Prenatally undernourished rats show increased preference for wheel running v. lever pressing for food in a choice task

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Maternal nutrition during pregnancy has a significant influence in establishing patterns of metabolism and postnatal behaviours in offspring, and therefore shapes their risk of developing disorders in later life. Although it is well established that a mismatch between food consumption and energy expenditure leads to obesity and metabolic dysregulation, little research has investigated the biological origin of such behaviour. We conducted the present experiments to investigate effects of early-life nutrition on preference between wheel running and lever pressing for food during adult life. To address this issue we employed a well-established experimental approach in the rat which has shown that offspring of mothers undernourished during pregnancy develop obesity and metabolic disorders when kept under standard laboratory conditions. Using this experimental approach, two studies were conducted where offspring of ad libitum-fed dams and dams undernourished throughout pregnancy were given the choice between wheel running and pressing a response lever for food. Across subsequent conditions, the rate at which the response lever provided food was varied from 0.22 to 6.0 (study 1) and 0.19 to 3.0 (study 2) pellets per min. Compared with the control group, offspring from dams undernourished during pregnancy showed a consistently greater preference for running over lever pressing for food throughout both experiments of the study. The results of the present study provide experimental evidence that a mother’s nutrition during pregnancy can result in a long-term shift in her offspring’s lifestyle choices that are relevant to obesity prevention. Such a shift, if endorsed, will have substantial and wide-ranging health consequences throughout the lifespan.

Choice behaviour: Food: Exercise: Obesity: Prenatal nutrition

Obesity and its co-morbidities are commonly related to overindulgence in food and a sedentary lifestyle. The worldwide increase in the incidence of obesity has been so rapid that it cannot be explained by genetic factors alone, and suggests a central role for environmental factors and lifestyle choices that promote energy storage (1,2). It has been suggested that early-life environmental influences can have long-lasting effects on health (3–5). Nutrition during early life is one such factor that may determine developmental pathways and health outcomes in adulthood. Experimentally, prenatal undernutrition has been widely used to investigate biological mechanisms that link prenatal events with long-term health consequences in later life, such as metabolic dysregulation, obesity and CVD (3,5).

Much less is known about effects of the early-life nutrition on behaviour, cognitive function and lifestyle choices during later life (for a review, see Landon et al. (6)). Epidemiological data suggest a positive relationship between birth weight and cognitive function until early adulthood (7,8). Additionally, animal studies have confirmed that early-life events influence behavioural and cognitive development, although there have been conflicting reports on the nature and extent of such differences (6).

Because obesity results from an imbalance between food consumption and energy expenditure, an understanding of its aetiology requires research into the factors that lead to different lifestyle choices. To date, research on the effects of prenatal nutrition on physical activity levels has been equivocal. Using an open-field test with rats, there have been reports that maternal undernutrition both increases (9) and decreases (10) adult levels of apparent locomotor activity. Procedures that arrange concurrently available physical activity and food may be a more precise way of measuring the choice between these commodities. Measures of preference can be obtained by arranging different alternatives and calculating the ratio of the number of responses emitted at each alternative (11).

Abbreviations: AD, offspring of dams fed ad libitum throughout pregnancy; UN, offspring of dams undernourished during pregnancy.
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The aim of the present study was to investigate whether the level of prenatal nutrition influences choice between eating and running in adult life using an operant choice procedure. To reach our goal we employed a well-established experimental approach in the rat which has shown that offspring of mothers which were undernourished during pregnancy develop obesity and altered metabolic regulation in adulthood when food supply is unlimited \(^5,12–15\). Using this experimental approach, two studies were conducted during which offspring of ad libitum-fed dams and of dams undernourished throughout pregnancy were given the choice between wheel running and pressing a response lever for food.

**Methods**

**Animals**

Offspring were bred in accordance with previously published experimental procedures \(^14–16\). Virgin Wistar rats were mated at a mature age of 100 ± 5 d using a rat oestrous cycle monitor (Fine Science Tools, Foster City, CA, USA) to assess the stage of oestrus of the female rats before introducing the male \(^17\). The colony of Wistar rats and the breeders were isolated to prevent a carry-over effect of prenatal undernutrition to the immediate neonatal period, pups from undernourished mothers were cross-fostered onto dams that had received ad libitum feeding throughout pregnancy (undernourished (UN) group). To prevent a carry-over effect of prenatal undernutrition to the immediate neonatal period, pups from undernourished mothers were cross-fostered onto dams that had received ad libitum feeding throughout pregnancy. Thus, UN offspring were provided with the same nutrient-rich environment after birth as the control offspring. This experimental approach generates UN offspring of a significantly reduced birth weight without affecting litter size \(^12,13,15,16,19\).

To allow a direct comparison with our previous studies investigating physiological, metabolic and endocrine consequences of maternal undernutrition during gestation, we generated identical conditions to those reported by our laboratory before \(^20–23\).

Litter size was adjusted to eight pups per litter in all groups to standardise and optimise nutrition of the neonates until weaning. After weaning at 22 d, rats were housed in pairs with ad libitum access to water and chow. All rats were exposed to a 12 h light–dark cycle with lights on at 06.00 hours. The present study was approved by the University of Auckland Animal Ethics Committee.

**Apparatus**

Ten conventional operant chambers for rats (model ENV-007; Med Associates Inc., St Albans, VT, USA) with dimensions 305 × 241 × 292 mm were situated in sound- and light-attenuating shells. The chambers were fitted on the right wall with one retractable response lever and a pellet dispenser.
Across sessions, the average time between food availability was gradually increased to variable-interval 30 s (i.e. on average two food reinforcers delivered per min).

**Experimental procedure.** The experimental procedure consisted of six phases, summarised in Fig. 2. Preference between exercise and food was measured across a range of conditions by allowing unlimited access to the running wheel and varying the rate at which food was delivered for lever pressing. Each phase differed in the rate of food delivery, with the rate arranged in an irregular order across phases. All phases continued until at least twenty sessions had been completed, and/or session ratios of running to lever pressing had stabilised. In addition, replicate conditions were used to determine consistency of responding across phases. Daily behavioural sessions lasted until 45 min had elapsed or until the animal had received the maximum of 60 food pellets, whichever occurred first.

**Body composition and endocrine analysis**

Upon completion of the final experimental phase (see Fig. 2), all animals were placed under halothane anaesthesia to enable body-length measurement, and body composition analysis using dual-energy X-ray absorptiometry scanning (Lunar Prodigy; GE Medical Systems, Madison, WI, USA). Rats were then culled by decapitation under halothane anaesthesia. Blood was collected into heparinised vacutainers and stored on ice (4°C) until centrifugation and removal of supernatant fractions for analysis. Fasting insulin and leptin levels in plasma were measured by ELISA and rat-specific RIA kits (Mercodia AB, Uppsala, Sweden; sensitivity 0·07 mU/l; intra-assay variation 1·2%; inter-assay variation 3·6%) and RL-83K (Linco, St Charles, MO, USA; sensitivity 0·04 ng/ml; intra-assay variation 1·88%; inter-assay variation 3·31%), respectively.

**Statistical analyses**

In order to determine the effect of prenatal undernutrition on preference between running and responding for food, taking food reinforcer rate as a continuous variable, we conducted regression analyses for each individual animal between preference (measured by the log10 of the ratio of cm run to number of lever presses for food) and the log10 of the obtained food reinforcers rates per min for that animal. We fitted a mixed model with prenatal nutrition level (AD, UN) and the log10 of the obtained food rate as fixed terms, and rat within treatment and study as a random term using the REMEL algorithm in the package GENSTAT® (VSN International Ltd, Hemel Hempstead, UK). This model assumes a different regression line for each animal. The slopes and intercepts for the regression lines for individual rats within a treatment group are assumed to vary randomly about the parameters for the group regression lines. The value of $R^2$ of each group regression line was calculated to determine goodness-of-fit. Physiological data were analysed by one-way ANOVA with post hoc analysis using Fisher’s protected least significant difference test using Stat-View software (Abacus Concepts Inc., Berkeley, CA, USA). All data are presented as mean values with their standard errors unless otherwise stated. Values of $P<0·05$ were considered statistically significant.

**Results**

**Body weight, food intake and plasma analyses**

Physiological parameters from both studies are presented in Table 1. Consistent with previous studies using this model of fetal undernutrition, UN offspring from both studies were significantly shorter and lighter than AD offspring at birth ($P<0·001$). Furthermore, UN offspring remained shorter than their AD counterparts throughout life.

No differences in body fat mass before cull, as assessed by dual-energy X-ray absorptiometry, were found (Table 1). Food intake per g body weight during adult life did not differ between the two prenatal nutrition groups (representative food intake data are presented at day 150 in Table 1).

Although plasma insulin concentrations appeared to be slightly elevated in UN offspring from both studies (see Table 1), no statistically significant differences were observed in either plasma insulin or leptin levels when analysing the two studies independently. However, when the two studies were combined and analysed by two-way ANOVA, prenatal undernutrition was associated with hyperinsulinaemia ($P<0·05$). In addition, when using the combined analysis,
Table 1. Physiological parameters and plasma endocrine analyses* 
(Mean values with their standard errors)

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>UN</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td><strong>Study 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>Mean</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>6.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Weaning (day 22)</td>
<td>50.1</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Body length</td>
<td>502.7</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>Nose–tail length</td>
<td>515</td>
<td>2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Body fat content (%)</td>
<td>38.9</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Food intake at day 150 (kJ/g body weight per d)</td>
<td>0.518</td>
<td>0.015</td>
<td>0.545</td>
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<tr>
<td><strong>Study 2</strong></td>
<td></td>
<td></td>
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<tr>
<td>Body weight (g)</td>
<td>Mean</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>6.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Weaning (day 22)</td>
<td>50.1</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Body length</td>
<td>502.7</td>
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</table>

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There was no ‘between-study effect’ on plasma insulin (P=0.54). No significant difference in plasma leptin between the combined AD and UN groups was observed (P=0.17).

Wheel running

Fig. 3 shows cm run per min in each phase of the experiment. Running rates in the UN groups were consistently higher than those for the AD groups in both study 1 (Fig. 3 (a); P<0.0005) and study 2 (Fig. 3 (b); P<0.0005). Running rates during phases with identical food delivery rates (i.e. phases 1 and 6 of study 1, and phases 2 and 6 of study 2) did not differ significantly from each other, thus providing repeatability of the behavioural measures. There was no systematic relationship between body weight and running, and there was no effect of running rate on food intake at any time during either study (data not presented).

Lever pressing for food

Fig. 4 shows lever presses per min in each phase of the experiment. Rates of lever pressing increased with increasing rates of food delivery during both study 1 (Fig. 4 (a); P<0.0001) and study 2 (Fig. 4 (b); P<0.001). In addition, the lever pressing rates of the UN groups were consistently higher than those for the AD groups in both studies (study 1, P<0.05; study 2, P<0.05).

Preference between wheel running and lever pressing for food

The mean data and mean fitted regression lines for each group of animals are shown in Fig. 5. Because we fitted a mixed model linear regression, each rat had its own regression line, and within each treatment group, the regression lines are clustered about a common mean and intercept. The use of a mixed model with random slopes and intercepts for each individual within a treatment group results in the mean slopes and intercepts experiencing shrinkage toward zero. Consequently, the mean slopes (plotted as continuous lines on Fig. 5) will appear flatter than a line fitted to the plotted points. The fitted regression lines maximised R², with 88.28 and 99.04% of the variance explained by the regression for the AD and UN groups, respectively. The degree of preference for running v. lever pressing for food decreased with increasing food rates for both groups of animals, shown by the regression slopes being significantly less than 0 (P<0.001). The way in which these preferences changed across food reinforcer rates did not differ significantly between AD and UN offspring (AD slope = -0.99; UN slope = -0.91; P=0.383). Importantly, preference for wheel running over lever pressing for food was significantly higher for the UN offspring compared with AD offspring across the entire range of food reinforcer rates (P=0.002). This is illustrated by the vertical shift between the fitted regression lines shown in Fig. 5. Results from study 1 and study 2 were similar in these respects. The obtained reinforcer rates (the rate at which food pellets were delivered) did not differ between the AD and UN groups (P=0.79).

Discussion

We previously established a robust animal model in which maternal undernutrition leads to low-birth-weight offspring that develop obesity and metabolic dysregulation during adult life. However, in these studies animals were maintained under laboratory conditions where exercise was limited to movement around a standard laboratory cage. The present results show that, when rats were offered a choice between exercise (wheel running) and lever pressing for food, the offspring of undernourished mothers exhibited a greater preference for wheel running compared with offspring of normally nourished mothers. The expression of choice between wheel running and lever pressing for food was assessed over an extended period of time and under a broad range of parametric manipulations, spanning a near 30-fold range of food reinforcement rates. Furthermore, behaviour
was assessed at stability and excludes the possibility of novelty effects commonly seen in brief assessments\(^6\). The long-lasting and stable preference for exercise observed in the present studies provides the first direct experimental evidence that prenatal nutrition may influence later lifestyle choices throughout adult life.

The results of the present study indicate that, despite an apparent predisposition to develop obesity\(^{15}\) when environmental conditions during postnatal life do not encourage exercise, prenatally undernourished offspring have an increased preference for exercise over feeding when the opportunity to express this choice is available. Furthermore, with the ability to express choice, UN offspring exercised more than controls during the daily 1 h sessions and did not develop the obesity and hyperleptinaemia that is consistently observed under standard (sedentary) laboratory conditions\(^{5,14-16,27}\). We speculate that this shift in lifestyle choice, if encouraged, is likely to have major health benefits and may prevent the onset of apparently pre-programmed obesity. However, it is not clear from the data of the present study whether the lack of obesity development and its associated metabolic dysregulation were directly related to the increase in physical activity. Future behavioural nutrition research should address this possibility by using standardised exercise protocols. Combining the results of the two present studies, the UN offspring maintained hyperinsulinaemia irrespective of exercise. We have made similar observations in a previous study where elevated insulin secretion was maintained despite an energy-restricted diet, and this was associated with enhanced insulin action\(^{15}\). It is tempting to speculate that this prenatally induced hyperinsulin secretion and enhanced insulin action in UN offspring may increase their ability to sustain exercise, although more work is required to investigate muscle metabolic function in this setting\(^{28}\).

The change in choice behaviour observed in the present study is in agreement with a recent study conducted in our laboratory, which found offspring of undernourished mothers to be less adaptive to change in relative (food) reinforcer rates.
an increased choice for exercise on long-term potentiation requires further exploration\(^{37}\).

While it is generally accepted that maternal nutrition during gestation can influence metabolic regulation in offspring throughout later life\(^{3,27}\) the present study provides experimental evidence that maternal nutrition during pregnancy can also influence choice behaviour that is relevant to obesity prevention. When given the choice, offspring of dams undernourished during pregnancy showed consistently higher preference for wheel running over lever pressing for food compared with controls, even though they resembled controls in body fat composition. These results suggest that nutritional or dietary factors during early development may result in a permanent shift in the nature of lifestyle choices. Such a shift will have substantial and wide-ranging health consequences throughout the lifespan. The present study also highlights that the availability and endorsement of exercise is a vital factor for the prevention of obesity and metabolic dysregulation. Therefore, the present study generally suggests the need for careful consideration of the availability of exercise for the interpretation of experimental studies that investigate dietary or pharmacological treatment or prevention of obesity. The present study also suggests that public health initiatives that intend to curb the global obesity epidemic should pay close attention to increasing the availability and the endorsement of exercise.

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References


