Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 55 (2006) 642–649

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Maternal protein intake in the pregnant rat programs the insulin axis and body composition in the offspring

William D. Rees[a,*], Susan M. Hay[a], Morven Cruickshank[a], Brigitte Reusens[b], Claude Remacle[b], Christos Antipatis[a,1], George Grant[a]

[a]*Division of Energy Balance and Obesity, The Rowett Research Institute, Greenburn Rd, Bucksburn, AB21 9SB Aberdeen, UK*
[b]*Laboratoire de Biologie Cellulaire, Institut des Sciences de la Vie Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium*

Received 6 August 2005; accepted 7 December 2005

Abstract

Evidence to support an association between early nutrition and the development of obesity in the rat is equivocal. In this study we have investigated the postnatal growth, glucose tolerance, and adipocyte function of the offspring from pregnant rats fed with diets containing either 20% or 8% protein during gestation. By 25 weeks of age, the female offspring of dams fed with the diet containing 8% protein had a significantly lower adult body weight due in part to a decrease in body fat. The peak concentration of insulin after oral administration of a glucose dose was significantly lower in both the male and female offspring of the dams fed with the diet containing 8% protein. However, the ability of insulin to stimulate lipogenesis or suppress lipolysis in fat cells isolated from the offspring was not influenced by the prenatal diet. Hepatic phospho*enol*pyruvate carboxykinase activity was reduced in female offspring of dams fed with the diet containing 8% protein. These results show that adult body composition is determined during the prenatal period as a result of programming of the insulin axis. This metabolic programming influences hepatic metabolism; however, there is no evidence for a programmed change in adipocyte function.

1. Introduction

In humans, reduced growth in early life is strongly linked with impaired glucose tolerance and the development of non–insulin-dependent diabetes [1]. Despite the good evidence for a linear relationship with respect to birth weight and the programming of glucose metabolism, the relationship between birth weight and adult body mass index is more complex. In some studies there is a positive linear relationship, whereas in others the relationship is U or J shaped [2,3]. These complex relationships may reflect prenatal and maternal influences on lean tissue rather than on fat with the postnatal environment also influencing the development of obesity in later life [4]. In animal models it is well established that the protein content of the maternal diet influences glucose tolerance, islet function, and organ development of the offspring [5,6]. For example, the changes in glucose metabolism in the offspring of the pregnant rat fed with a low-protein diet parallel the correlation between birth weight and the adult onset of disease observed in epidemiologic studies of human populations [7]. However, as with human studies, the evidence for long-term programming of body composition and obesity in these animal models is less well defined.

In rodent models the greatest changes in the insulin axis appear to occur when normal nutrition is restored after a period of malnutrition during gestation. In a number of studies, moderate protein restriction during pregnancy leads to a small reduction in the body weight of the offspring at birth, reduced fetal beta-cell proliferation, and insulin secretion [8,9]. Recent studies have suggested that this restriction does not change the capacity of preadipose cells to divide or to store fat and suggest that the rates of fat accretion in the offspring reflect a change in the neuroendocrine environment [10]. In both humans [11] and animals [12] it is unclear whether the fetal environment mediates changes in glucose metabolism through effects on insulin secretion or resistance, or is a combination of both factors.

* Corresponding author. Tel.: +44 01224 716637; fax: +44 01224 716622.
E-mail address: wdr@rri.sari.ac.uk (W.D. Rees).
[1] Present address: DSM Nutritional Products Ltd, CH-4002 Basel, Switzerland.

0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.12.006

In this study of the pregnant rat we have manipulated fetal development by varying the protein content of the maternal diet during gestation and reverted to normal nutrition during lactation. We have then investigated the postnatal consequences of this treatment on glucose tolerance and body composition in the offspring. The function of adipocytes and hepatic enzymes was also studied to evaluate the role of these tissues in determining body composition.

2. Materials and methods

2.1. Animals

The study used female rats of the Rowett hooded strain bred in the Institute's own colony. The colony was founded in 1929 with hooded rats from the Lister Institute for virology. These animals, also known as the Long-Evans strain, were originally developed by cross-breeding Wistar albino females with a wild gray male. The procedures were approved by the ethical review committee of the Rowett Research Institute and conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986. The semisynthetic casein-based diets described previously [10] were obtained from Hope Farms (Woerden, The Netherlands). Diet composition is given in Table 1. Groups of 5 dams were fed with the diets containing 20% and 8% casein (wt/wt) ad libitum commencing at 6 to 7 weeks of age. Two weeks later, when weighing approximately 230 to 240 g, the animals were mated with males of the same strain. Mating was confirmed by detection of a vaginal plug and this day was denoted day 0. The female rats were maintained on the same diets throughout pregnancy. Pups were delivered naturally, and, where possible, on postnatal day 1 the litters were culled to 8 pups per dam with 4 males and 4 females. Once they had given birth the dams were fed with stock laboratory chow (Rat and Mouse breeder and grower diet, Special Diet Services, Witham, Essex, UK) ad libitum until weaning was complete. The calculated analysis of protein, lipid, and carbohydrate content of the diet is shown in Table 1. The offspring were group-housed and weaned onto stock laboratory chow (Table 1), which was fed ad libitum for the remainder of the experiment. One subset of the offspring were killed at 7 to 10 weeks for the preparation of adipocytes and the measurement of lipogenesis and lipolysis. A further group of 10 male and 10 female offspring per prenatal treatment group were kept until 25 weeks of age. All animals were weighed twice weekly to monitor growth.

Table 1
Composition of the experimental and stock diets

	Hope Farms diet (g/kg of diet)		Stock diet (Special Diet Services breeder and grower)	
	20% Casein	8% Casein	Calculated analysis[a] (%)	
Casein	220[b]	90[c]	Digestible Protein	17.4
Dextrose	551	681	Sugars	4.7
Cellulose fiber	50	50	Total fiber	12.3
Cornstarch	80	80	Starch	44.8
Vitamin mix[d]	2.5	2.5		
Mineral mix[e]	47	48		
Soya oil	43	43	Oil	3.4
Choline chloride	4	4	Choline	0.95
DL-methionine	2	0.8		

[a] Analysis provided by the manufacturer.
[b] Equivalent to 19.2% digestible protein.
[c] Equivalent to 8.1% digestible protein.
[d] The vitamin mix contained (per kg): thiamine 20 mg, pyridoxine 13 mg, riboflavin 12 mg, folic acid 8 mg, biotin 315 mg, inositol 500 mg, cholecalciferol 50 μg, calciferol 50 μg, 1′ *N*-tocopherol 63 mg, retinol 5.4 mg, cyanocobalamin 0.5 mg, choline 1520 mg, menadione 10 mg, niacin 40 mg, pantothenic acid 15 mg.
[e] The mineral mix contained (per kg): $FeCO_3$ 80 g, $CuCO_3$ 12 g, MnO 40 g, ZnO 24 g, Na_2SeO_3 0.16 g, $CrCl_3 \cdot 6H_2O$ 1 g, NaF 0.8 g, $CaIO_3$ 0.24 g made up to 1 kg with cornstarch.

2.2. Glucose tolerance tests

Five animals from each of the 2 groups of 25-week-old animals were fasted overnight before the glucose tolerance test. A baseline blood sample of approximately 150 μL was taken from the tail vein. The animals were then given D-glucose (200 mg/100 g body weight) dissolved in 1 mL of water per 100 g of body weight and administered as a single dose by gavage. Over the following 90 minutes, six 150-μL blood samples were collected in a heparin-treated tube after venesection of the tail vein. Red cells were removed by centrifugation and plasma glucose was estimated with a KONE selective chemistry analyzer using the hexokinase/glucose-6-phosphate dehydrogenase method (kit no. 981300, Labmedics, Manchester, UK). Plasma insulin was measured by radioimmunoassay [13].

2.3. Carcass analysis

The offspring were killed and the internal organs were dissected without delay. After weighing, samples of the organs were frozen in liquid nitrogen and then stored at −80°C until used for analysis. The eviscerated carcasses were minced, lyophylized, and ground to a uniform, fine powder. The fat content was determined with a Soxtec fat extraction System (Tecator, Hoganas, Sweden), Nitrogen and ash were determined by combustion in an Automated Dumas system (Foss Electric [UK] Ltd, Warrington, Cheshire, UK).

2.4. Adipocytes

The incorporation of glucose into lipids provided a measure of the rates of lipogenesis in adipocytes [14]. Briefly, a suspension of adipocytes (8% fat) was prepared from epididymal and endometrial fat pads by collagenase digestion (1.25 mg/mL; Sigma Chemical Co, Poole, Dorset, UK). Aliquots of the suspension were incubated with 0.05 μCi (1.85 kBq) D-[2-^{3}H]glucose (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) for 90 minutes in the presence or absence of 3.6 mIU/mL bovine insulin. The reaction was stopped by the addition of 3 mL of toluene containing 0.3 g/L 1-4, bis-2-(5-phenyloxazolyl)benzene; PPO 2,5-di phenyloxazole (POPOP) and 5 g/L PPO.

Table 2
Maternal and neonatal growth

Diet	20% Casein (mean ± SEM)	8% Casein (mean ± SEM)
n	4	4
Dam		
Weight at start (day −31) (g)	204.2 ± 3.8	201.3 ± 5.7
Weight at mating (g)	226.3 ± 2.4	223.4 ± 3.9
Weight at day 7 of gestation (g)	252.9 ± 4.7	253.1 ± 2.8
Weight at day 16 of gestation (g)	292.5 ± 6.9	281.1 ± 3.4
Weight at day 20 of gestation (g)	335.8 ± 8.1	315.8 ± 4.6
Dam weight on postnatal day 3 (g)	274.8 ± 3.8	263.0 ± 7.5
Average litter size	12.5 ± 1.0	12.5 ± 1.2
Offspring		
Average pup weight on day 1 (g)	5.5 ± 0.2	5.8 ± 0.4
Weight at weaning (males)	38.3 ± 1.3	36.5 ± 0.7
Weight at weaning (females)	37.1 ± 1.5	37.2 ± 1.1

Radioactivity extracted into the organic layer was determined by counting in a liquid scintillation counter.

Lipolysis was estimated by incubating a suspension of adipocytes (20% fat) for 60 minutes in the presence or absence of 4 μmol isoprenaline (Sigma) [15]. The cell suspension was centrifuged at 13 000*g* for 5 minutes and a sample of the medium removed. The glycerol present in the medium was measured using a direct colorimetric procedure for the measurement of glycerol using a quinoneimine chromogen system in the presence of glycerol kinase, peroxidase, and glycerol phosphate oxidase (kit no. GL 105, Randox Laboratories, Crumlin, Co. Antrim, UK).

2.5. Liver enzyme activities

Glucokinase and phospho*enol*pyruvate carboxykinase (PEPCK) activities in homogenates of liver were measured by the assays described by Desai et al [16]. Briefly, glucokinase was determined in samples of liver, which were homogenized in 3 volumes of buffer containing 50 mmol/L HEPES, 100 mmol/L KCL, 5 mmol/L $MgCl_2$, 1 mmol/L EDTA, and 2.5 mmol/L DTT. The homogenate was centrifuged at 100 000*g* for 60 minutes and the supernatant used for the assay. The final assay mixture contained, in addition to the buffer, 0.5 mmol/L or 100 mmol/L D-glucose, 5.0 mmol/L adenosine triphosphate (ATP), and 0.5 mol/L NAD and 0.2 IU of glucose-6-phosphate dehydrogenase (*Leuconostoc mesenteroides*). Rates were determined by following the absorbance at 340 nm and corrected for the blank rates (−ATP). The glucokinase activity was corrected by subtracting the rate with 0.5 mmol/L glucose (hexokinase activity) from the total rate with 100 mmol/L glucose. One unit of activity is defined as the conversion of 1 μmol nicotinamide adenine dinucleotide per minute.

Phospho*enol*pyruvate carboxykinase activity was assayed in samples of liver, which were homogenized in 3 volumes of buffer containing 0.25 mol/L sucrose, 5 mmol/L Tris-Cl (pH 7.0), and 1 mmol/L 2-mercaptoethanol. The homogenate was centrifuged at 100 000*g* for 60 minutes and the supernatant used for the assay. The final assay mixture contained in addition to the buffer, 100 mmol/L $NaHCO_3$ (containing 2 μCi of $NaH^{14}CO_3$), 1.5 mmol/L phospho*enol*pyruvate, 1.25 mmol/L inosine diphosphate, and 0.5 mmol/L malate dehydrogenase plus liver extract in a volume of 1.0 mL. The reaction was started by the addition of extract and after incubation at 37°C stopped with 0.5 mL of 10% (wt/vol) trichloroacetic acid. Samples of the mixture were transferred to a scintillation vial and freeze dried. The residue was reconstituted in 0.5 mL of water, and 5 mL of liquid scintillator was added to the vial. Radioactivity was determined using a liquid scintillation counter, and 1 unit of activity is defined as the conversion of 1 μmol CO_2 per minute.

2.6. Statistics

Data were analyzed by 1-way analysis of variance or by Student *t* test (Genstat 6 statistical package, Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Herts, UK).

3. Results

The growth of the dams is shown in Table 2. During the premating period the weight gain of the animals in the 2 experimental groups were not significantly different. However, during the second and third weeks the weight gain of the group fed with the diet containing 8% protein was approximately 15% lower, although this did not cause a significant difference in the overall weight of the 2 experimental groups. Four of the 5 dams in both groups were pregnant, and the gestation time was 22.5 days in both groups. The protein intake did not significantly affect the average number of pups in the litter (Table 2) and these numbers were equivalent to those found with animals fed with stock diet (12.8 ± 0.7 pups per dam). Table 2 shows that there were no significant differences between the 2 groups in the average weight of the pups at birth. On postnatal day 1, pups were culled to 8 animals per litter and the remaining pups were successfully weaned without further losses. There were no significant differences in the

Table 3
Postweaning growth of offspring after protein restriction in utero

Maternal diet (% protein)	Sex	Weeks of age	7	11	15	19	22
20%	Male	Mean	224.7	390.3	471.2	528.7	575.9
		SEM	6.8	10.6	12.3	13.4	14.3
8%	Male	Mean	218.7	382.7	474.7	522.9	563.1
		SEM	4.5	6.2	7.8	10.6	10.3
20%	Female	Mean	152.6	233.0	275.1	298.2	300.7
		SEM	4.9	8.5	10.8	11.2	11.5
8%	Female	Mean	157.9	229.5	259.3	277.6*	282.5*
		SEM	2.3	3.2	3.5	3.4	4.1

Mean weights of 10 male and 10 female offspring from each group were expressed in grams. Data at each time point were analyzed by *t* test.

* $P < .05$, significantly different from the 20%-protein group.

Table 4
Organ weights of offspring

Maternal diet	Sex		Heart (g)	Kidney (g)	Liver (g)	Spleen (g)	Lungs (g)	Fat pads[a] (g)
20% Protein	Males	Mean	1.606	3.930	21.140	0.920	2.753	15.261
		SEM	0.069	0.156	1.965	0.034	0.300	1.104
8% Protein	Males	Mean	1.715	4.114	23.144	0.927	3.085	13.767
		SEM	0.102	0.107	1.947	0.031	0.242	1.329
20% Protein	Females	Mean	1.065	2.363	11.970	0.746	2.145	11.465
		SEM	0.041	0.052	0.947	0.050	0.167	1.165
8% Protein	Females	Mean	1.016	2.215	10.825	0.638	2.171	8.116*
		SEM	0.052	0.060	0.887	0.019	0.084	0.750

Two male and 2 female pups from each litter giving a total of 10 in each group were killed at 22 weeks (males) and at 25 weeks (females). The organs were dissected and weighed. Data in each group were analyzed by t test.

[a] Epididymal fat pads were taken from male animals and endometrial fat pads from the females.

* $P < .05$.

weights of the male or female offspring when the pups were weaned at day 21.

The postweaning growth of the offspring is shown in Table 3. There were no significant differences in the body weights of the males up to 22 weeks of age. In contrast, the body weights of the female offspring of dams fed with the diet containing 8% protein were, by approximately 16 weeks after weaning, significantly ($P < .05$) lower than the offspring of the dams fed with the diet containing 20% protein. It is also interesting to note that the growth of the offspring from dams fed with the higher-protein diet was more variable than those of dams fed with the lower-protein diet. This was true of both sexes; the coefficient of variation was 8.6% for the male offspring of dams fed with the diet containing 20% protein and 6.0% in the male offspring of dams fed with the diet containing 8% protein. The corresponding values for the female offspring were 12.1% and 5.1%, respectively. Table 4 shows the weights of the major internal organs from these animals. In the females the only significant differences were in the absolute weight of the endometrial fat pads, which were significantly smaller ($P < .05$) in the offspring of dams fed with the 8% protein diet during gestation. The relative weights of the fat pads were also significantly different (3.8% compared with 2.9%, $P < .05$). These results suggest that the female offspring of dams fed with the diet containing 8% protein deposit less fat, contributing to a decrease in body weight.

This observation was confirmed by an analysis of the remaining carcass as shown in Fig. 1. The fat content of the female offspring of dams fed with the diet containing 8% protein was significantly lower than the offspring of dams fed with the diet containing 20% protein. However, there is no significant difference in the total ash or nitrogen content, indicating that there was no effect of the prenatal diet on lean tissue and skeletal growth. Almost all of the change in body weight is accounted for by the fat content. These results show that feeding the dam a diet containing 8% protein during the prenatal period reduced subsequent fat accretion in both the intra-abdominal and subcutaneous fat depots in the offspring.

Before being killed, glucose tolerance tests were carried out on the offspring as shown in Fig. 2. After an overnight fast there were no significant differences in the basal plasma insulin or glucose levels in male or female offspring of either group. After the administration of the test dose, plasma glucose concentrations increased by approximately 2-fold (Fig. 2, left-hand panels A and C). The plasma

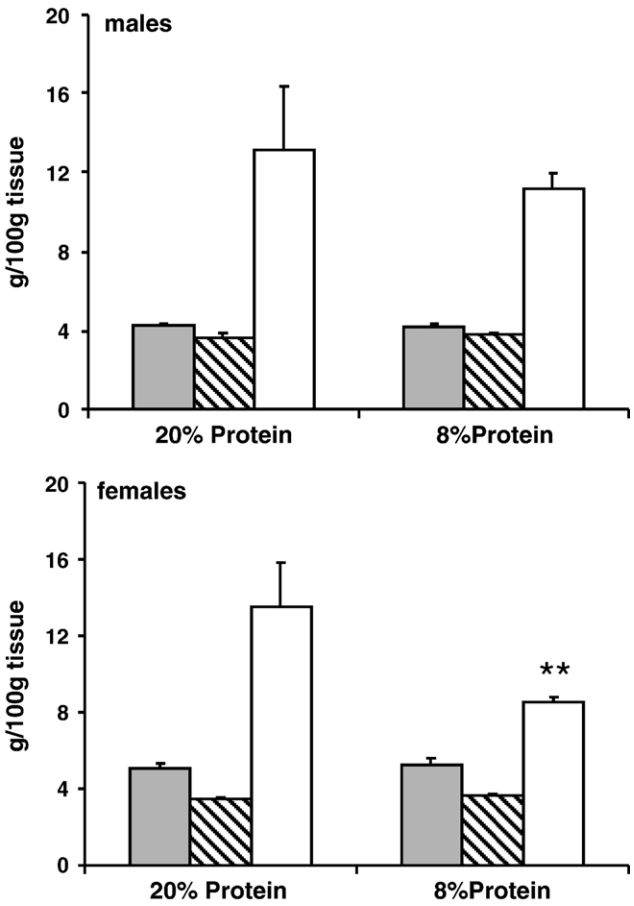

Fig. 1. The ash (closed bars), nitrogen (hatched bars), and fat (open bars) content of dried powder prepared from the carcass of the offspring. The male offspring were killed at 22 weeks and the females at 25 weeks after weaning. The upper panel shows the data for the male offspring of dams fed with diet containing 20% protein (n = 5) and 8% protein (n = 5). The lower panel show data for the female offspring of dams fed with diet containing 20% protein (20% HF, n = 6) and 8% protein (8% HF, n = 5) (error bars ± SEM). **$P < .01$.

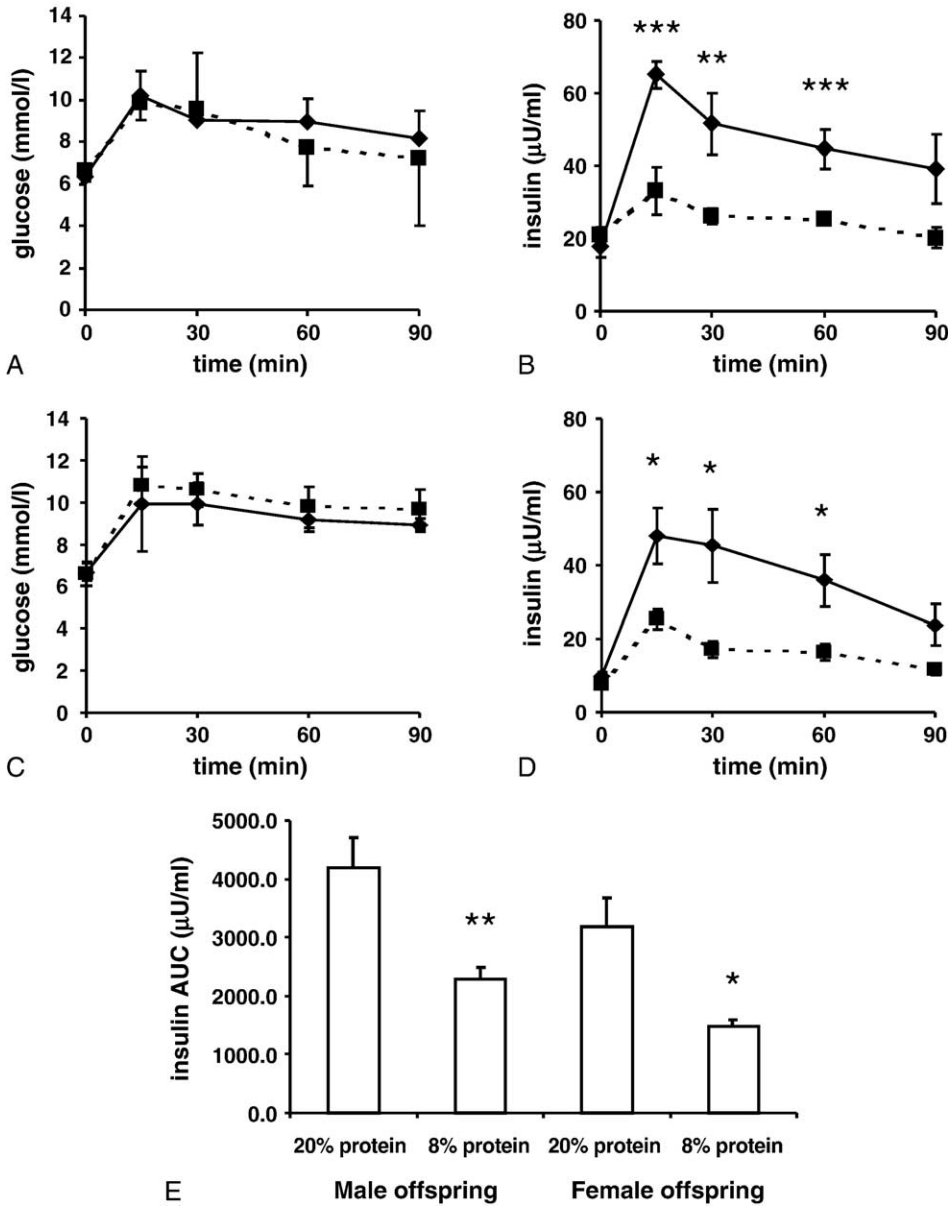

Fig. 2. Plasma glucose (left-hand panels A and C) and insulin concentrations (right-hand panels B and D) after the administration of a standard glucose dose to male (upper panels A and B) and female (lower panels C and D) offspring of dams fed with diet containing 20% protein (diamonds, solid line) and 8% protein (squares, dotted line). The cumulative insulin area under the curve is shown in E. Data are the average from 5 animals (error bars ± SEM). *$P < .05$; **$P < .01$; ***$P < .001$.

glucose profile did not differ significantly between the males from the 2 groups either in the peak height or the total area under the curve. In the female offspring, although the peak plasma glucose concentrations were not significantly different, there was a significant ($P < .05$) increase in the area under the curve in the offspring of dams fed with the 8% protein diet. The administration of glucose caused an immediate increase in plasma insulin concentrations. In both males and females the peak concentration was significantly lower in the offspring of the dams fed with the diets containing 8% protein (Fig. 2, right-hand panels B and D). In addition, insulin levels in the offspring of the dams fed with the low-protein diet returned to the fasting levels by 90 minutes, whereas in the offspring of dams fed with the high-protein diet the levels remained elevated for longer. These changes are also reflected by significant changes in the area under the curve in both sexes (Fig. 2E).

Lipogenesis and lipolysis rates were measured in fat cells isolated from the subgroup of offspring killed at 7 to 10 weeks of age. Cells isolated from the epididymal fat pads of male offspring had a lower activity than the cells from the endometrial fat of the female offspring. Both basal and insulin-stimulated glucose uptake rates were higher in the

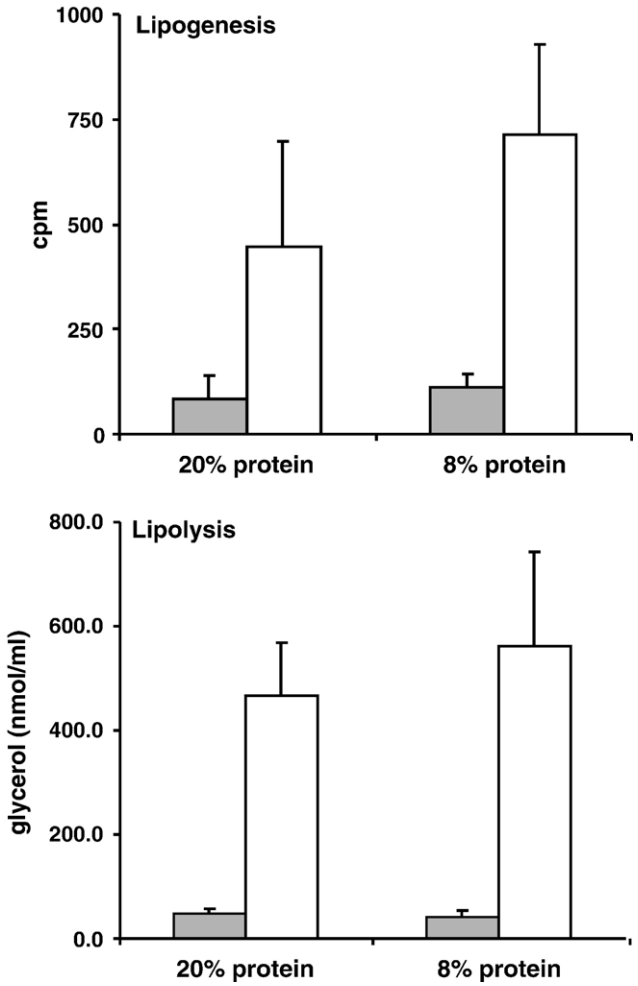

Fig. 3. Lipogenesis (upper panel) and lipolysis (lower panel) in adipocytes prepared from the endometrial fat pads of female offspring of dams fed with diet containing 20% protein (n = 5) and 8% protein (n = 5). Cells were either basal (closed bars) or stimulated with insulin or isoprenaline (open bars). Error bars ± SEM.

endometrial adipocytes isolated from the offspring of dams fed with the diet containing 8% protein (Fig. 3, upper panel). However, there is no significant difference in the fold stimulation caused by a maximum dose of insulin. Insulin stimulated glucose uptake by 5.2 ± 2.4-fold in the cells isolated from the offspring of dams fed with the diet containing 20% protein and by 6.4 ± 0.8-fold in the offspring of dams fed with the diet containing 8% protein. Fig. 3 (lower panel) shows the release of glycerol by the same cells. Treating the cells with isoprenaline caused a similar fold stimulation of lipolysis in both groups, 10.3 ± 2.4-fold in the cells isolated from the offspring of dams fed with the high-protein diet and by 15.2 ± 8.8-fold in the low-protein group. It is difficult to get accurate measurements of cell numbers for isolated adipocytes because of the propensity of the cells to lyse, and the cell preparations were normalized to give the same fat content [17]. The difference in absolute values for both lipogenesis and lipolysis probably reflects the increased size of the fat pads in the offspring of dams fed with the high-protein diet. It is likely that the cells from these animals are larger with a higher fat content. Therefore, a preparation containing a given concentration of fat will contain fewer cells resulting in a lower rate of lipogenesis or lipolysis when they are expressed per unit of fat.

Enzyme extracts were prepared from the livers of the animals killed at 25 weeks of age. Extracts from the group of animals, which were fed normally before euthanasia, were assayed for glucokinase and PEPCK. The upper panel of Fig. 4 shows that there were no significant differences in glucokinase activity in livers from male or female offspring from either group. The lower panel shows that hepatic PEPCK activity was also not significantly different in the male offspring. However, the activity was significantly increased in the livers of female offspring of the dams fed with diets containing 8% protein. The small differences in

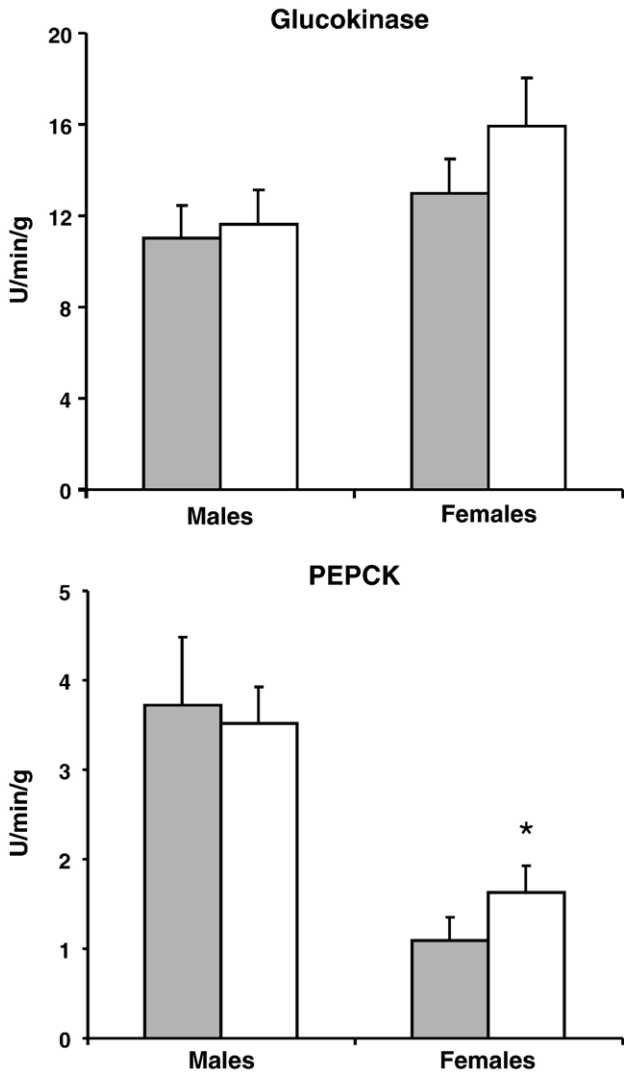

Fig. 4. Glucokinase (upper panel) and PEPCK activities in liver homogenates prepared from the offspring of dams fed with diet containing 20% protein (closed bars) and 8% protein (open bars). Data are the average from 5 animals (error bars ± SEM). *$P < .05$; **$P < .01$; ***$P < .001$.

the liver weight between the 2 groups did not account for the difference, and the total PEPCK activity was also significantly different increasing from 10.08 ± 5.1 to 21.3 ± 7.1 U ($P < .01$, n = 5). Extracts were also prepared from the livers of animals fasted overnight for the glucose tolerance test. The activity was increased to 5.63 ± 1.98 U/g protein in livers of female offspring in the high-protein group and 5.13 ± 1.20 U/g protein in the low-protein group, (n = 5 for both groups). Although fasting the animals significantly increased the PEPCK activity ($P < .001$), there was no significant difference between the offspring from the high- and low-protein groups.

4. Discussion

This study shows that the protein content of the maternal diet has a long-lasting effect on insulin regulation of glucose metabolism and that this is accompanied by a change in fat accretion by the female offspring. The offspring of dams fed with a diet containing 8% protein had approximately 30% less total body fat than those from dams fed with diets containing 20% protein. The change in fat deposition and body weight induced by the prenatal treatment only becomes significant when lean tissue growth slows. Both males and females show significant differences in plasma insulin in response to an oral glucose load; therefore, at present, it is not clear whether there is a sex-specific effect on postnatal obesity.

The offspring of dams fed with the diet containing 8% protein only release about one third of the amount of insulin released by the offspring of dams fed with the diet containing 20% protein in response to a similar glucose challenge. Despite this difference the animals in both groups are still able to maintain almost normal plasma glucose concentrations. This suggests that there is a relative difference in the insulin sensitivity of the peripheral tissues; those from the offspring of dams fed with the high-protein diet are less sensitive to insulin than those of the low-protein group. Analysis of the adipocytes isolated from animals in the 2 groups showed that there were no significant differences in the magnitude of the response to maximal stimulation by insulin in vitro. These results are consistent with the observation that a maternal diet low in protein has no effect on adipocyte development in vitro [10]. This suggests that other tissues such as liver [18] and muscle [19] may be more important targets for prenatal programming. In addition to a lower peak level after a glucose challenge, plasma insulin is elevated for a much shorter period in the offspring of dams fed with the low-protein diets. This may also change the period when glucose and lipid metabolism is perturbed after a meal directly as well as having the potential to interfere indirectly through factors such as appetite.

It is interesting to note that feeding diets with a very high protein content (40% protein) also leads to an increase in adiposity [20]. This would suggest a direct relationship between dietary protein intake during pregnancy and adiposity in the offspring and that the increase in adiposity caused by increasing the maternal protein content from 8% to 20% is part of a continuum. Regardless of whether the dam is fed with a diet containing 20% protein or 8% protein there is no significant difference in the birth weight of the pups, suggesting little or no overall relative fetal growth retardation in the latter group. However, the reduced protein supply did affect the growth of the dams, and by the end of gestation the weight gained by the dams fed with 8% protein was 18.6% less than that of those fed with the diet containing 20% protein. This implies that the dams fed with the low-protein diet are able to maintain fetal growth at the expense of their own tissues. High levels of dietary protein are actually required to support lactation. Diets containing a minimum of 5% to 7% protein in the diet are required to support lactation [21]; however, these cause a reduction in lean tissue growth leading to irreversible long-term growth restriction [22]. Optimal performance, measured in terms of dam and offspring weights at weaning, is achieved with diets containing 15% to 20% protein [23,24]. This is in contrast to the results of this study, which show that low-protein diets fed to the dam in the prenatal period do not affect birth weight and do not have a long-lasting effect on the lean tissue mass of the offspring. This raises an interesting question with regard to the appropriate protein content of the diet during the prenatal period. These data suggest that the diet containing 8% protein is sufficient for fetal growth per se and that higher protein levels are required for the lactation period only. High levels of protein in the maternal diet prenatally up-regulates the release of insulin and leads to a corresponding decrease in insulin sensitivity of the peripheral tissues in the offspring. This programming of the insulin axis in the offspring resulting from manipulation of the maternal protein intake may therefore not be related to fetal growth retardation, but result from interactions between the maternal and fetal metabolism.

Acknowledgment

This work was supported by the European Union Fifth Framework programme NUTRIX (QLK1-2000-00083) and the Scottish Executive, Environment and Rural Affairs Department as part of the Rowett Research Institute core funding.

We thank M Annand, C Horrocks, K Simpson, and D Wallace for their skilled technical assistance.

References

[1] Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 1991;303:1019-22.

[2] Oken E, Gillman MW. Fetal origins of obesity. Obes Res 2003; 11:496-506.

[3] Rogers I. The influence of birthweight and intrauterine environment on adiposity and fat distribution in later life. Int J Obes Relat Metab Disord 2003;27:755-77.

[4] Sayer AA, Syddall HE, Dennison EM, et al. Birth weight, weight at 1 y of age, and body composition in older men: findings from the Hertfordshire Cohort Study. Am J Clin Nutr 2004;80:199-203.

[5] Remacle C, Bieswal F, Reusens B. Programming of obesity and cardiovascular disease. Int J Obes Relat Metab Disord 2004; 28(Suppl 3):S46-S53.
[6] Armitage JA, Khan IY, Taylor PD, et al. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J Physiol 2004;561:355-77.
[7] Godfrey KM, Barker DJ. Fetal nutrition and adult disease. Am J Clin Nutr 2000;71:1344S-52S.
[8] Petrik J, Reusens B, Arany E, et al. A low protein diet alters the balance of islet cell replication and apoptosis in the fetal and neonatal rat and is associated with a reduced pancreatic expression of insulin-like growth factor–II. Endocrinology 1999;140:4861-73.
[9] Ozanne SE. Metabolic programming in animals. Br Med Bull 2001;60:143-52.
[10] Bieswal F, Hay SM, McKinnon C, et al. Prenatal protein restriction does not affect the proliferation and differentiation of rat preadipocytes. J Nutr 2004;134:1493-9.
[11] Prentice AM. Intrauterine factors, adiposity, and hyperinsulinaemia. BMJ 2003;327:880-1.
[12] Hales CN, Ozanne SE. For debate: fetal and early postnatal growth restriction lead to diabetes, the metabolic syndrome and renal failure. Diabetologia 2003;46:1013-9.
[13] MacRae JC, Bruce LA, Hovell FD, et al. Influence of protein nutrition on the response of growing lambs to exogenous bovine growth hormone. J Endocrinol 1991;130:53-61.
[14] Moody AJ, Stan MA, Stan M, et al. A simple free fat cell bioassay for insulin. Horm Metab Res 1974;6:12-6.
[15] Shepherd PR, Crowther NJ, Desai M, et al. Altered adipocyte properties in the offspring of protein malnourished rats. Br J Nutr 1997;78:121-9.
[16] Desai M, Byrne CD, Zhang J, et al. Programming of hepatic insulin-sensitive enzymes in offspring of rat dams fed a protein-restricted diet. Am J hysiol 1997;272:G1083-90.
[17] Gliemann J, Rees WD. The insulin sensitive hexose transport system in adipocytes. Curr Top Membr Transp 1983;339-79.
[18] El I, Khattabi F, Gregoire C, et al. Isocaloric maternal low-protein diet alters IGF-I, IGFBPs, and hepatocyte proliferation in the fetal rat. Am J Physiol Endocrinol Metab 2003;285:E991-E1000.
[19] Ozanne SE, Olsen GS, Hansen LL, et al. Early growth restriction leads to down regulation of protein kinase C zeta and insulin resistance in skeletal muscle. J Endocrinol 2003;177:235-41.
[20] Daenzer M, Ortmann S, Klaus S, et al. Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. J Nutr 2002;132:142-4.
[21] Zeman FJ. Effect of the young rat of maternal protein restriction. J Nutr 1967;93:167-73.
[22] Desai M, Hales CN. Role of fetal and infant growth in programming metabolism in later life. Biol Rev Camb Philos Soc 1997;72:329-48.
[23] Reeves PG, Nielsen FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939-51.
[24] Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 1997;127:838S-41S.