Early nutrition and leptin concentrations in later life1–3

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ABSTRACT

Background: Formula feeding or overweight in infancy may increase the later risk of obesity, but the mechanisms involved are uncertain. Because obesity is associated with high leptin concentrations relative to fat mass, programming of leptin concentrations may be one mechanism by which early nutrition influences later obesity.

Objective: We tested the hypothesis that high nutrient intake or formula feeding in infancy programs greater leptin concentrations relative to fat mass in later life.

Design: Serum leptin concentrations were measured by radioimmunoassay in 197 adolescents aged 13–16 y who were born preterm and randomly assigned at birth to receive either a nutrient-enriched preterm formula or banked donated breast milk (trial 1) or a preterm formula or a standard formula (trial 2). Fat mass was estimated with the use of bioelectrical impedance analysis.

Results: After combining the results of trials 1 and 2 as planned, the ratio of leptin to fat mass was significantly greater in the children who received the preterm formula (geometric mean: 0.84 μg·L⁻¹·kg⁻¹) than in those who received standard formula or banked breast milk (0.62 μg·L⁻¹·kg⁻¹; mean difference: 30.8%; 95% CI for difference: 8.4%, 53.2%; P = 0.007). The difference between the diet groups remained significant after adjustment for age, sex, Tanner stage, social class, and fat mass. Human milk intake was significantly associated with lower leptin concentrations relative to fat mass in adolescence (P = 0.023), independent of potential confounding factors.

Conclusion: Programming of relative leptin concentrations by early diet may be one mechanism that links early nutrition with later obesity. Am J Clin Nutr 2002;75:993–9.

KEY WORDS  Leptin, obesity, breast-feeding, formula feeding, adolescents, preterm infants, fat mass

INTRODUCTION

The global epidemic of childhood obesity is a major public health issue (1). Although a positive energy balance is likely to be a final common pathway for obesity, the determinants of this imbalance are debated. Secular changes in eating behavior and genetic susceptibility are factors that have received much recent attention. However, programming, the process by which factors acting during early life may have a longer-term effect on health, has been suggested as a further potentially important mechanism that could contribute to the development of obesity (2). For instance, adults who were conceived during the Dutch famine and whose mothers experienced poor nutrition in the first and second trimesters of their pregnancies were more likely to be obese (and those whose mothers experienced poor nutrition in the last trimester, leaner) than were their peers whose mothers did not experience poor nutrition (3, 4). The greater propensity to obesity in later life seen in children who are heavier at birth (5–9) and the greater central fat distribution seen in children with low birth weight (10–13) also suggest that fetal life is a critical window for programming of later body fatness.

However, the contribution of the early postnatal environment, particularly early nutrition, to the development of obesity has received relatively little attention. An effect of nutrition is suggested by the association between greater weight gain in infancy and a slightly higher risk of obesity in adulthood (14), by the relation between the early introduction of solid foods and increased body fatness at 7 y of age (15), and by the finding that breast-feeding is associated with a lower risk of later obesity (16–18). The association between a vigorous breast-feeding style, which increases energy intake, with greater adiposity at 6 y of age (19) suggests a role for overnutrition in the programming of obesity. Similarly, the relation between early catch-up growth, which may be associated with increased food intake, and later obesity (20) is consistent with the hypothesis that overnutrition in infancy increases the later propensity to obesity. Experimental data from animal models support this underlying hypothesis; rats that were food restricted before weaning were permanently lighter regardless of how much food was available after weaning (21), whereas baboons overfed before weaning were more likely to be obese in early adult life (22).

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2 Formulas were a gift from Farley Health Products (a division of HJ Heinz Company Ltd, Stockley Park, Uxbridge, United Kingdom).

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The mechanisms by which early factors influence later obesity are poorly understood, however. Hypothalamic dysfunction rather than abnormalities of adipocytes has been suggested to affect later obesity (4, 23), and leptin, a key regulator of body fatness (24–28), has been proposed as a potential candidate for this hormonal programming (29, 30). Because obesity is associated with high relative leptin concentrations (27), we tested the hypothesis that high nutrient intake in infancy programs greater leptin concentrations relative to fat mass in later life. A cohort of children born preterm and randomly assigned at birth to receive different diets provided a unique opportunity to test our hypothesis in an experimental study with prospective follow-up (31).

SUBJECTS AND METHODS

Subjects consisted of a subset of a large cohort of infants born preterm who had participated in randomized trials that investigated the influence of early diet on later cognitive function (32) and cardiovascular risk factors (33). Between 1982 and 1985, infants who were free of major congenital anomalies and who weighed <1850 g at birth were recruited in 5 centers (Norwich, Cambridge, Sheffield, Ipswich, and King’s Lynn) into 1 of 2 parallel randomized trials. In trial 1 subjects received either a nutrient-enriched preterm formula (Farley’s Osterprem; Farley Health Products (a division of HJ Heinz Company Ltd, Stockley Park, Uxbridge, United Kingdom) or breast milk donated by unrelated lactating women, and in trial 2 subjects received either the same preterm formula or a standard term formula (Farley’s Ostermilk; Crookes Health Care). Within each trial the diets were randomly assigned in 2 strata: either by themselves (stratum A) or, for mothers who elected to express their own milk, as supplements to the mothers’ milk (stratum B) (Figure 1).

To compare the nutrient-enriched preterm formula with the standard diets as originally planned (34), trials 1 and 2 (and strata A and B within each trial) were combined as a balanced addition, thereby preserving randomization. Subjects were randomly assigned to diet groups within 48 h of birth with the use of opaque sealed envelopes as described previously (31, 32). Ethical approval for the study was obtained from each center, and informed consent was obtained from each parent (no parent refused consent).

The assigned diets were given until the infants weighed 2000 g or were discharged to their homes. Compared with the standard formula (15 g protein/L and 38 g fat/L), the preterm formula was enriched in protein and fat (20 g protein/L and 49 g fat/L) but not carbohydrate (70 g/L in both formulas) (31–33). The preterm formula was also enriched in vitamins, zinc, and copper (32, 33). For infants fed banked donated milk, protein and energy intakes were estimated from 600 donor milk pools collected from multiple donors (≈11 g protein, 20 g fat, and 70 g carbohydrate/L). The composition of the mothers’ own expressed milk was measured in 4935 complete 24-h collections (≈15 g protein, 30 g fat, and 70 g carbohydrate/L).

Extensive demographic, social, anthropometric, biochemical, and clinical data were collected throughout the hospital admission as previously described (31–33). Infants were weighed daily by trained staff, and the weights were expressed as absolute values and as SDs from expected weights (z scores) with the use of birth-weight centiles from preterm infants (35). The z score for weight at discharge (or at discontinuation of the assigned diet) was also derived and represented both intrauterine and extrauterine growth. Social class was assessed on the basis of the occupation of the parent providing the main financial support for the family (or, if both parents worked, on the father’s occupation) in accordance with the Registrar General’s Classification (33).
Follow-up

The follow-up at age 13–16 y involved separate comparisons of cardiovascular outcomes in trials 1 and 2 and a comparison of leptin concentrations relative to fat mass between the subjects fed the preterm formula and those fed 1 of the 2 available standard diets (trials 1 and 2 combined). Our planned minimum target recruitment was estimated to exclude a one-half SD difference in outcomes between the diet groups in each of the 2 trials. To detect this difference (with 2 parallel trials), we required a maximum sample of ≈250 subjects at 80% power and 5% significance and a minimum sample of ≈200 subjects at 70% power and 5% significance (34). A subsample of 216 subjects agreed to participate in our first recruitment drive and was shown to be representative of the whole cohort (36). No further recruitment was undertaken because this sample was sufficient to meet our minimum criteria and to exclude a one-third SD difference between the diet groups in the ratio of leptin to fat mass at 80% power and 5% significance (34). Ethical approval for the follow-up was obtained from national and local research ethical committees, and written consent was obtained from all the adolescents and their guardians.

Fat mass and anthropometry

All researchers and subjects were blinded to the original dietary assignments. After subjects fasted overnight and were supine for 15–20 min, their fat mass was determined with the use of bioelectric impedance analysis (EZ Comp 1500; Fitness Concepts Inc, Park City, UT). Electrodes were attached, in 2 pairs, to the right hand and foot in a tetrapolar arrangement in accordance with the manufacturer’s instructions. The percentage of body fat was derived from impedance, height, weight, sex, and age with the use of the manufacturer’s internal algorithm.

Height was measured with the use of a portable stadiometer (Holtain Ltd, Crymych, United Kingdom) accurate to 1 mm; weight, with the use of an electronic scale accurate to 0.1 kg (Seca, Hamburg, Germany); and skinfold thicknesses, with the use of Holtain calipers and standard procedures. Most (99%) measurements were made by 1 of 2 observers trained in the techniques involved. Equipment was calibrated before each field site visit, and the measuring technique of observers was monitored throughout. The z score for body mass index, calculated with the use of standard centile charts, was used in the analyses. Subjects assessed their Tanner stage themselves in private with the use of standard Tanner stage photographs, and the mean of the Tanner stage for pubic hair and genitalia was used in the analyses (37).

Leptin

Blood was obtained by venipuncture between 0900 and 1100 after subjects fasted overnight. Serum was separated immediately, stored initially at −20°C and then at −80°C, and thawed only once immediately before analysis. Serum leptin concentrations were determined with the use of a commercially available radioimmunoassay (Linco Research Inc, St Charles, Mo) with a detection limit of 0.5 μg/L (intraassay and interassay CVs of 2% and 5%, respectively).

Statistical analysis

The ratio of leptin to fat mass was compared between the diet groups. The appropriateness of this ratio was assessed with the use of a log-log regression of leptin against fat mass, which gave a slope of 0.7. We repeated the analysis by using leptin/fat mass0.7 and also after adjusting analyses of ln (leptin/fat mass) for ln fat mass. Comparisons of normally distributed variables between the diet groups were made with Student’s t test (fat mass was transformed by taking the square root). The distribution of leptin concentration per kilogram fat mass (38) (or per percentage of fat mass; 39) was ln transformed and then multiplied by 100. Therefore, comparisons between the diet groups represented the mean percentage difference between groups (40). Regression analyses were used to adjust for possible baseline differences, with the coefficient representing the percentage change in leptin relative to fat mass per unit change in independent variable (40). Statistical significance was assumed as P < 0.05 for significance tests, which were all two-tailed. All data were analyzed with the use of SPSS (version 8.0; SPSS Inc, Chicago).

RESULTS

Preliminary analyses

Representativeness of sample

The numbers of subjects followed up in the 2 trials of dietary intervention are given in Figure 1. For both trials, the children who were followed up at age 13–16 y did not differ significantly from those who were not followed up in terms of their birth weight, gestation, social class, birth weight or discharge weight z scores, or clinical variables (eg, number of days of ventilation) (Table 1). Thus, no significant selection bias in our study sample was detected (36).

Leptin and fat mass

As expected, ln leptin concentrations were significantly related to fat mass in the whole group (regression coefficient: 8.5% increase/1-kg increase; 95% CI: 7.3%, 9.7%; P < 0.001) and in the children randomly assigned to receive the standard diets (regression coefficient: 8.7% increase/1-kg increase; 95% CI: 6.7%, 10.8%; P < 0.001) or the nutrient-enriched diet (regression coefficient: 8.3% increase/1-kg increase; 95% CI: 6.9%, 9.7%; P < 0.001).

Comparisons between the diet groups

The characteristics of the subjects who were followed up in adolescence are compared in Table 2. Fewer children were followed up in trial 2 than in trial 1. As expected, the children who received nutrient-enriched formula at birth had greater neonatal weight gain, as indicated by their discharge weight z score, than did those who received the standard diets (Table 2). However, there were no significant differences in weight, fat mass (Table 2), or Tanner stage (mean: ≈4) between the diet groups at follow-up.

The trial-by–diet group interaction for leptin concentrations relative to fat mass was not significant, which justified combining trials 1 and 2. In the combined analysis, the leptin concentration relative to fat mass and to the percentage of fat mass was greater in the children who received the preterm formula than in those who received 1 of the 2 standard diets (Table 2). However, there were no significant differences in weight, fat mass (Table 2), or Tanner stage (mean: ≈4) between the diet groups at follow-up. The ratio of leptin to fat mass was also greater in the children who received the preterm formula than in those who received term formula (trial 2), but the difference between those who received the preterm formula and those who received banked breast milk (trial 1) was not significant (Table 3). Similar observations were made for leptin expressed relative to the percentage of fat mass (Table 3).
Comparison of subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trials 1 and 2 combined</th>
<th>Trial 1</th>
<th>Trial 2</th>
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<td></td>
<td>Preterm formula (n = 106)</td>
<td>Standard diet (n = 110)</td>
<td>Preterm formula (n = 64)</td>
</tr>
<tr>
<td>At follow-up</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
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<td>15.0 ± 0.9</td>
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<tr>
<td>Weight (kg)</td>
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<td>55.8 ± 10.0</td>
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<td>Height (cm)</td>
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<td>161.8 ± 9.7</td>
<td>160.8 ± 9.4</td>
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<tr>
<td>BMI (kg/m(^2))</td>
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<td>21.3 ± 3.8</td>
<td>21.1 ± 3.9</td>
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<tr>
<td>Fat mass (kg(^d))</td>
<td>9.7 ± 1.4</td>
<td>10.5 ± 1.1</td>
<td>9.8 ± 1.3</td>
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<tr>
<td>Percentage of fat (%)</td>
<td>19.9 ± 1.3</td>
<td>20.4 ± 1.0</td>
<td>19.7 ± 1.18</td>
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<td>Neonatal Growth</td>
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<tr>
<td>Birth weight (kg)</td>
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<td>1.4 ± 0.3</td>
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<td>Gestation (wk)</td>
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<td>30.9 ± 2.7</td>
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<td>Birth weight z score</td>
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<tr>
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<td>−2.2 ± 0.9(^d)</td>
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<td>Apgar score at 5 min</td>
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<td>7.9 ± 1.8</td>
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<td>No. of infections(^d)</td>
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<td>1.0 (1.0)</td>
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</tr>
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</table>

\(^a\) SD reported as the square root.
\(^b\) Significantly different from preterm formula, \(P < 0.05\).
\(^c\) Median; interquartile range in parentheses.
\(^d\) Based on the number of courses of antibiotics.
Similar results were obtained for the ratio of leptin to percentage of fat mass (data not shown).

**Analyses without randomization**

**Human milk intake and later relative leptin concentrations**

The volume and percentage of human milk (mother’s expressed breast milk plus banked donated breast milk) consumed in the neonatal period were significantly and negatively related to later leptin concentration relative to fat mass (for volume, regression coefficient: −3% change/1-L increase in human milk intake; 95% CI: −6%, −0.4%; P = 0.023; for percentage, regression coefficient: −3.2% change/10% increase in human milk intake; 95% CI: −5.8%, −0.5%; P = 0.018; [Figure 2]). The association of leptin concentration relative to fat mass with the volume of human milk intake remained significant after adjustment for potential confounding factors [age, sex, pubertal stage, and social class (as above) together with fat mass, body mass index z score, and mother’s education] (regression coefficient: −3% change/1-L increase in human milk intake; 95% CI: −5%, 0%; P = 0.020), but the association with the percentage of human milk intake failed to reach significance (regression coefficient: −2.3% change/10% increase in human milk intake; 95% CI: −5.0%, 0.0%; P = 0.09). Similar associations were obtained between the volume and percentage of human milk intake and the ratio of leptin to the percentage of fat mass (data not shown).

**Birth weight and later leptin concentrations**

Leptin concentrations relative to fat mass were not significantly related to birth weight z score (regression coefficient: 4.0% change per birth weight z score increase; 95% CI: −5.6%, 13.6%) or to discharge weight z score (regression coefficient: 2.0% change per birth weight z score increase; 95% CI: −9.5%, 13.6%).

**DISCUSSION**

The leptin concentration relative to fat mass was 30% greater in the children who received a preterm formula at birth than in those who received 1 of the 2 standard diets. This difference was independent of population differences at birth or in adolescence, and given the experimental design, strongly supported an influence of early diet on later leptin concentrations. Therefore, infancy, at least in preterm infants, could be a critical window for programming later leptin physiology and by inference the risk of obesity.

Associations of low birth weight with higher leptin concentrations relative to fat mass suggest that leptin physiology may be programmable (29, 30). However, unlike the results of previous studies with children born at term, low birth weight in our preterm cohort was not associated with higher leptin concentrations in adolescence, possibly because the antenatal programming of leptin concentration, like the programming of obesity (4), differs according to the timing of the in-utero insult. Alternatively, higher leptin concentrations as a consequence of puberty may have obscured any association between birth weight and later leptin concentrations in the present study, and this may also explain why the present study found higher leptin concentrations relative to fat mass than did studies with adults (38).
In contrast to prenatal factors, the influence of early postnatal nutrition on later leptin concentrations or obesity has not been researched extensively. Moreover, the results of previous epidemiologic studies that showed associations between early nutrition and later obesity may have been confounded by environmental factors (such as socioeconomic status) that affect both early diet and the later risk of obesity. The novelty of the present study therefore lies in its experimental design, in which infants were randomly assigned at birth to receive diets with different nutrient compositions. Thus, controlling for possible confounding factors, we found that dietary manipulation for an average of only 1 mo markedly influenced leptin concentrations relative to fat mass up to 16 y later. These differences were seen in the combined comparison between the nutrient-enriched and standard diets, in the comparison between the preterm formula and the term formula (trial 2), and in the comparison between the preterm formula and banked donated breast milk (trial 1), although the difference in the latter comparison was not significant. Our further observational analysis that showed an association between consumption of human milk (maternal plus banked breast milk) and lower leptin concentrations relative to fat mass was consistent with the hypothesis that leptin concentrations are programmable by early diet.

Potential limitations

We studied a preterm population; whether our findings can be generalized to infants born at term requires confirmation. Our studies with children born at term are in progress. Nevertheless, associations between diet and later relative hyperleptinemia in the present study and between birth weight and later obesity in earlier studies (9, 10, 13) were independent of gestation. Although we studied only a subsample of our original study population, our sample proved representative of those recruited at birth, and the subject characteristics of the diet groups did not differ significantly. As discussed previously, our population was similar to the general population in terms of social class (33), a key consideration in studies of programming of body fatness. Furthermore, although by necessity we had an indirect and relatively imprecise technique for measuring body fat, bioelectrical impedance analysis allowed us to compare our results with those of previous studies that used a similar technique (38, 39) and also provided a measure of total fat mass rather than just subcutaneous fat mass, with the measurement of skinfold thickness. We found concentrations of leptin relative to fat mass similar to those published previously (38, 39). Finally, the experimental design of the present study should have reduced any bias associated with errors in the measurement of body fat.

Possible mechanisms

Hypothalamic and endocrine regulatory mechanisms, which may be programmable (41), have been proposed as a mechanism underlying the programming of obesity (4, 23). We postulate that the higher leptin concentrations associated with greater body fatness early in postnatal life program the leptin-dependent feedback loop such that the regulation of body fat is less sensitive to leptin in later life. The physiologic mechanisms for this may involve down-regulation of hypothalamic leptin receptors or postreceptor effector mechanisms. Such mechanisms would be analogous to those seen in animal studies, in which hypothalamic leptin receptors are up-regulated when there is a lack of functioning leptin (42) and in which expression of receptors within the hypothalamus of food-restricted ewes is greater than that in well-fed ewes, probably because of down-regulation of leptin receptors in well-fed ewes (43). Therefore, if exposed to an environment favoring a positive energy balance, persons who had high leptin concentrations and greater body fatness early in postnatal life would be more susceptible to greater body fatness, particularly when there is physiologic leptin insensitivity and an increase in body fat such as at puberty.

Implications

The influence of relative hyperleptinemia on the propensity for obesity in a nonobese population is uncertain. The tendency to gain weight in some (29, 44) but not all (45, 46) nonobese populations with high leptin concentrations at baseline may represent leptin insensitivity (or leptin resistance) or be an early indicator of positive energy balance. However, despite their higher leptin concentrations relative to fat mass, adolescents previously fed the nutrient-enriched diet were not fatter at age 13–16 y and whether they will become so is a key aspect for further follow-up. Nonetheless, it seems likely that factors acting in childhood may promote obesity in early adulthood (5), and we postulate therefore that insensitivity to leptin in persons who receive a nutrient-enriched diet may program the commonest onset of obesity, which is that seen in early adult life. The lack of an influence of early diet on obesity at age 7–8 y in the same cohort (47) is consistent with our hypothesis and with animal data that suggest that the programming effects of early diet on later obesity may not emerge until adult life (22). Furthermore, the lower leptin resistance of infants fed human milk may provide one potential mechanism for the long-term benefit of breast-feeding on adiposity (16–18).

Animal models support an effect of the preweaning diet on the later propensity to obesity. Greater early food intake in rats (48) and baboons (22) increased the later risk of adiposity, and, as in humans, this effect may be mediated by long-term programming of metabolism by a nutrient-enriched diet in early life (49). In addition, consistent with our data in humans, rats overfed before weaning were shown to be overweight and hyperleptinemic and to have hypothalamic leptin resistance in later life (50).

Conclusions

Although there was no difference in fat mass, leptin concentrations relative to fat mass were greater in the 13–16-y-old adolescents who were randomly assigned at birth to receive a nutrient-enriched diet than in those who were randomly assigned to receive 1 of the 2 standard diets. We postulate that leptin concentrations can be programmed by early diet and that this is one mechanism that links diet in infancy to the risk of obesity in adult life.

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