 1). A similar diet given during an equivalent period of 22 d to non-pregnant rats of comparable age did not affect the body-weight gain. The major impact of such a diet on the weight gain during lactation, also, was apparent. The lactating mothers deprived of protein had no weight gain, in contrast to the control lactating rats, during the initial 20 d. In the LP group the total food intake was slightly higher during gestation but lower during lactation. The insulin content of the maternal endocrine pancreas in relation to the total body or total pancreas weight was reduced significantly by 50% at 21.5 d of gestation in the LP group compared with the controls. In non-pregnant adult females receiving a low-protein diet for 22 d, the insulin content of the endocrine pancreas was similar to that of controls (A. Snoeck, C. Remacle and J. J. Hoet, unpublished results). The lower weight gain in the LP pregnant group could be related to the observed reduction in the insulin content of the endocrine pancreas, although the maternal fasting glucose and insulin levels were similar in both groups. At

Table 1. *Weight gain (g) during an equivalent period of gestation in adult non-pregnant (22 d), pregnant or lactating rats (20 d) in the control (C) and the low-protein (LP) groups†*

(Values are means with their standard errors)

Dietary group . . .	C		LP	
	Mean	SE	Mean	SE
Non-pregnant	32.1	1.8	28.9	2.3
<i>n</i>	33		19	
Pregnant	95.7	3.7	85.4**	1.7
<i>n</i>	29		39	
Lactating	19.3	4.7	-0.1**	3.1
<i>n</i>	12		7	

Mean values were significantly different from those of C group: ** $P < 0.001$.

† C and LP groups received *ad lib.* 200 and 80 g protein/kg diet respectively from the 1st day to the end of gestation and during lactation or for an equivalent period for non-pregnant rats.

21.5 d glucose levels (mg/l) were 634 (SE 29, n 21) and 621 (SE 22, n 20) and the insulin levels (ng/ml) were 1.11 (SE 0.34, n 17) and 0.82 (SE 0.21, n 16) in the C and LP groups respectively.

Histological studies highlighted the enlargement of islet volume due to increased number of β cells and hypertrophy of the individual β cell also. This was associated with an enhancement of the secretory activity of the individual β cell. In pregnant rats with a normal diet the adaptation of the endocrine pancreas to normal pregnancy needs an increase in the total β cell mass (Van Assche *et al.* 1980) which involves a multiplication of β cells and an increase in insulin content as well as in its secretion. With a low-protein diet, the maternal endocrine pancreas seems unable to adapt normally to pregnancy and to provide a normal insulin content in the endocrine pancreas.

The progeny and its growing phase. Progeny were divided into three groups and raised until they reached an adult age (84 d); the C and LP groups received *ad lib.* 200 and 80 g protein/kg diet respectively during fetal life, the suckling period and after weaning until adult; the recuperation group (R) received the low-protein diet only during fetal life and the control diet during the suckling period and after weaning until adult. The pups born from mothers fed on the low-protein diet during gestation weighed less than the controls (Snoeck *et al.* 1990). When nursed by a normal mother they regained normal body weight soon after birth.

The body-weight gains up to 84 d (Fig. 1) were significantly lower in males and females of the LP group than in those of the C and R groups. Feeding the low-protein diet after birth seemed to reduce weight gain more in males than in females. The body-weight gains were similar for the R and C groups. Absolute weekly food intake was lower for the LP group than those for the C and R groups which had similar food intakes. However, when food intake was expressed as a proportion of body weight (relative food intake) male and female LP rats consumed more food up to 70 d. The fasting insulin levels and pancreatic insulin contents at 84 d of the male and female rats of the LP group were reduced by 29 and 39% respectively compared with those of the controls (Table 2).

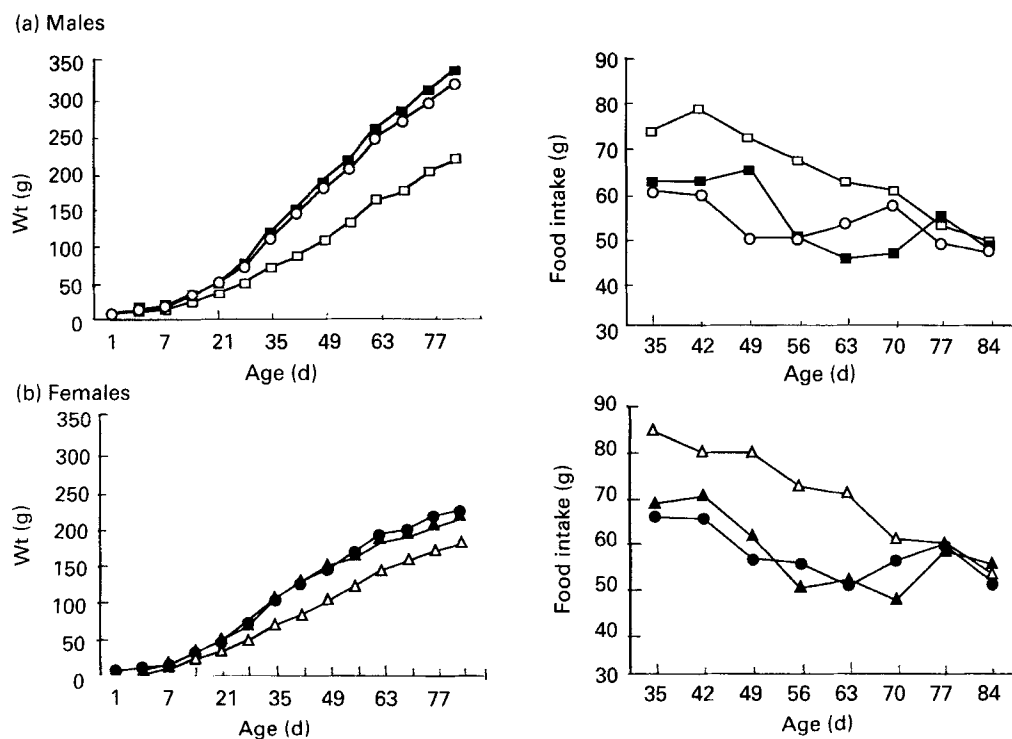


Fig. 1. Body-weight gain (g) and weekly food intake (g) in (a) male (■, □, ○) and (b) female (▲, △, ●) rats in control (C; ■, ▲), low-protein (LP; □, △) and recuperation (R; ○, ●) groups during growing phase. C and LP groups received *ad lib.* 200 and 80 g protein/kg diet respectively during fetal life, the suckling period and after weaning until adult; R group received the low-protein diet only during fetal life and the control diet during the suckling period and after weaning until adult.

Table 2. *Islet size, pancreatic insulin content and fasting plasma glucose and insulin levels in the control (C), low-protein (LP) and recuperation (R) groups of rats at 84 d†*

Dietary group . . .	C		LP		R	
	Mean	SE	Mean	SE	Mean	SE
Islet size (μm^2)	6340	422	8026**	446	8034*	572
No. of islets		425		451		415
Pancreatic insulin content ($\mu\text{g}/\text{kg}$ body wt)	335.2	26	204.3**	19	294.0*	9.9
No. of pancreas		6		7		8
Fasting plasma glucose level (mg/l)	1030	48	1020	51	1060	45
No. of animals		34		37		29
Fasting plasma insulin level (ng/ml)	0.65	0.04	0.46**	0.05	0.66	0.05
No. of animals		30		38		23

Mean values were significantly different from those for C group: * $P < 0.05$, ** $P < 0.001$.

† C and LP groups received *ad lib.* 200 and 80 g protein/kg diet respectively during fetal life, the suckling period and after weaning until adult; R group received the low-protein diet only during fetal life and the control diet during the suckling period and after weaning until adult.

Reductions in the concentrations of insulin, an essential growth-promoting factor, in plasma and in the endocrine pancreas might be responsible for lack of adequate nutritional intake.

Adaptation of the endocrine pancreas of progeny born from mothers fed on a low-protein diet

Changes in the development of rat endocrine pancreas at birth. When the mother was fed on the low-protein diet during gestation, the development of the endocrine pancreas of the progeny was abnormal at birth. Neonatal islet cell proliferation was reduced by 12% when analysed *in vivo* (Snoeck *et al.* 1990). Its reduction was more pronounced in the head than in the tail of the pancreas. The reduction in islet cell proliferation was confirmed by *in vitro* cell culture in which the labelling index (%) for group C after 7 d was 11.9 (SE 0.59, *n* 34) and for the LP group was 5.23 (SE 0.6, *n* 19). The islet size was significantly decreased in the pancreatic head in the LP group (Snoeck *et al.* 1990).

When these fetal islets of the LP group were challenged *in vitro* with secretagogues, such as leucine, arginine and/or theophylline the insulin response was depressed (Dahri *et al.* 1991). When these islets were challenged with glucose with or without amino acids (arginine or leucine), cAMP content was significantly reduced in the LP group compared with the control group (Dahri *et al.* 1994). In addition a major reduction of more than 50% in the vascularization of the endocrine pancreas in the neonates of the LP group (Snoeck *et al.* 1990) was observed in the head and tail of the pancreas. This contrasts with the change in the islet size which occurs essentially in the head of the pancreas. These results indicate that a low-protein diet during gestation transmits signals to the intra-uterine milieu which impairs the normal maturation of two major cell types, the β cell with its growth-promoting hormone (insulin) and the endothelial cell.

Changes in the endocrine pancreas in the adult. When the endocrine pancreas of the progeny at maturity (84 d) was examined differences between the C, LP and R groups were apparent. In the R group where the low-protein diet was limited only to fetal life, the islet size was significantly increased but the pancreatic insulin content was significantly decreased (-12%) and the fasting plasma glucose and insulin levels were normal (Table 2). When these islets were challenged *in vitro* with glucose or leucine, insulin secretion after 120 min was comparable with that of the control islets. On the other hand if these islets were challenged with arginine or arginine plus theophylline, their insulin secretion was significantly depressed compared with the control islets (Dahri *et al.* 1994). When the low-protein diet was maintained after birth until 84 d (LP), islet size was also increased but pancreatic insulin content was dramatically decreased (-39%) compared with the control group. Fasting plasma insulin levels were decreased in the presence of a normal fasting plasma glucose level (Table 2). When the islets of this group were challenged *in vitro* with glucose, amino acids (arginine or leucine), forskolin or 5-tetradecanoyl-13-phorbol acetate, insulin response was significantly depressed compared with control islets (Dahri *et al.* 1994). These findings indicate that alterations observed at birth do remain when a low-protein diet is maintained until adulthood. A normal diet given immediately after birth only partially restores the insulin secretory pattern induced by specific secretagogues *in vitro*.

When rats of the LP group were challenged *in vivo* with oral glucose, an abnormal glucose tolerance with low insulin levels was observed in males as well as in females (Fig. 2). However, in the R group normal glucose tolerance, together with satisfactory insulin

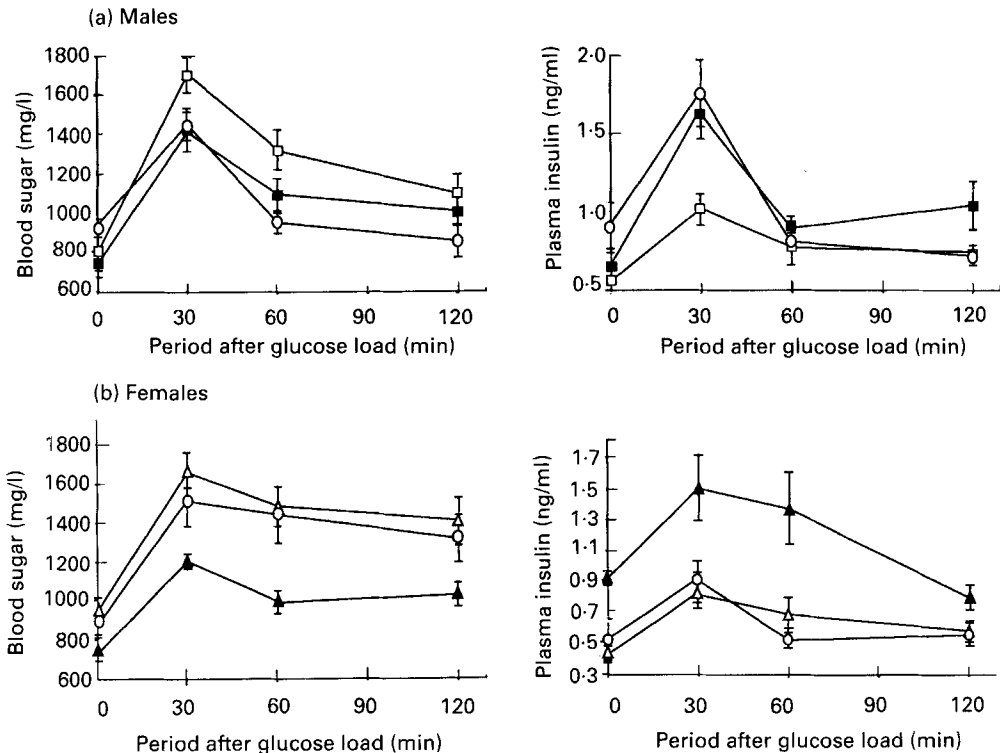


Fig. 2. Glucose tolerance and insulin response 30, 60 and 120 min after oral glucose challenge at 70 d in (a) male and (b) female rats of the control (C; ■, ▲), low-protein (LP; □, △) and recuperation (R; ○) groups. C and LP groups received *ad lib.* 200 and 80 g protein/kg diet respectively during fetal life, the suckling period and after weaning until adult; R group received the low-protein diet only during fetal life and the control diet during the suckling period and after weaning until adult. Points are means with their standard errors represented by vertical bars.

response, was observed only in male adult rats. In contrast, the female rats of the R group showed higher blood glucose levels and lower insulin levels than control animals. Therefore, male animals seem able to recuperate with a normal diet after birth. Female animals, even with a normal diet after birth, seem unable to recover.

Adaptation of the endocrine pancreas to pregnancy. Female rats of the first generation of the three groups were submitted to pregnancy. The glucose tolerance and insulin responses of pregnant rats at 18.5 d of gestation (Fig. 3) show that the basal fasting glucose level was significantly higher in the LP mothers (LPM) and the R mothers (RM) compared with the control mothers (CM). When glucose was administered, plasma glucose levels remained higher in LPM and RM groups at each of the time intervals ($P < 0.05$). The plasma insulin responses of the LPM and RM are inadequate for the glucose challenge. At each time-point, the plasma insulin levels of the LPM and RM were significantly lower ($P < 0.05$) than those of CM. These findings show that a low-protein diet given during fetal life and adult life hinders the adaptation of the endocrine pancreas to pregnancy. This inability leads to abnormal glucose tolerance. When a low-protein diet is limited to fetal life only, some of the sequelae acquired *in utero* in the endocrine pancreas remain at the adult stage and lead to glucose intolerance, especially during pregnancy, such as in gestational diabetes.

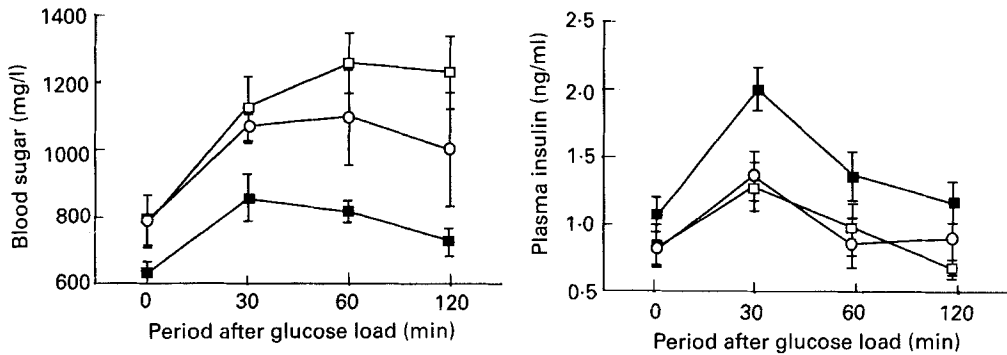


Fig. 3. Glucose tolerance and insulin response 30, 60 and 120 min after oral glucose challenge in pregnant offspring of the control (C; ■), low-protein (LP; □) and recuperation groups (R; ○) at 18.5 d of gestation. C and LP groups received *ad lib.* 200 and 80 g protein/kg diet respectively during fetal life, the suckling period and after weaning until adult; R group received the low-protein diet only during fetal life and the control diet during the suckling period and after weaning until adult. Points are means with their standard errors represented by vertical bars.

Consequences for the second generation

Fetal pancreatic insulin content of the second generation was analysed at 21.5 d of gestation and is reported for the three groups of fetuses, those of mothers fed on the control diet during their fetal life and after birth (CF), those of mothers fed on the low-protein diet during their fetal life and after birth (LPF) and those of mothers fed on the low-protein diet only during fetal life (RF). The relative pancreatic insulin content ($\mu\text{g/g}$ body weight) was significantly higher in the LPF (1.25 (SE 0.09, n 23)) and in the RF (1.11 (SE 0.15, n 14)) than in the CF (0.62 (SE 0.08, n 32); $P < 0.001$). These findings, together with those obtained in the mother, demonstrate that a low-protein diet given only during fetal life or during fetal life and adult life leads to gestational diabetes with its usual consequences for the progeny. The changes in the endocrine pancreas, however, could be different in the LPF rat and the RF rat, as suggested by preliminary findings (S. Dahri, C. Remacle and J. J. Hoet, unpublished results) showing a higher proportion of larger islets in the RF than in the CF and LPF and fewer small sized islets in the RF. Reduced protein availability during development may affect the number of cells able to replicate and may have damaged permanently the capacity of the β cell to enter into the proliferative cycle.

GENERAL PERSPECTIVES

Reduction in food intake during pregnancy may have multiple consequences for the evolution of pregnancy as well as for the early and late development of the progeny in man as well as in animals. The effect of malnutrition limited to protein deficiency only, while allowing an appropriate amount of energy, has been studied seldom during pregnancy. The objective of our current study was to verify the specific role of a low-protein-normal-energy diet during pregnancy on the weight gain of mother and progeny in rats and on specific changes in their endocrine pancreas. Maternal weight gain was affected but only during the last 2 d of pregnancy in our experiments. Various

observations have been reported but experimental conditions have not always been comparable because of differences in pregestational diet, source of proteins used (vegetable or meat origin) or energy content of the diet. In our experiments the food intake was similar in both groups of animals and the source of protein was casein in the experimental and control groups. Lederman & Rosso (1980) observed reduced weight gain by pregnant animals fed on a protein-deficient diet (75 g protein/kg) compared with those fed on a control diet (220 g/kg). In our experiments reduction in body weight during pregnancy could be associated with a diminution of pancreatic insulin content in the LP group. No effect of a low-protein-normal-energy diet on the pancreatic insulin content was observed when given for 22 d to non-pregnant female rats. This demonstrates the role of proteins during normal pregnancy in the hyperplasia and hypertrophy of the endocrine pancreas.

The offspring of mothers fed on a low-protein diet and kept on a similar diet until adults (LP group) gained less weight than controls. However, their food intake per g body weight was greater than that of the controls. Increased intake of food with inadequate protein will not achieve normal weight gain. This diet reduced the weight gain more in males than females, which may be attributed to the more intense growth in males than in females in early adulthood. The weight gain of rats receiving a low-protein-normal-energy diet only during fetal life (R group) was normal in males and females. However, in other experiments undernutrition early in gestation has affected weight gain and adiposity differently in males and females later in life. Male rats born to mothers undernourished in the first 2 weeks of gestation became hyperphagic and obese as adults; female rats did not become obese but both sexes showed abnormal adipocyte development (Jones & Friedman, 1982; Anguita *et al.* 1993). Observations in humans seem to indicate that the early phases of development might be more susceptible to the deleterious effects of famine; its effects differ with sex also. In young men, obesity was more prevalent when they were exposed to famine early in fetal life than later (Ravelli *et al.* 1976). Females exposed to famine *in utero* during the first and second trimesters had offspring with birth weights lower than those of offspring of mothers not exposed to famine. The birth weight of the progeny exposed to famine during the third trimester was reduced while their own offspring had a normal birth weight (Lumey, 1992). The foregoing observations on famine were made in pregnant, previously well-nourished women. In less-privileged societies continuous or cyclic chronic energy deprivation is occurring. Offspring with reduced birth weight are born from mothers who themselves exhibit chronic malnutrition before pregnancy, as assessed by lower final height and weight. Total weight gain during pregnancy may be related to the body-weight gain of the mother herself, the placenta and/or the fetus. The relative distribution of the nutrients between mother, placenta and fetus is partly responsible for the regulation and the phenotypic expression of fetal growth, which is influenced by the development of the fetal endocrine pancreas and its secretion. Some genes are expressed fully during postnatal life when the intra-uterine influences will have disappeared. In chronic malnutrition throughout generations mothers may gain weight while their infants have a low birth weight (Kusin *et al.* 1993). Proper growth-promoting factors such as insulin may be lacking in these infants. It is noteworthy that poor fetal islet cell maturation evident in reductions in β cell numbers, islet size, proliferation capacity or islet insulin content was observed in our experiments with the low-protein diet during pregnancy in rats. Postnatal protein deficiency may also induce a reduction in the neonatal β cell mass

First generation

Fetal stage * Fetal weight is reduced
 * Fetal β cell proliferation is reduced
 * Fetal β cell sensitivity to secretagogue is altered

Adult stage

If a low-protein diet is maintained after birth:

Low-protein group
 Islet cell mass and pancreatic insulin content are reduced
 β cell response to secretagogues is altered
 Basal blood insulin level and insulin response to glucose challenge are reduced \rightarrow glucose intolerance in males and females

If a normal diet is given immediately after birth:

Recuperation group
 Islet cell mass is partially reduced
 β cell response to secretagogues is partially altered
 Glucose intolerance only in females

Gestation

Islet cell mass and pancreatic insulin content are reduced
 Plasma glucose level is increased
 Plasma insulin level is decreased
 Insulin response to oral glucose is depressed \rightarrow glucose intolerance

Pancreatic insulin content is slightly reduced
 Plasma glucose level is increased
 Insulin response to oral glucose is depressed \rightarrow glucose intolerance

Second generation

Fetal stage Plasma glucose and insulin levels are increased
 Pancreatic insulin content is increased
 Changes in islet size distribution

Adult stage Type 2 diabetes and its complications
 More prevalent in females

Fig. 4. Consequences of a low-protein-normal-energy diet (80 g protein/kg) during pregnancy on subsequent generations maintained on the low-protein diet or given a normal-protein diet (200 g protein/kg; control) immediately after birth.

which persists during adult life (Swenne, 1992). The proliferative function of the β cell is altered during the developmental stage and hinders the normal adaptation to requirements of later life events. In addition these observations in animals and humans indicate the extent to which food intake and fat tissue distribution during life are programmed already *in utero* by maternal nutrition during pregnancy.

Our findings highlight the importance of an adequate amount of protein during pregnancy in order to ensure normal development of the fetal endocrine pancreas. The latter was programmed by the low-protein diet during development in a way different from the control group. During pregnancy these structural and functional lesions led to abnormal oral glucose tolerance with blood insulin levels lower than those of the controls. The low-protein diet during intra-uterine life, therefore, resulted in diabetogenic conditions with its consequences for the subsequent generations. The consequences of the effect of poor protein initially are summarized in Fig. 4. With reduced

protein the progeny will develop changes in the endocrine pancreas leading to glucose intolerance and possibly diabetes in adult males and females if the low-protein diet is maintained. Females becoming pregnant feature gestational diabetes with a consequent specific imprint on the endocrine pancreas of their own progeny. The later exposure to high blood sugars is associated with protein deprivation. In the recuperation group given the low-protein diet only during fetal life, females were affected before and also during pregnancy; their progeny were exposed to high blood sugars in the presence of an adequate intake of proteins which allowed the endocrine pancreas of the fetus to adapt to maternal hyperglycaemia with islet cell hyperplasia.

It is of interest to note that females were affected more than males in our experiments. In humans, female monozygotic or dizygotic twins have a higher prevalence of type 2 diabetes than males (Kaprio *et al.* 1992; Hales, 1994). The same observation can be made in female and male twins regarding the prevalence of type 1 diabetes. Type 2 diabetes and its complications seem to be more prevalent in females than in males (Mitchell *et al.* 1993), especially in less-privileged populations. This indicates the greater sensitivity of the fetal endocrine pancreas of females and offers an explanation for the role of maternal environment in the transmission of diabetes. Both features are highlighted by our animal investigation. In the presence of the low-protein diet during pregnancy there was vertical transmission of diabetes to the next generation without calling on genetic factors or thrifty genotypes for its explanation. Different adaptation of males and females to nutritional changes is illustrated in the apparent need of females to increase islet size in order to maintain their metabolic status when dietary changes are imposed (Tejning, 1947). When our current findings are analysed the thrifty phenotype hypothesis (Hales & Barker, 1992) gains further ground. The latter calls on changes in maternal environment influencing the programming of the female progeny and the maturation of its β cell. Protein availability may be essential for the latter.

Our observations highlight the effect of a low-protein diet on the development of vascularization in the head and the tail of the endocrine pancreas. Endothelial cells as well as β cells seem to need an adequate amount of protein during fetal life for both cell types to achieve proper development. When a normal diet is restored immediately after birth, the vascular network in the endocrine pancreas seems to recover by adulthood; this does not happen with β cells (Dahri *et al.* 1993). However, the structure and the function of the adult blood vessels may still have defects at this stage. It is not known what relationships may exist in the fetus between blood vessels and β cell maturation or how blood vessel anomalies may impede β cell development.

The effect of a low-protein diet during pregnancy on the blood vessels is not limited to those located in the islets of Langerhans. Preliminary findings indicate a reduction in blood vessel density in organs such as brain when protein intake is inadequate during pregnancy (N. Bennis, C. Rémacle and J. J. Hoet, unpublished results). Muscle biopsies have shown that a lower density of capillaries exists when insulin resistance is present (Lilliojas *et al.* 1980). Furthermore, other organs also are affected by a low-protein diet administered during gestation. In primates it reduced the weight of the neonatal kidney, which did not occur with a normal-protein-low-energy diet (Cheek & Hill, 1975). Intra-uterine growth retardation by uterine vessel ligation in rats leads to kidneys with fewer glomeruli (Merlet-Benichou *et al.* 1992), and protein restriction during pregnancy induces more immature glomeruli in the pups (Zeman, 1968). In the latter, hypertension may develop (Langley-Evans *et al.* 1994) which may be associated with changes in the renin-angiotensin status.

In humans, birth weight predicts the occurrence of elevated blood pressure in early postnatal life (Launer *et al.* 1993). Epidemiological observations indicate that diabetes, hypertension and renal disease are frequent in less-privileged societies (Wetterhall *et al.* 1992). These associations could be due to lack of proper nutrition during pregnancy and to poor maternal health. The triad of diabetes and vascular and renal diseases is more prevalent in Afro-American women than men, indicating the susceptibility of women to degenerative disease possibly due to early programming.

Our investigations indicate the need to integrate into studies on epidemiology of diabetes the health status of the previous generation and, more precisely, the well-being of the mother, her nutritional status and dietary intake. Objective experimental evidence now indicates that this is imperative and primary prevention of diabetes should be its principal motivation.

In conclusion, reduced birth weight, consequential to lack of adequate protein availability in the maternal diet, is associated with alterations in early programming which affects the development of the fetal endocrine pancreas, endothelial cells and nephrons. Diabetes and hypertension develop in the progeny in animals and in humans.

Our current investigation on the structure and function of the β cells confirms these observations in population studies and emphasizes the channels which may explain the prevalence of diabetes and gestational diabetes in less-privileged societies. It may suggest also new experimental approaches based on early programming to explain heart, kidney and brain damage later in life, especially in relation to diabetes.

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