

Early nutrition and leptin concentrations in later life¹⁻³

Atul Singhal, I Sadaf Farooqi, Stephen O'Rahilly, Tim J Cole, Mary Fewtrell, and Alan Lucas

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 \bar{x} : $0.84 \mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg}^{-1}$) than in those who received standard formula or banked breast milk ($0.62 \mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg}^{-1}$; 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 impor-

tant 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).

¹From the MRC Childhood Nutrition Research Center (AS, MF, and AL) and the Department of Pediatric Epidemiology and Biostatistics (TJC), Institute of Child Health, London, and the University Department of Medicine, Addenbrooke's Hospital, Cambridge, United Kingdom (ISF and SOR).

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

³Reprints not available. Address correspondence to A Singhal, Institute of Child Health, 30 Guilford Street, WC1N 1EH London, United Kingdom. E-mail: a.singhal@ich.ucl.ac.uk.

Received April 5, 2001.

Accepted for publication July 10, 2001.

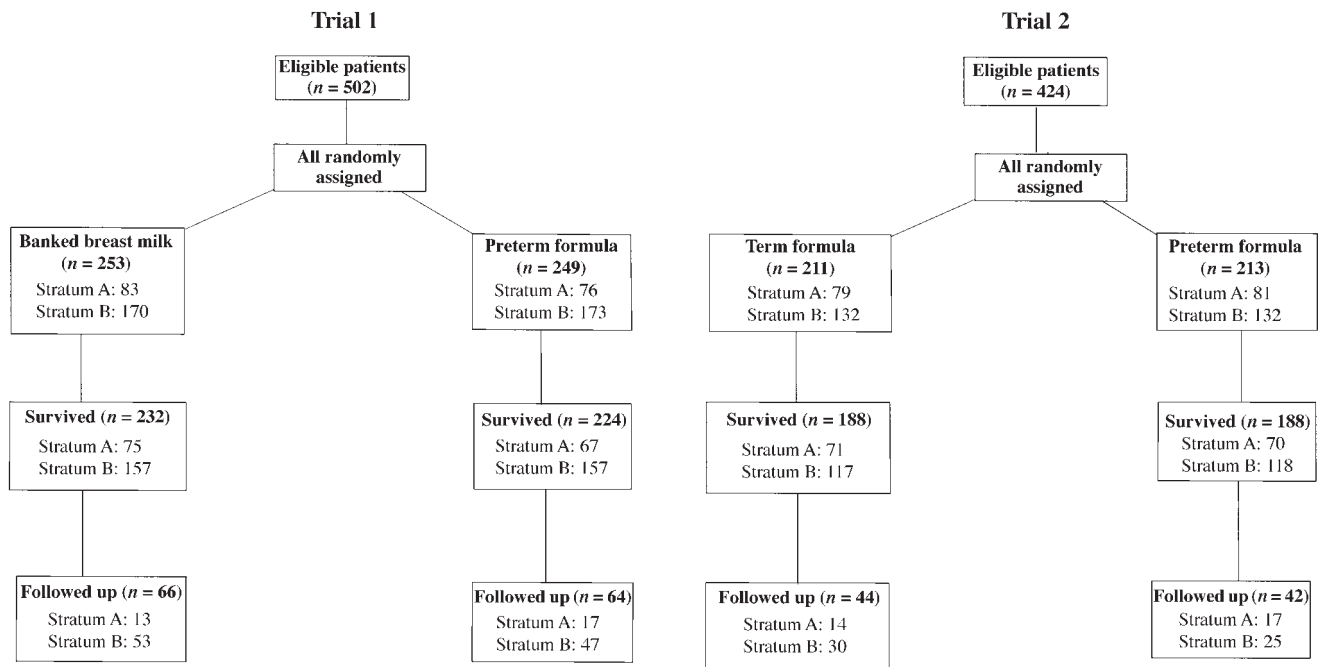


FIGURE 1. Schematic of trials 1 and 2. In stratum A, subjects received the assigned diet only. In stratum B, subjects received the assigned diet as a supplement to their mothers' expressed breast milk.

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 Osterpremi; 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 (\approx 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 (\approx 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, Croswell, Crymch, 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 $\mu\text{g/L}$ (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

mass^{0.7} 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 3). 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).

TABLE 1
Some characteristics of the children followed up and not followed up in adolescence¹

| Variable | Trial 1 (preterm formula or banked breast milk) | | Trial 2 (preterm formula or term formula) | |
|----------------------------------|---|------------------|---|------------------|
| | Followed up | Not followed up | Followed up | Not followed up |
| Growth | | | | |
| Birth weight (kg) | 1.4 ± 0.3 ² [130] | 1.4 ± 0.3 [372] | 1.4 ± 0.3 [86] | 1.4 ± 0.3 [338] |
| Gestation (wk) | 31.1 ± 2.6 [130] | 30.7 ± 2.9 [372] | 30.7 ± 2.8 [85] | 30.8 ± 2.9 [338] |
| Birth weight z score | -1.0 ± 1.2 [130] | -0.7 ± 1.3 [372] | -0.8 ± 1.1 [85] | -0.7 ± 1.3 [338] |
| Discharge weight z score | -2.1 ± 1.0 [130] | -2.0 ± 1.1 [367] | -2.1 ± 1.0 [85] | -2.1 ± 1.0 [338] |
| Demographic and clinical | | | | |
| Social class | 3.4 ± 1.5 [130] | 3.6 ± 1.9 [360] | 3.5 ± 1.6 [84] | 3.8 ± 1.8 [324] |
| Apgar score at 5 min | 8.3 ± 1.7 [124] | 8.0 ± 1.9 [353] | 7.8 ± 1.8 [80] | 8.0 ± 2.0 [323] |
| Ventilation (d) ³ | 0.0 (4.0) [130] | 1.0 (5.0) [370] | 1.0 (4.0) [86] | 1.0 (6.0) [338] |
| No. of infections ^{3,4} | 1.0 (1.0) [130] | 1.0 (1.0) [370] | 1.0 (1.0) [86] | 1.0 (1.0) [338] |

¹The total number of subjects in the cohort was 926. *n* in brackets. There were no significant differences between groups in either trial.

² $\bar{x} \pm$ SD.

³Median; interquartile range in parentheses.

⁴Based on the number of courses of antibiotics.

Analysis by sex

The interaction between sex and diet was not significant, which justified combining the sexes in our analyses.

Adjustment for potential confounding factors

Leptin concentrations relative to fat mass in the children who received the preterm formula remained significantly greater than in those who received 1 of the 2 standard diets after adjustment for potential confounders (age, sex, pubertal stage, and social class); the adjusted means for the children who received the preterm formula and for those who received the standard diets were 0.82 and 0.61 $\mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg}^{-1}$, respectively ($P = 0.009$). The difference between the diet groups remained significant

after adjustment for potential confounders (as above) together with fat mass (the adjusted means for the children who received the preterm formula and for those who received the standard diets were 0.78 and 0.62 $\mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg}^{-1}$, respectively; $P = 0.019$), skinfold thickness (the adjusted means were 0.82 and 0.61 $\mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg}^{-1}$, respectively; $P = 0.009$), or body mass index z score (the adjusted means were 0.82 and 0.60 $\mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg}^{-1}$, respectively; $P = 0.009$). The difference in leptin concentration relative to fat mass also remained significant after adjustment for potential confounders (as above) together with gestation (the adjusted means for the children who received the preterm formula and for those who received the standard diets were 0.82 and 0.61 $\mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg}^{-1}$, respectively; $P = 0.010$).

TABLE 2
Comparison of subject characteristics

| Variable | Trials 1 and 2 combined | | Trial 1 | | Trial 2 | |
|----------------------------------|--------------------------------------|------------------------------------|-------------------------------------|--|-------------------------------------|----------------------------------|
| | Preterm formula (<i>n</i> = 106) | Standard diet (<i>n</i> = 110) | Preterm formula (<i>n</i> = 64) | Banked breast milk (<i>n</i> = 66) | Preterm formula (<i>n</i> = 42) | Term formula (<i>n</i> = 44) |
| At follow-up | | | | | | |
| Sex: males (<i>n</i> [%]) | 45 [42] | 52 [57] | 32 [50] | 32 [49] | 13 [31] | 20 [45] |
| Age (y) | 15.0 ± 0.9 ¹ | 15.0 ± 0.9 | 15.1 ± 1.0 | 15.2 ± 0.9 | 14.8 ± 0.8 | 14.8 ± 0.8 |
| Weight (kg) | 55.0 ± 11.3 | 55.8 ± 10.0 | 55.0 ± 12.2 | 53.9 ± 9.9 | 54.9 ± 10.1 | 58.6 ± 9.7 |
| Height (cm) | 161.2 ± 8.6 | 161.8 ± 9.7 | 160.8 ± 9.4 | 161.3 ± 10.2 | 161.9 ± 7.3 | 162.5 ± 8.9 |
| BMI (kg/m ²) | 21.0 ± 3.6 | 21.3 ± 3.8 | 21.1 ± 3.9 | 20.8 ± 3.9 | 20.9 ± 3.2 | 22.2 ± 3.5 |
| Fat mass (kg) ² | 9.7 ± 1.4 | 10.5 ± 1.1 | 9.8 ± 1.3 | 8.9 ± 1.0 | 9.6 ± 1.5 | 13.2 ± 1.0 |
| Percentage of fat (%) | 19.9 ± 13.1 | 20.4 ± 10.2 | 19.7 ± 11.8 | 18.0 ± 9.5 | 20.1 ± 15.2 | 23.8 ± 10.2 |
| Neonatal | | | | | | |
| Growth | | | | | | |
| Birth weight (kg) | 1.4 ± 0.3 | 1.4 ± 0.3 | 1.3 ± 0.3 | 1.4 ± 0.3 | 1.4 ± 0.4 | 1.3 ± 0.3 |
| Gestation (wk) | 31.1 ± 2.7 | 30.9 ± 2.7 | 31.2 ± 2.6 | 31.1 ± 2.5 | 30.9 ± 2.8 | 30.6 ± 2.9 |
| Birth weight z score | -0.9 ± 1.2 | -0.8 ± 1.2 | -1.1 ± 1.2 | -0.8 ± 1.2 | -0.7 ± 1.1 | -0.8 ± 1.1 |
| Discharge weight z score | -1.9 ± 1.0 | -2.2 ± 0.9 ³ | -2.0 ± 1.0 | -2.1 ± 1.0 | -1.8 ± 0.9 | -2.3 ± 0.8 ³ |
| Social class | 3.4 ± 1.4 | 3.5 ± 1.7 | 3.5 ± 1.3 | 3.4 ± 1.7 | 3.4 ± 1.7 | 3.6 ± 1.6 |
| Apgar score at 5 min | 8.3 ± 1.8 | 7.9 ± 1.8 | 8.4 ± 1.9 | 8.2 ± 1.6 | 8.2 ± 1.5 | 7.5 ± 1.9 |
| Ventilation (d) ⁴ | 0.0 (4.0) | 0.0 (4.0) | 0.0 (4.0) | 0.0 (3.0) | 1.0 (4.0) | 1.0 (4.0) |
| No. of infections ^{4,5} | 1.0 (2.0) | 1.0 (1.0) | 1.0 (1.0) | 1.0 (1.0) | 1.0 (1.0) | 1.0 (2.0) |

¹ $\bar{x} \pm$ SD.

²SD reported as the square root.

³Significantly different from preterm formula, $P < 0.05$.

⁴Median; interquartile range in parentheses.

⁵Based on the number of courses of antibiotics.

TABLE 3
Early diet and later leptin concentration relative to fat mass (FM)

| Variable | Diet | | Mean difference (95% CI) | P |
|---|--------------------------|----------------------------|--------------------------|-------|
| | Preterm formula | Other than preterm formula | | |
| | | | % | |
| Trials 1 and 2 combined ¹ | | | | |
| Leptin ($\mu\text{g/L}$) | 5.9 (101.9) ² | 5.6 (97.5) | 4.3 (-23.7, 32.3) | 0.76 |
| Leptin/FM ($\mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg FM}^{-1}$) | 0.84 (76.2) | 0.62 (80.9) | 30.8 (8.5, 53.2) | 0.007 |
| Leptin/percentage of FM ($\mu\text{g} \cdot \text{L}^{-1} \cdot \% \text{FM}^{-1}$) | 0.45 (76.4) | 0.34 (79.9) | 28.3 (6.1, 50.5) | 0.01 |
| Trial 1 ³ | | | | |
| Leptin ($\mu\text{g/L}$) | 5.5 (103.2) | 4.7 (96.3) | 15.1 (-20.4, 50.6) | 0.40 |
| Leptin/FM ($\mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg FM}^{-1}$) | 0.79 (85.3) | 0.60 (90.3) | 26.5 (-5.2, 58.2) | 0.10 |
| Leptin/percentage of FM ($\mu\text{g} \cdot \text{L}^{-1} \cdot \% \text{FM}^{-1}$) | 0.42 (84.9) | 0.32 (88.7) | 27.1 (-4.3, 58.4) | 0.09 |
| Trial 2 ⁴ | | | | |
| Leptin ($\mu\text{g/L}$) | 6.7 (99.8) | 7.9 (91.4) | -17.5 (-62.2, 27.2) | 0.44 |
| Leptin/FM ($\mu\text{g} \cdot \text{L}^{-1} \cdot \text{kg FM}^{-1}$) | 0.93 (58.6) | 0.64 (61.6) | 37.3 (9.0, 65.5) | 0.01 |
| Leptin/percentage of FM ($\mu\text{g} \cdot \text{L}^{-1} \cdot \% \text{FM}^{-1}$) | 0.51 (59.7) | 0.38 (60.9) | 29.0 (0.7, 57.4) | 0.04 |

¹Children who received preterm formula ($n = 95$) were compared with those who received a standard diet ($n = 102$).

²Geometric mean and CV (%).

³Children who received preterm formula ($n = 58$) were compared with those who received banked breast milk ($n = 66$).

⁴Children who received preterm formula ($n = 37$) were compared with those who received term formula ($n = 36$).

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).

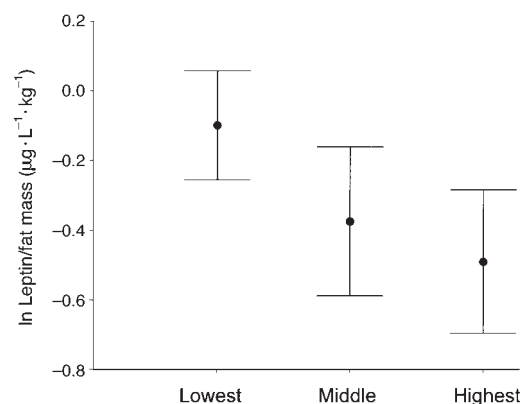


FIGURE 2. The distribution of leptin concentration relative to fat mass (geometric \bar{x} and 95% CI) by tertiles of the percentage of human milk intake ($n = 191$; P for trend = 0.006).

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, bioelectric 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, as 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. 

We thank the staff at the special-care baby units at Cambridge, Ipswich, King's Lynn, Sheffield, and Norwich for their assistance and cooperation; MF Bamford, JFB Dossetor, P Crowle, A Boon, and R Pearse for help and collaboration; Farley Health Products for technical assistance; Ruth Morely for the earlier tracing and follow-up of this cohort; Mia Kattenhorn, Eve Smith, Emma Sutton, Kathy Kennedy, and Ian Merryweather for technical assistance; and the children who participated in this project and their families.

REFERENCES

1. Freedman DS, Srinivasan SR, Valdez RA, Williamson DF, Berenson GS. Secular increases in relative weight and adiposity among children over two decades: the Bogalusa Heart Study. *Pediatrics* 1997;99: 420–6.
2. Dietz W. Critical periods in childhood for the development of obesity. *Am J Clin Nutr* 1994;59:955–9.



3. Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. *N Engl J Med* 1976;295:349–53.
4. Ravelli ACJ, van der Meulen JHP, Osmond C, Barker DJP, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr* 1999;70:811–6.
5. Parsons TJ, Power C, Logan S, Summerbell CD. Childhood predictors of adult obesity: a systematic review. *Int J Obes Relat Metab Disord* 1999;23(suppl):S1–107.
6. Seidman DS, Laor A, Gale R, Stevenson DK, Danon YL. A longitudinal study of birth weight and being overweight in late adolescence. *Am J Dis Child* 1991;145:782–5.
7. Curhan GC, Chertow GM, Willet WC, et al. Birth weight and adult hypertension and obesity in women. *Circulation* 1996;94:1310–5.
8. Curhan GC, Willet WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus and obesity in US men. *Circulation* 1996;94:3246–50.
9. Sorensen HT, Sabroe S, Rothman KJ, Gillman M, Fischer P, Sorensen TIA. Relation between weight and length at birth and body mass index in young adulthood: cohort study. *BMJ* 1997;315:1137.
10. Law CM, Barker DJP, Osmond C, Fall CHD, Simmonds SJ. Early growth and abdominal fatness in adult life. *J Epidemiol Community Health* 1992;46:184–6.
11. Fall CHD, Osmond C, Barker DJP, et al. Fetal and infant growth and cardiovascular risk factors in women. *BMJ* 1995;310:428–32.
12. Malina RM, Katzmarzyk PT, Beunen G. Birth weight and its relationship to size attained and relative fat distribution at 7 to 12 years of age. *Obes Res* 1996;4:385–90.
13. Barker M, Robinson S, Osmond C, Barker DJP. Birth weight and body fat distribution in adolescent girls. *Arch Dis Child* 1997;77:381–3.
14. Charney E, Goodman HC, McBride M, Lyon B, Pratt R. Childhood antecedents of adult obesity. Do chubby infants become obese adults? *N Engl J Med* 1976;295:6–9.
15. Wilson AC, Forsyth JS, Greene SA, Irvine L, Hau C, Howie PW. Relation of infant diet to childhood health: seven year follow up of cohort children in Dundee infant feeding study. *BMJ* 1998;316:21–5.
16. Kramer MS. Do breast-feeding and delayed introduction of solid foods protect against subsequent obesity? *J Pediatr* 1981;98:883–7.
17. Elliott KG, Kjøhede CL, Gournis E, Rasmussen KM. Duration of breastfeeding associated with obesity during adolescence. *Obes Res* 1997;5:538–41.
18. Von Kries R, Koletzko B, Sauerwald T, et al. Breast feeding and obesity: cross sectional study. *BMJ* 1999;319:147–50.
19. Agras WS, Kraemer HC, Berkowitz RI, Hammer LD. Influence of early feeding style on adiposity at 6 years of age. *J Pediatr* 1990;116:805–9.
20. Ong KKL, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ* 2000;320:967–71.
21. McCance RA, Widdowson EM. The determinants of growth and form. *Proc R Soc Lond B Biol Sci* 1974;185:1–17.
22. Lewis DS, Bertrand HA, McMahan CA, McGill HC, Carey KD, Masoro EJ. Prewaning food intake influences the adiposity of young adult baboons. *J Clin Invest* 1986;78:899–905.
23. Proietto J, Thorburn AW. Animal models of obesity—theories of aetiology. *Baillieres Clin Endocrinol Metab* 1994;8:509–25.
24. Lonngqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese (*ob*) gene in adipose tissue of human obese subjects. *Nat Med* 1995;1:950–3.
25. Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nat Med* 1995;1:1155–61.
26. Hamiliton BS, Paglia D, Kwan AYM, Deitel M. Increased obese mRNA expression in omental fat cells from massively obese humans. *Nat Med* 1995;1:953–6.
27. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal weight and obese humans. *N Engl J Med* 1996;334:292–5.
28. Klein S, Coppack SW, Mohamed-Ali V, Landt M. Adipose tissue leptin production and plasma leptin kinetics in humans. *Diabetes* 1996;45:984–7.
29. Lissner L, Karlsson C, Lindroos AK, et al. Birth weight, adulthood BMI, and subsequent weight gain in relation to leptin levels in Swedish women. *Obes Res* 1999;7:150–4.
30. Phillips DIW, Fall CHD, Cooper C, Norman RJ, Robinson JS, Owens PC. Size at birth and plasma leptin concentrations in adult life. *Int J Obes Relat Metab Disord* 1999;23:1025–9.
31. Lucas A, Gore SM, Cole TJ, et al. A multicentre trial on feeding of low birthweight infants: effects of diet on early growth. *Arch Dis Child* 1984;59:722–30.
32. Lucas A, Morley R, Cole TJ. Randomized trial of early diet in preterm babies and later intelligence quotient. *BMJ* 1998;317:1481–7.
33. Lucas A, Morley R. Does early nutrition in infants born before term programme later blood pressure? *BMJ* 1994;309:304–8.
34. Gore SM. Statistical considerations in infant nutrition trials. *Lipids* 1999;34:185–97.
35. Freeman JV, Cole TJ, Chinn S, Jones PRM, White EM, Preece MA. Cross-sectional stature and weight reference curves for the UK, 1990. *Arch Dis Child* 1995;73:17–24.
36. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomized trials. *Lancet* 2001;357:413–9.
37. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. *Pediatrics* 1980;66:918–20.
38. Bennett FI, McFarlane-Anderson N, Wilks R, Luke A, Cooper RS, Forrester TE. Leptin concentration in women is influenced by regional distribution of adipose tissue. *Am J Clin Nutr* 1997;66:1340–4.
39. Havel PJ, Kasim-Karakas S, Mueller W, Johnson PR, Gingerich RL, Stern JS. Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. *J Clin Endocrinol Metab* 1996;81:4406–13.
40. Cole TJ. Sympercents: symmetric percentage differences on the 100 log_e scale simplify the presentation of log transformed data. *Stat Med* 2000;19:3109–25.
41. Clark PM, Hindmarsh PC, Shiell AW, Law CM, Honour JW, Barker DJP. Size at birth and adrenocortical function in childhood. *Clin Endocrinol* 1996;45:721–6.
42. Huang XF, Lin S, Zhang R. Upregulation of leptin receptor mRNA expression in obese mouse brain. *Neuroreport* 1997;8:1035–8.
43. Dyer CJ, Simmons JM, Matteri RL, Keisler DH. Leptin receptor mRNA is expressed in the ewe anterior pituitary and adipose tissues and is differentially expressed in hypothalamic regions of well-fed and feed-restricted ewes. *Domest Anim Endocrinol* 1997;14:119–28.
44. Chessler SD, Fujimoto WY, Shofer JB, Boyko EJ, Weigle DS. Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. *Diabetes* 1998;47:239–43.
45. Lindross A, Lissner L, Carlsson B, et al. Familial predisposition for obesity may modify the predictive value of serum leptin concentrations for long-term weight change in obese women. *Am J Clin Nutr* 1998;67:1119–23.
46. Ravussin E, Pratley R, Maffei M, et al. Relatively low plasma concentrations precede weight gain in Pima Indians. *Nat Med* 1997;3:238–40.
47. Morley R, Lucas A. Randomized diet in the neonatal period and growth performance until 7.5–8 y of age in preterm children. *Am J Clin Nutr* 2000;71:822–8.
48. Czajka-Narins DM, Hirsch J. Supplementary feeding during the preweaning period. Effect on carcass composition and adipose tissue cellularity of the rat. *Biol Neonate* 1974;25:176–185.
49. Rolland-Cachera MF, Deheeger M, Akrouf M, Bellisle F. Influence of macronutrients on adiposity development: a follow study of nutrition and growth from 10 months to 8 years of age. *Int J Obes Relat Metab Disord* 1995;19:573–8.
50. Plagemann A, Harder T, Rake A, et al. Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats. *J Neuroendocrinol* 1999;11:541–6.