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Long-term Effects on Glucose Tolerance and Insulin Secretory Response to Glucose Following a Limited Period of Severe Protein or Energy Malnutrition in Young Rats

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ABSTRACT

The long-term effects on growth, glucose tolerance and insulin secretory response to glucose of temporary malnutrition early in life have been investigated. Rats were weaned onto either normal diet (18% protein), a protein-restricted diet (5% protein) or a diet adequate in protein but restricted in amount to equal the energy intake of protein-restricted rats ("energy restriction"). From 6 weeks of age and onwards all rats were fed normal diet. Body weight gain was inhibited by both protein and energy restriction but growth was resumed when rats were transferred to normal diet. Protein restriction impaired glucose tolerance and blunted insulin secretory response to glucose. Following refeeding glucose tolerance was normalized but insulin secretory response remained impaired at 12 weeks of age. Energy restriction did not initially affect glucose tolerance and insulin secretion. However, after refeeding male energy restricted rats developed a delayed and exaggerated insulin secretory response to glucose without concomitant deterioration of glucose tolerance. It is suggested that temporary protein restriction at a young age impairs pancreatic B-cell function and decreases peripheral sensitivity to insulin. By contrast, temporary energy restriction does not directly affect B-cell function but confers insulin resistance and compensatory increases of the insulin secretory response to glucose later in life. These models of malnutrition offer possibilities to further study long-term effects of early nutritional insults.

INTRODUCTION

Protein deficiency in early life stunts growth, impairs glucose tolerance and reduces insulin secretion both in man (1,2,6,8) and laboratory animals (13,16,20). Following nutritional rehabilitation growth is resumed, glucose tolerance normalized but insulin secretion may remain impaired (11,13,16). This has led to the proposition that early protein deficiency has an etiological role in the development of malnutrition-related diabetes, a form of diabetes not uncommon in developing countries (15,21). However, experimental animals presented food with a reduced

protein content lower their total intake which results in a deficiency of both protein and energy (3,10). It is therefore not clear whether the metabolic and hormonal pertubations observed in rats fed low-protein diets are caused by protein or energy deficiency. We have presently compared the effects on growth, glucose tolerance and insulin secretion in rats subjected to either severe protein-energy restriction or the same degree of energy restriction with an only moderate concomitant decrease in protein intake.

MATERIAL AND METHODS

Wistar rats from the University of Sheffield breeding colony were caged individually with free acess to a standard rat chow ("N diet"; protein content 18%; Labsure, Croydon, UK) and water throughout breeding and pregnancy. Spontanous delivery took place on day 22 of pregnancy. Large litters were reduced to 12 pups and litters with less than 6 pups were not used. At 3 weeks of age pups were weaned, caged in groups of 2-4 of the same sex and fed according to one of three dietary regimes. Normal rats ("N rats") were fed N diet throughout the experiment. A second group of rats were weaned onto a low protein diet which was available ad libitum ("LP rats"; diet provided by Dr RDE Rumsey, Dept of Human Gastro-enterology, Physiology and Nutrition, University of Sheffield, UK; protein content 5%, for detailed composition see Syme (19)). A third group of rats were weaned onto a diet made of the same constituents and isocaloric with the low protein diet but with 15% protein. This diet was provided in rations of 3 g/day (low energy; "LE rats") which is equal to the amount of chow consumed by LP rats fed ad libitum. On these dietary regimes the energy intake of LP and LE rats was 79% and 77%, respectively, of that of N rats when calculated on the basis of body weight. The protein intake was reduced to 16% in the LP rats and to 47% in the LE rats. At 6 weeks of age LP and LE rats were transferred to N diet for the rest of the experiment. Body weights were registered longitudinally in the same groups of rats, all other data refer to separate groups of rats.

Intraperitoneal glucose tolerance tests (GTT; 2 g glucose/kg body weight) were performed in rats fasted overnight at 6, 12 and 48 weeeks of age, as previously described (16). Blood samples were obtained from the cut tip of the tail, serum separated and stored at -20^{o} until assayed for glucose using a glucose oxidase method (Yellow Springs Analyser, Clandon, Aldershot, UK) and insulin using a radio-immunoassay (Immuno-Diagnostics, Washington, Tyne & Wear, UK). At the end of the experiments the rats were killed by chloroform inhalation and nose to tail-tip length measured.

Differences between the three groups of rats were analyzed using Student's two-tailed t-test for independent observations.

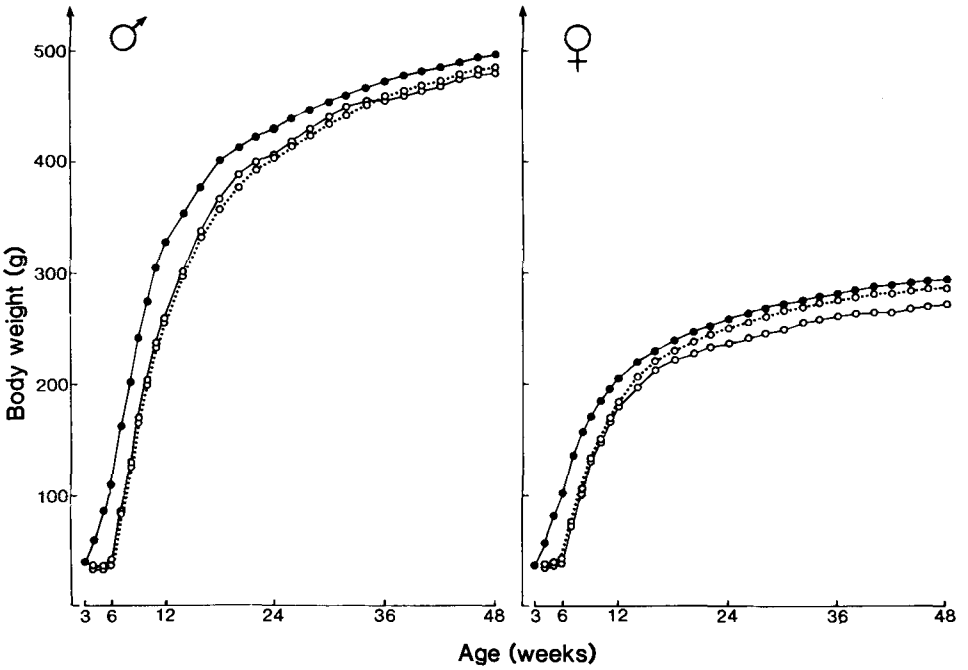

Fig 1. Weight gain of rats fed different diets between 3 and 6 weeks of age. Three-week-old rats were weaned onto normal diet fed ad libitum (18% protein; ●——●), low protein diet fed ad libitum (5% protein; ○•••○) or diet adequate in protein but restricted in amount to equal the energy intake of rats on low protein diet (energy restriction; ○——○). At 6 weeks of age all rats were transferred to normal diet. Values are given as means for at least 11 rats.

RESULTS

During the period on experimental diet neither LP nor LE rats gained weight (Fig 1). When they were refed N diet they resumed growth but remained smaller than N rats. Catch-up growth was achieved by LP rats of both sexes and by male LE rats which beyond 18 week of age were not significantly lighter than N rats. Female LE rats remained lighter than N rats throughout the experiment ($p < 0.05$).

Growth in body length, a measure of skeletal growth, was retarded but not completely inhibited in both LP and LE rats (Fig 2). LE rats were less severly affected and thus longer than LP rats ($p < 0.001$) at 6 weeks of age. Following refeeding with N diet rapid growth ensued and LE rats had caught up with N rats at 12 weeks of age. LP rats remained shorter than N and LE rats at 12 weeks of age ($p < 0.05$) but had caught up in body length at 24 weeks of age.

Glucose tolerance and insulin secretory response to glucose did not differ between sexes at 6 and 12 weeks of age and data were pooled. Results from 48-week-old rats are, however, givenseparatly by sex. In N rats there was a progressive impairment with age of the glucose tolerance with increasing serum glucose concentrations after fasting and during the GTT (Fig 3).

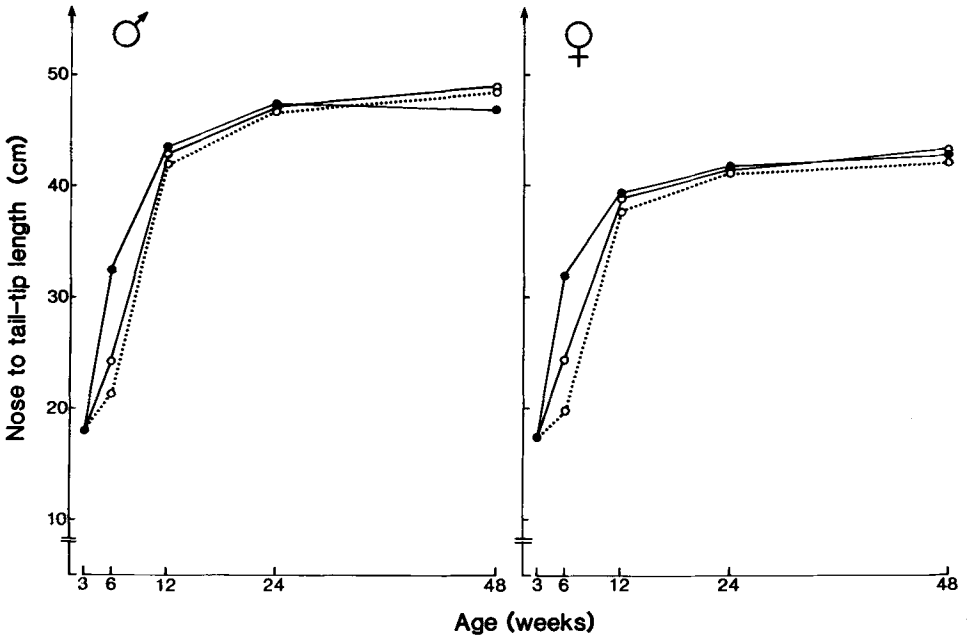

Fig 2. Body length of rats fed different diets between 3 and 6 weeks of age. Three-week-old rats were weaned onto normal diet fed ad libitum (18% protein; ●——●), low protein diet fed ad libitum (5% protein; ○•••○) or diet adequate in protein but restricted in amount to equal the energy intake of rats on low protein diet (energy restriction; ○——○). At 6 weeks of age all rats were transferred to normal diet. Nose to tail-tip length was measured in rats killed by chloroform inhalation. Values are given as means for at least 6 rats.

At 48weeks of age this was most evident in male N rats. In 6-week-old LP rats glucose tolerance was impaired with a very high peak serum glucose concentration and a rapid return to lower concentrations at the end of the GTT. Following refeeding glucose tolerance had normalized at 12 weeks of age and subsequently showed an impairment with age similar to that of N rats. Six-week-old LE rats had a fasting serum glucose concentration higher ($p < 0.001$) than that of N rats but otherwise a similar glucose tolerance curve. The development with age of the glucose tolerance was similar in LE and N rats.

The insulin secretory response to glucose of N rats peaked at 30 min after the glucose injection (Fig 3). The peak response was highest in 12-week-old N rats and had decreased ($p < 0.05$) in 48-week-old rats of both sexes. The 6-week-old LP rats did not exhibit a significant insulin secretory response to glucose. After refeeding a small insulin secretory response could be demonstrated at 12 weeks of age but it was less prominent than that of N rats ($p < 0.001$). The insulin secretory response did not change in either sex up to 48 weeks of age but since the response of N rats had decreased the two groups were not significantly different at this age. In 6--week-old LE rats the insulin secretory response to glucose did not differ significantly from that of N rats of the same age. In 12-week-old LE rats the insulin secretory response was lower than that of N rats at 30 min

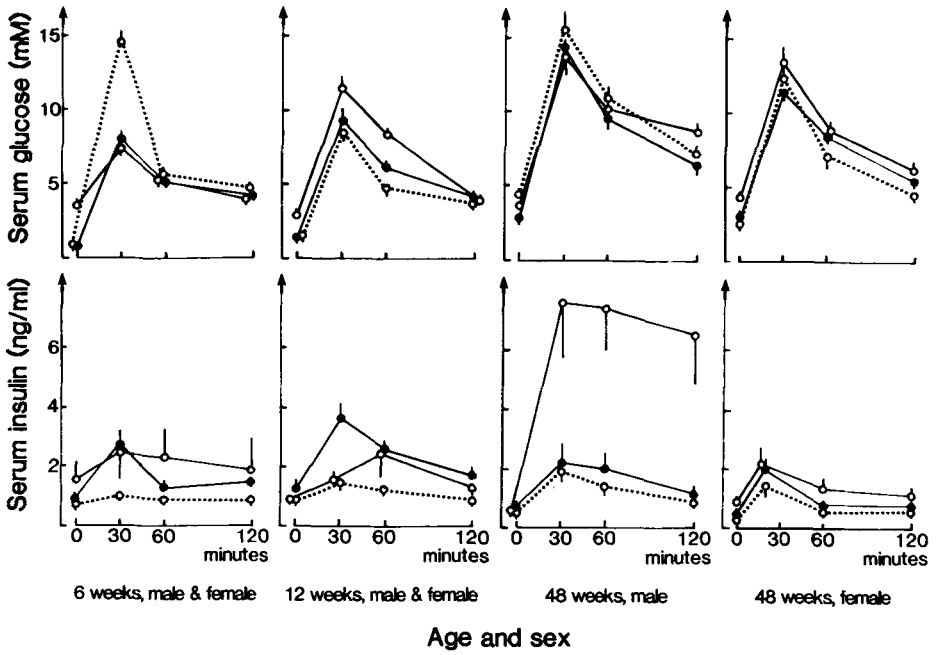

Fig 3. Glucose tolerance and insulin secretory response to glucose of rats fed different diets between 3 and 6 weeks of age. Three-week-old rats were weaned onto normal diet fed ad libitum (18% protein; ●——●), low protein diet fed ad libitum (5% protein; ○•••○) or diet adequate in protein but restricted in amount to equal the energy intake of rats on low protein diet (energy restriction; ○——○). At 6 weeks of age all rats were transferred to normal diet. Intraperitoneal glucose tolerance tests (2 g glucose/kg body weight) were performed at 6 weeks of age, whith rats still on the different experimental diets, and following refeeding with normal diet at 12 and 48 weeks of age. For details see Material & Methods section. Values are given as means ± S.E.M. for 9-16 rats.

after the glucose injection ($p<0.001$). However, a delayed peak response reaching the corresponding level of N rats, was seen 60 min after the injection. The insulin secretory response in 48-week-old female LE rats was similar to that of N and LP rats of the same age. By contrast, the peak response at 30 min in 48-week-old male LE rats was almost four-fold that of N rats and the serum insulin concentrations remained at this high level throughout the GTT.

DISCUSSION

The present investigation confirms a previous study by Okitolonda et. al. (14) in that during the acute phase of malnutrition insulin secretion is more affected by protein deficiency than by energy restriction. This forms a parallel with similar findings in children with kwashiorkor and marasmus, the human diseases of protein and energy malnutrition (1,6). In the present study it was, however, also possible to demonstrate long-term consequences of temporary malnutrition despite what appeared to be adequate nutritional rehabilitation.

In refed LP rats, the ability to normalize serum glucose concentrations during GTT despite

a blunted insulin secretory response to glucose suggests a persisting B-cell defect combined with a lowered peripheral resistance to insulin. Indeed, LP rats have a decreased total B-cell mass (18), isolated islets have an impaired insulin secretory response to glucose in vitro (17) and there is evidence of an increased peripheral sensitivity to insulin (4,5).

On the other hand, the development of first a delayed and later an increased insulin secretory response to glucose in male LE rats indicates preserved B-cell function which maintains glucose tolerance despite increasing peripheral resistance to insulin. The present investigation offers no explanation of the considerable difference between male and female 48-week-old LE rats. It is, however, notable that male LE rats continue a considerable weight gain after 6 months of age when growth in length is small. During this period they accumulate considerable amounts of adipose tissue and may therefore become more insulin resistant than females. However, direct measurements of insulin sensitivity remain to be performed.

This difference between sexes in old LE rats forms a parallel with the offspring of diabetic rats when investigated for possible late effects of the diabetic intrauterine milieu. Interestingly, adult male, but not adult female, offspring of diabetic rat mothers have an increased insulin secretory response to glucose and increased serum insulin concentrations during GTT. These findings have also been interpreted to indicate an increased peripheral resistance to insulin in ageing male offspring (7).

In neither LP nor LE rats did overt diabetes, with fasting hyperglycemia and/or glucosuria, develop. It should in this context be noted that rats, unlike man, grow throughout life. B-cell mass expands up to at least a year of age (9,12) and it is conceivable that rats would be capable of considerable compensatory growth. The lack of difference in insulin secretory response between the oldest N and LP rats and the increased insulin secretory response in the old male LE rats may reflect such growth and compensation for the persisting effects of early malnutrition. It is possible that diabetes would develop should such adaptive mechanisms be insufficient. The present animal models of malnutrition nevertheless offer the possibility of studies of long-term effects on growth and metabolism of early nutritional insults.

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