Early programming of weight gain in mice prevents the induction of obesity by a highly palatable diet

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Abstract

Poor early growth is associated with Type II diabetes, hypertension and other features of the metabolic syndrome in adulthood. It has been suggested that this results from the development of a thrifty phenotype by a malnourished fetus. Such a phenotype would predispose the offspring to the development of obesity if born into conditions of over-nutrition. The present study aimed to determine if early nutrition affected subsequent development of obesity. Mice were established as follows: (a) controls (offspring of control dams), (b) recuperated (offspring of dams fed a low-protein diet during pregnancy, but nursed by control dams) and (c) postnatal low-protein (offspring of control dams nursed by low-protein-fed dams). Mice were weaned on to standard laboratory chow or a cafeteria diet. Recuperated offspring, although smaller at birth ($P < 0.01$), caught up and exceeded the weight of control offspring by 7 days of age ($P < 0.001$). Postnatal low-protein offspring were smaller than controls by 7 days of age ($P < 0.001$). Recuperated animals gained more weight than controls when given free access to a highly palatable diet ($P < 0.01$). Postnatal low-protein animals showed no additional weight gain when given a highly palatable diet compared with chow-fed litter-mates. These results suggest that the early environment has long-term consequences for weight gain. These programmed responses are powerful enough to block excess weight gain from a highly palatable diet and, thus, have major implications for the drug-free regulation of food intake and obesity.

Introduction

Obesity is a major problem in the developed world and an increasing problem in the developing world. In the U.S.A., 22.5% of adults are obese (defined by a body mass index > 30) [1]. Perhaps even more alarming is the observation that 11% of children are now obese [2]. Obesity is strongly linked to adult diseases such as Type II diabetes and cardiovascular disease [3]. The rapidly increasing amount of obesity is thought to be a major underlying factor in the increasing prevalence of these diseases. The increase in childhood obesity is resulting in the early appearance of Type II diabetes and has large implications for healthcare costs. An enormous effort is, therefore, devoted to understanding the mechanisms by which obesity may arise and to the development of drugs which may reduce appetite and, thus, prevent the development of obesity.

A large number of epidemiological studies have demonstrated that there is a relationship between poor fetal and early growth and the subsequent development of adult diseases such as Type II diabetes and the metabolic syndrome [4]. This relationship has been observed in a wide range of populations world-wide, thus there is little doubt that the relationship exists. However, the mechanistic basis of this relationship and the relative roles of genes and the environment remains the subject of much debate. It has been proposed that poor fetal nutrition leads to programming of metabolism in a manner beneficial to survival under conditions of poor postnatal nutrition [5].

Key words: appetite, catch-up growth, diet, growth restriction, obesity, Type II diabetes.

Abbreviation: SGA, small for gestational age.

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This would give rise to a thrifty phenotype, which would become detrimental if the fetus was born into conditions of either adequate or over-nutrition and became obese. It may also increase the risk of the development of obesity. Indeed, studies of humans have suggested that adults who were growth-restricted in utero have increased body fat [6] and, in particular, increased central fat [7].

The maternal low-protein rat model has been used extensively to study the importance of the early environment in determining susceptibility to future development of Type II diabetes and the metabolic syndrome (for review, see [8]). In this model, pregnant and/or lactating rat dams are fed a diet containing a little under half the protein content of a control diet (8 % compared with 20 %). Maternal protein restriction leads to growth restriction in utero. If such offspring are nursed by low-protein-fed dams, permanent growth restriction occurs, even if the animals are weaned on to a standard laboratory chow and fed ad libitum. Low-protein offspring have been shown to become diabetic [9], insulin resistant [9] and hypertensive [10]. Other models of early growth restriction, including maternal calorie restriction [11], maternal anaemia [12], intrauterine artery ligation [13] and fetal exposure to glucocorticoids [14], have also been shown to result in the development of features of the metabolic syndrome. The phenotypic outcomes of these different insults have been remarkably similar, suggesting that these act through a common pathway.

Few studies have focused on the long-term consequences of poor early nutrition on susceptibility to obesity. The aim of the present study, therefore, was to determine if poor early nutrition has long-term effects on weight gain and the development of obesity in response to a highly palatable diet.

**MATERIALS AND METHODS**

**Diets**
The 20 % and 8 % protein diets given to the pregnant and lactating mice were purchased from Hope Farm (Woerden, The Netherlands). These two diets are iso-caloric and their composition has been described previously [15]. The control diet fed from weaning (LAD1) was purchased from Special Diet Services (Witham, Essex, U.K.).

**Establishment of experimental mouse groups**
All procedures involving animals were conducted under the British Home Office Animals Act (1986). Virgin female C57bl/6 mice (initial weight, 25–30 g) used for the study were housed individually and were maintained at 22°C on a 12 h light/12 h dark cycle. They were mated and day 0 of gestation was taken as the day on which vaginal plugs were expelled. Mice were then fed either a control (20 % protein) or an isocaloric low-protein (8 % protein) diet. At birth, the control dams were either continued on the control diet with their own litter culled to eight offspring (‘control’) or received and suckled four randomly selected male offspring from the litter of a low-protein dam (‘recuperated’). The purpose of culling to only four males in the recuperated group was to maximize the plane of nutrition during suckling and, therefore, the rate of catch-up growth. Low-protein dams, which had donated their offspring to a control dam, received that dam’s litter unculled (‘postnatal low-protein’; litter size, 8.6 ± 0.3). The purpose of leaving the postnatal low-protein animals unculled was to minimize the quantity of nutrition during suckling and, therefore, the rate of growth. At weaning (21 days), the male mice from each group were weaned on to either a control diet or a highly palatable diet which was fed ad libitum. The composition of the highly palatable diet was as described by Petry et al. [15]. This diet consists of ground laboratory chow, sugar and condensed milk and leads to excessive weight gain in control rodents [15]. The consistency of this diet means that it has to be given to mice in food pots that are placed inside their cages, rather than via a standard dispenser. Due to the consistency of the diet and the manner by which it is given, it is therefore impossible to record food intake. Body weights of individual mice were monitored weekly.

**Statistical analysis**
Body weights and percentage weight gain was analysed by two-way ANOVA, followed by Duncan post-hoc testing where appropriate. All results are expressed as means ± S.E.M.

**RESULTS**

**Early growth**
The offspring of dams fed a low (8 %)-protein diet were significantly (*P* < 0.01) smaller at birth than offspring of control dams fed a diet containing 20 % protein (1.13 ± 0.03 g compared with 1.57 ± 0.06 g). When these offspring were suckled by normally fed dams (‘recuperated’), their weight caught up with, and exceeded, that of control offspring by 7 days of age (*P* < 0.001; Table 1). In contrast, control offspring cross-fostered to mothers fed the low-protein diet (‘postnatal low-protein’) were growth-restricted and, thus, significantly (*P* < 0.001) lighter than controls by day 7 (Table 1).

**Growth following weaning on to standard chow**
When weaned on to the control diet, recuperated animals gained more weight than controls (*P* < 0.01 from week 5). This difference in body weight was apparent throughout
Table 1  Body weights

PNLP, Postnatal low-protein. ***P < 0.001 compared with controls. n = 24 per group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.77 ± 0.03</td>
<td>3.82 ± 0.04</td>
<td>6.53 ± 0.05</td>
<td>7.75 ± 0.14</td>
</tr>
<tr>
<td>PNLP</td>
<td>1.77 ± 0.03</td>
<td>3.10 ± 0.09***</td>
<td>4.47 ± 0.07***</td>
<td>5.96 ± 0.20***</td>
</tr>
<tr>
<td>Recuperated</td>
<td>1.70 ± 0.04</td>
<td>4.23 ± 0.06***</td>
<td>8.13 ± 0.10***</td>
<td>9.33 ± 0.15***</td>
</tr>
</tbody>
</table>

the study period (Figure 1a). In contrast, despite being weaned on to a control diet at 21 days of age, postnatal low-protein animals gained less weight than controls (P < 0.01 at each time point; Figure 1a).

Growth following weaning on to a highly palatable diet

When given unlimited access to a highly palatable diet, the recuperated animals remained heavier than control mice fed a highly palatable diet (P < 0.01 from 5 weeks of age) throughout the study. The postnatal low-protein group fed the highly palatable diet, however, remained lighter than control mice fed a highly palatable diet (P < 0.001 at each time point; Figure 1b). The striking difference in weight gain was even more apparent when weights of highly palatable diet-fed animals were compared with those of their chow-fed litter-mates. The recuperated animals showed a greater percentage weight gain in response to the highly palatable diet than the control offspring in response to the highly palatable diet (P < 0.05 from week 5; Figure 1c). In marked contrast, the postnatal low-protein group showed no additional weight gain compared with their chow-fed litter-mates when given the highly palatable diet for up to 10 weeks.

DISCUSSION

The detrimental effects of rapid postnatal catch-up growth to adult health have been shown in a number of recent animal and human studies [16–19]. Experiments of a similar design to those described in the present study have shown that rats which are growth-restricted in utero by maternal protein restriction, but then undergo rapid catch-up growth during lactation by suckling control dams, have a reduced longevity compared with control offspring [16]. This reduction in longevity has been related to increased rates of telomere shortening in kidneys [16]. In contrast, rats who were growth-restricted during the suckling period were shown to have an increased longevity and displayed reduced rates of telomere shortening [16]. In human studies, particular attention has been drawn to the apparent metabolic conflict that occurs when early growth restriction is followed by rapid postnatal catch-up growth and/or obesity. Studies of men in Sweden revealed that those individuals who were born small but were of above average height in adulthood and became obese were hypertensive [17]. Thinness at birth, followed by catch-up growth to an average or above-average body mass index by 7 years of age in males, has also been shown to be associated with an increased risk of death from coronary heart disease [18]. More recently, studies in Finland have shown that individuals who developed diabetes were short or thin at birth, had a low growth rate during
infancy and showed accelerated growth (in terms of both weight and height) after 7 years of age [19].

It is relatively well established that obesity is required for the full expression of the thrifty phenotype. However, little attention has been paid to the possibility that obesity may be a consequence of early growth restriction, followed by rapid catch-up growth. It has been shown recently [20] that infants who were growth-restricted in utero and underwent postnatal catch-up growth between birth and 2 years of age were fatter and had more central fat than other children.

In the present study, we show that maternal protein restriction in mice at defined time windows had long-term (‘programmed’) effects on weight gain. Mice that were growth-restricted in utero and then underwent rapid postnatal catch-up growth remained heavier than control offspring throughout the study period. In contrast, mice who were growth-restricted during the lactation period remained permanently smaller than control offspring. We attribute these changes, at least in part, to programmed changes in appetite, as we [21] and others [22,23] have demonstrated in rats by measures of food consumption. Similar observations have been made in humans, where increased rates of postnatal weight gain have been associated with reduced satiety in small for gestational age (SGA) infants (as assessed by volume of milk consumed by bottle-fed infants) [24]. The mechanistic basis of this programming of appetite is not known; however, leptin may play a key role. In humans, it has been shown [25] that cord blood leptin is inversely related to rates of growth during infancy and that SGA infants have lower leptin levels. Changes in energy expenditure may also contribute to changes in weight gain observed in offspring who were growth-restricted in utero [26]. As well as changes in overall body growth, there is also evidence that early growth restriction is associated with changes in body composition. Offspring of protein-restricted rat dams have been shown [27] to have a reduced muscle mass at weaning. Studies in humans have also suggested that low birth weight is associated with reduced lean mass [28]. It is, therefore, possible that poor early nutrition is associated with changes in body composition, which reduce the capability of an individual to perform physical activity.

One of the most striking observations in the present study was the finding that the early programming affected the response of the animals to a highly palatable diet and, therefore, their susceptibility to obesity. Postnatal low-protein animals weaned on to a cafeteria-style diet showed no additional weight gain compared with Chow-fed litter mates. This suggests that the programming effect of poor nutrition during lactation is powerful enough to suppress the effect of a highly palatable diet to increase food consumption in the postnatal low-protein animals. Almost equally powerful was the effect of poor nutrition in utero, followed by a high plane of nutrition during lactation to induce excessive weight gain post-weaning (as observed in the recuperated group).

The potential implications of the present findings for the voluntary drug-free control of weight gain and for the obesity-inducing effects of early nutrition are of great interest and importance.

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REFERENCES