Outcomes 8 years after preterm birth

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Chapter 7

Leptin and IGF-1 in relation to body composition and bone mineralization of preterm-born children from infancy to 8 years

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ABSTRACT

Objective Preterm birth has been associated with altered body composition, especially increased fat mass (FM) and decreased bone mineralization, and leptin and IGF-1 have been suggested to be involved in the regulation of both. We aimed to study the interplay between leptin, IGF-1, FM, and bone mineralization measured in infancy and childhood of children born preterm.

Design Observational study.

Subjects Seventy-nine (40 boys) preterm-born children (gestational age ≤ 32 weeks and/or birth weight ≤ 1500 g) aged 8 years.

Measurements Serum leptin and IGF-1 were measured at term age, at 3 and 6 months corrected age (CA), and 8 years. Body composition (fat and lean mass) and bone parameters (bone area, mineral content and density) were measured by Dual-energy X-ray Absorptiometry (DXA) at term age, 6 months CA and 8 years.

Results Leptin was positively associated with FM at all time points and with bone parameters at term age and 6 months CA. IGF-1 was associated with body composition and bone density at most of the time points. Explained variation in bone mineralization increased significantly by adding bone area and height to the models.

Conclusions During infancy and childhood, leptin and IGF-1 were associated with body composition in preterm-born children. In addition, leptin was associated with bone parameters in early infancy, but not in childhood. It is hypothesized that a complicated interplay between multiple pathways, which most likely changes over time, is involved in regulation of body composition and bone mineralization of preterm infants.

INTRODUCTION

Preterm birth has been associated with a number of long-term sequelae, including increased risks of cardiovascular disease and osteoporosis.[1,2] The link between prematurity and cardiovascular disease might be explained by altered fat tissue development, as preterm-born children were found to have an increased fat mass (FM) at term equivalent age compared to term-born peers.[3] This pattern was reported, although not unequivocally, to persist beyond infancy.[4-8] Likewise, some studies showed a lower bone mass or bone mineral density (BMD) after preterm birth around term age, but also at school-age, which could not be confirmed by others.[2] A multifactorial mechanism, where endocrine systems play an important role, might underlie these differences.

Insulin-like growth factor 1 (IGF-1) is a key regulator of growth and affects metabolic processes such as glucose and lipid metabolism, and thereby body composition. In addition, IGF-1 regulates longitudinal bone growth through stimulation of chondrocytes in the growth plate and increases BMD.[9]

Leptin is a regulator of food intake and energy expenditure through its effects on the central nervous system.[10-12] The association between leptin and FM has been extensively described, although predominantly in adult, obese populations. In term-born children at school age, leptin has also been positively associated with FM index (i.e., FM adjusted for height^2).[13,14] We showed that this association was also present in preterm-born children at term age and 6 months corrected age (CA, i.e., calculated from term age).[15]

More recently, leptin has been suggested to be involved in bone metabolism, although its exact role has yet to be determined. A commonly adopted hypothesis encompasses a combination of two pathways: a central pathway via hypothalamic activation of the sympathetic nervous system that inhibits bone formation and a peripheral pathway through leptin receptors on osteoblastic cells that increases bone formation.[16,17] Data on this association in children are scarce and current literature is inconclusive, with studies reporting positive, negative or no associations.[14,18] In children born preterm (between 32 and 37 weeks of gestation), no relation was found between cord blood leptin and lumbar spine BMD at the age of 2 years.[19] To our knowledge, no studies have investigated associations between leptin and bone metabolism in children born before 32 weeks of gestation.

Considering the positive association between leptin and FM found during infancy in our cohort, the aim of this study was to investigate whether this association persists, and to explore the association between leptin and body composition, including bone parameters, from infancy to age 8 years. In addition, we studied whether IGF-1 was associated with leptin, body composition and bone parameters.

SUBJECTS AND METHODS

The study protocol was approved by the Ethics Committee of the VU University Medical Center Amsterdam, The Netherlands, and all parents of participants gave written informed consent.

Subjects

All subjects included in this study originally participated in a nutritional RCT (Study Towards the Effects of Postdischarge nutrition, STEP), which has been described in detail before.[20] For this study, infants were randomized at term age to receive either an isocaloric protein- and mineral-enriched postdischarge formula (PDF) or standard term formula (TF) and were fed this diet until 6 months CA. A control group of children fed human milk (HM) was also included. Subjects visited the outpatient clinic at term age, at 3 and 6 months CA, and, for the follow-up study (STEP-2), at chronological age 8 years. Of the original cohort of 152 children, 21 were excluded or lost to follow-up at age 8 years, and 52 refused to participate or could not be traced, resulting in 79 children who were included in the follow-up study. Exclusion criteria were severe physical impairment or conditions known to affect growth or body composition. Participants and nonparticipants of the follow-up were comparable in terms of perinatal and demographic characteristics as described previously.[21] The cohort retrieval is described in detail elsewhere.[21]

Measurements

Weight and length/height were measured during each study visit using standard methods,[20,21] and absolute values were converted to standard deviation scores (SDSs) with the use of appropriate reference data.[22,23] Children with a weight and/or length at birth ≤ -2 SDS were classified as small-for-gestational-age (SGA).

Laboratory parameters

Leptin and IGF-1 were measured in venous blood samples drawn at term age, at 3 and 6 months CA, and 8 years. Samples were stored at -80°C and thawed only once just before the analyses were performed. Leptin (μg/L) was analyzed by radioimmunoassay (Linco Research Inc., St. Charles Missouri USA; inter-assay coefficient of variation [CV] 6%, lower detection limit 0.5 μg/L). IGF-1 (nmol/L) was analyzed using 3 different methods as the assay changed during the course of the study. At term age and at 3 and 6 months CA, IGF-1 was analyzed by chemiluminescence immunoassay (Advantage, Nichols Diagnostics, San Juan Capistrano USA; inter-assay CV ranged from 5% to 8%, lower detection limit 6

nmol/L) or immunometric assay (Immulite 2500, Siemens Diagnostics, Los Angeles USA; inter-assay CV 8%, lower detection limit 3.2 nmol/L). Comparison between these methods showed excellent agreement.[24] At age 8 years IGF-1 was analyzed using chemiluminescence immunoassay (Liaison DiaSorin S.p.A., Italy; inter-assay CV ranged from 7.4% to 16%, lower detection limit 3.2 nmol/L). A comparison of the methods by regression analysis, as described by Passing and Bablok,[25] showed strong agreement (Liaison = 1.040*Immulite + 1.785). We therefore decided that, in spite of the different assays, IGF-1 values obtained throughout the study were comparable and could be analyzed concurrently.

Dual-energy X-ray Absorptiometry (DXA)

At term age, 6 months CA and 8 years a DXA-scan (Hologic QDR 4500A, Hologic Inc., Bedford, Mass., USA) was performed, providing information on total body fat mass (FM, kg) and lean mass (LM, kg), as well as total body bone area (BA, cm^2), bone mineral content (BMC, g), and bone mineral density (BMD, g/cm^2). DXA-scans were analyzed using Infant Whole Body Software version 12.3.3 at term age and 6 months CA, and Apex Software version 13.3.3 at 8 years.

Statistical analyses

Normally distributed data were presented as means ± SDs and skewed data as medians [interquartile ranges, IQRs]. Skewed outcome variables were natural log-transformed before use in linear regression analyses.

Cross-sectional associations between leptin or IGF-1, and FM, LM, BMC, and BMD were analyzed using linear regression analyses, and longitudinal associations were analyzed using linear mixed models with fixed effects and a diagonal repeated covariance type.

All analyses were adjusted for sex, as this is a known confounder in analyses with leptin and body composition. Linear regression analyses with BMC as outcome variable were additionally adjusted for simultaneously measured BA and length/height.[26] Outcomes of linear regression analyses are reported as β (95% confidence interval (CI)), *P* value, and, if appropriate, adjusted R^2 (R^2_{adj}, i.e., the proportion of the variance in the dependent variable that is predictable from the independent variable(s)). Because of strong collinearity between FM and leptin, the analyses with either of those parameters as covariable were not adjusted for the other.

All analyses were adjusted for type of feeding (i.e., PDF, TF and HM) in addition to sex, as this was a post hoc analysis of a nutritional RCT. Previous analyses in this cohort showed that body composition at 6 months CA differed between the feeding groups.[20] Moreover,

nutrition (in particular protein) is a known regulator of IGF-1 and possibly also of leptin.

All statistical analyses were performed with IBM SPSS Statistics version 22. Statistical significance was defined as a P value of < 0.05.

RESULTS

A total of 79 children were included in the follow-up study at a mean age of 7.9 (range 7.1–8.6) years. Leptin and IGF-1 concentrations in combination with DXA-parameters were available for 70, 68 and 55 children at term age, 6 months CA and 8 years, respectively. Leptin at 3 months CA was available for 73 children.

Leptin and IGF-1 in infancy and at age 8 years

Girls had significantly higher leptin, but similar IGF-1 levels compared with boys at all 4 time points (**Table 7.1**). Leptin was similar between the feeding groups at all time points (all $P > 0.1$). IGF-1 was significantly different at 3 and 6 months CA between the feedings groups (**Table 7.1**).

Leptin measured in infancy was not associated with leptin at 8 years. IGF-1 measured in infancy was positively associated with IGF-1 at 8 years: at 3 months CA β 0.84, 95% CI 0.41; 1.26, $P < 0.001$ and R^2_{adj} 27%, and at 6 months CA β 0.96, 95% CI 0.42; 1.50, $P = 0.001$ and R^2_{adj} 27% (**Figure 7.1**). A positive association was found between IGF-1 and leptin for each time point separately, with the exception of 6 months CA (data not shown).

Leptin – body composition and bone parameters

At term age, 6 months CA and 8 years, leptin was associated with FM ($P < 0.001$, R^2_{adj} 30%–66%). Leptin at 3 months CA was associated with FM at 6 months CA ($P < 0.001$, R^2_{adj} 39%). At 8 years, leptin was associated with LM, $P = 0.002$, R^2_{adj} 17% (**Table 7.2**). In longitudinal analyses, leptin was associated with FM (β 0.16, 95% CI 0.13; 0.19, $P < 0.001$, **Figure 7.2A**) and LM (β 0.10, 95% CI 0.01; 0.19, $P = 0.029$) over time.

At term age and 6 months CA leptin was positively associated with BMC and BMD (**Table 7.2**). Adjusting the associations between leptin and BMC for BA and length/height, in addition to sex and type of feeding, resulted in weaker, but still significant associations, and increased the explained variation in BMC (i.e., an increase in R^2_{adj}): at term age from 30% to 81% and at 6 months CA from 19% to 84%.

Table 7.1. Characteristics of the study population

	Birth	Term age	3 months CA	6 months CA	8 years
n	79	70	73	68	55
Boys	40 (50.6)	36 (51.4)	37 (50.7)	34 (50.0)	30 (54.5)
Gestational age, wks	30.7 [29.3; 31.6]	30.7 [29.3; 31.6]	30.6 [29.1; 31.4]	30.7 [29.0; 31.5]	30.6 [29.3; 31.4]
SGA	17 (21.5)	14 (20.0)	14 (19.2)	14 (20.6)	13 (23.6)
Weight, kg	1.3 ± 0.3	3.1 ± 0.5	5.5 ± 0.9	7.2 ± 1.0	25.8 ± 4.2
SDS	-0.4 ± 1.0	-1.2 ± 1.2	-0.5 ± 1.3	-0.5 ± 1.2	-0.6 ± 1.1
Length/height, cm	38.0 ± 3.1	48.4 ± 2.3	58.9 ± 2.7	66.3 ± 2.7	129.8 ± 5.7
SDS	-0.8 ± 1.3	-1.2 ± 1.3	-0.7 ± 1.2	-0.4 ± 1.1	-0.3 ± 0.9
Endocrine parameters					
Leptin, µg/L*	–	1.8 [1.3; 2.5]	3.6 [2.5; 5.0]	3.0 [2.3; 4.2]	3.5 [2.2; 4.8]
Boys	–	1.6 [1.3; 1.9]	3.2 [2.4; 4.7]	2.4 [1.8; 3.7]	2.8 [2.1; 3.8]
Girls	–	2.0 [1.3; 3.3]	4.2 [2.6; 6.7]	3.5 [2.9; 4.7]	4.4 [2.7; 6.1]
IGF-1, nmol/L**	–	6.1 ± 1.6	9.4 ± 4.2	8.1 ± 3.0	22.7 ± 7.4
PDF	–	6.4 ± 1.4	11.0 ± 4.8	9.6 ± 3.5	23.8 ± 9.2
TF	–	5.8 ± 2.0	9.2 ± 3.5	7.8 ± 2.9	21.1 ± 5.7
HM	–	6.1 ± 1.5	7.2 ± 2.6	7.1 ± 2.6	22.8 ± 6.3
Body composition and bone parameters					
FM, kg	–	0.3 ± 0.2	–	1.9 ± 0.7	6.8 ± 2.2***
FM, %	–	8.8 ± 5.0	–	24.3 ± 6.2	25.6 ± 4.8***
LM, kg	–	3.0 ± 0.4***	–	5.7 ± 0.7***	18.5 ± 2.7
BA, cm^2	–	310.3 ± 43.1	–	614.4 ± 72.2	1222.2 ± 73.3
BMC, g	–	45.6 ± 10.4	–	140.1 ± 25.7	895.6 ± 103.1
BMD, g/cm^2	–	0.15 ± 0.02	–	0.23 ± 0.02	0.73 ± 0.05

Data are presented as mean ± SD, *n* (%) or median [IQR]. BA, bone area; BMC, bone mineral content; BMD, bone mineral density; CA, corrected age; FM, fat mass; HM, human milk; IGF-1, insulin-like growth factor 1; LM, lean mass; PDF, postdischarge formula; SDS, standard deviation score; SGA, small-for-gestational-age; TF, term formula. * Male vs female $P = 0.015$, $P = 0.046$, $P = 0.001$, and $P = 0.006$, for increasing ages, respectively. ** IGF-1 compared between the feeding groups of the original RCT: PDF vs. HM 3 months CA $P = 0.002$, 6 months CA $P = 0.016$. *** Male vs female $P < 0.05$, data not shown separately.

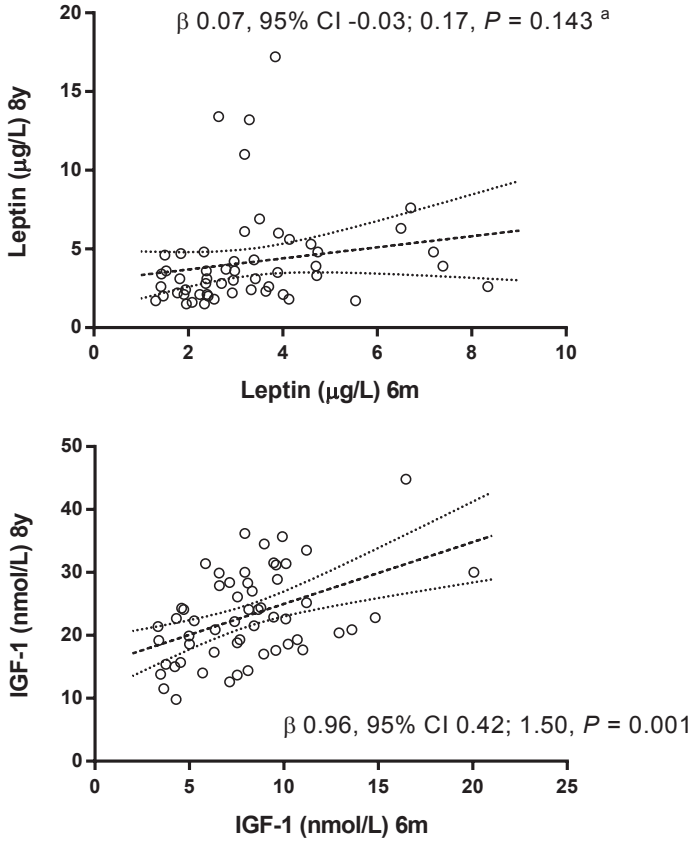

Figure 7.1. Association between leptin/IGF-1 measured at 6 months corrected age (CA) and age 8 years. Results of regression analyses were adjusted for sex and type of feeding from term age to 6 months CA. [a] Results for natural log-transformed leptin at age 8 years. Dotted lines = regression line with 95% confidence interval.

Leptin measured at 3 months CA was associated with BMC and BMD at 6 months CA (R^2_{adj} 84% and 27%, respectively, when adjusted for sex, type of feeding, BA, and length). In crude and adjusted analyses, leptin, regardless of time point, was not associated with BMC or BMD at 8 years (**Table 7.2**).

In longitudinal analyses, leptin was associated with BMC (β 7.13, 95% CI 5.07; 9.20, $P <$ 0.001; **Figure 7.2A**) and BMD (β 0.01, 95% CI 0.01; 0.01, $P < 0.001$) over time.

IGF-1 – body composition and bone parameters

At term age, 6 months CA and 8 years IGF-1 was associated with FM (R^2_{adj} 6%–13%) (**Table 7.3**). IGF-1 at 3 months CA was positively associated with FM at 6 months CA (R^2_{adj} 6%).

Table 7.2. Associations of leptin with body composition and bone parameters from infancy to 8 years

	Term age	3 months CA	6 months CA	8 years
Leptin, µg/L	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
FM, kg				
Term age	0.14 (0.11; 0.17)*	–	–	–
6 months CA	0.07 (-0.10; 0.24)	0.18 (0.12; 0.23)*	0.24 (0.15; 0.33)*	–
8 years	0.30 (-0.28; 0.88)	0.15 (-0.09; 0.40)	0.07 (-0.30; 0.44)	0.55 (0.43; 0.66)*
LM, kg				
Term age	0.10 (0.01; 0.20)**	–	–	–
6 months CA	0.06 (-0.09; 0.21)	0.07 (0.01; 0.13)**	0.01 (-0.09; 0.10)	–
8 years	0.39 (-0.30; 1.08)	0.21 (-0.07; 0.49)	0.02 (-0.43; 0.47)	0.39 (0.15; 0.62)**
BA, cm^2 [a]				
Term age	11.15 (5.29; 17.02)*	–	–	–
6 months CA	2.02 (-9.85; 13.89)	5.31 (0.20; 10.42)**	5.72 (-1.74; 13.17)	–
8 years	5.44 (-5.55; 16.43)	-0.97 (-5.48; 3.54)	-0.55 (-7.57; 6.47)	-4.67 (-8.63; -0.71)**
BMC, g[b]				
Term age	1.46 (0.17; 2.75)**	–	–	–
6 months CA	3.16 (0.47; 5.85)**	1.72 (0.48; 2.97)**	2.28 (0.54; 4.02)**	–
8 years	10.61 (-2.18; 23.38)	3.58 (-1.95; 9.11)	0.10 (-8.33; 8.53)	3.65 (-1.57; 8.86)
BMD, g/cm^2				
Term age	0.008 (0.005; 0.012)*	–	–	–
6 months CA	0.007 (0.002; 0.012)**	0.004 (0.002; 0.006)*	0.006 (0.002; 0.009)***	–
8 years	0.008 (-0.004; 0.020)	0.005 (0.000; 0.010)	0.001 (-0.007; 0.009)	0.004 (0.000; 0.009)

Linear regression analyses adjusted for sex and type of feeding from term age to 6 months CA. [a] Additionally adjusted for length/height; [b] Additionally adjusted for bone area and length/height. BA, bone area; BMC, bone mineral content; BMD, bone mineral density; CA, corrected age; CI, confidence interval; FM, fat mass; LM, lean mass.
* $P < 0.001$, ** $P < 0.05$, *** $P = 0.001$.

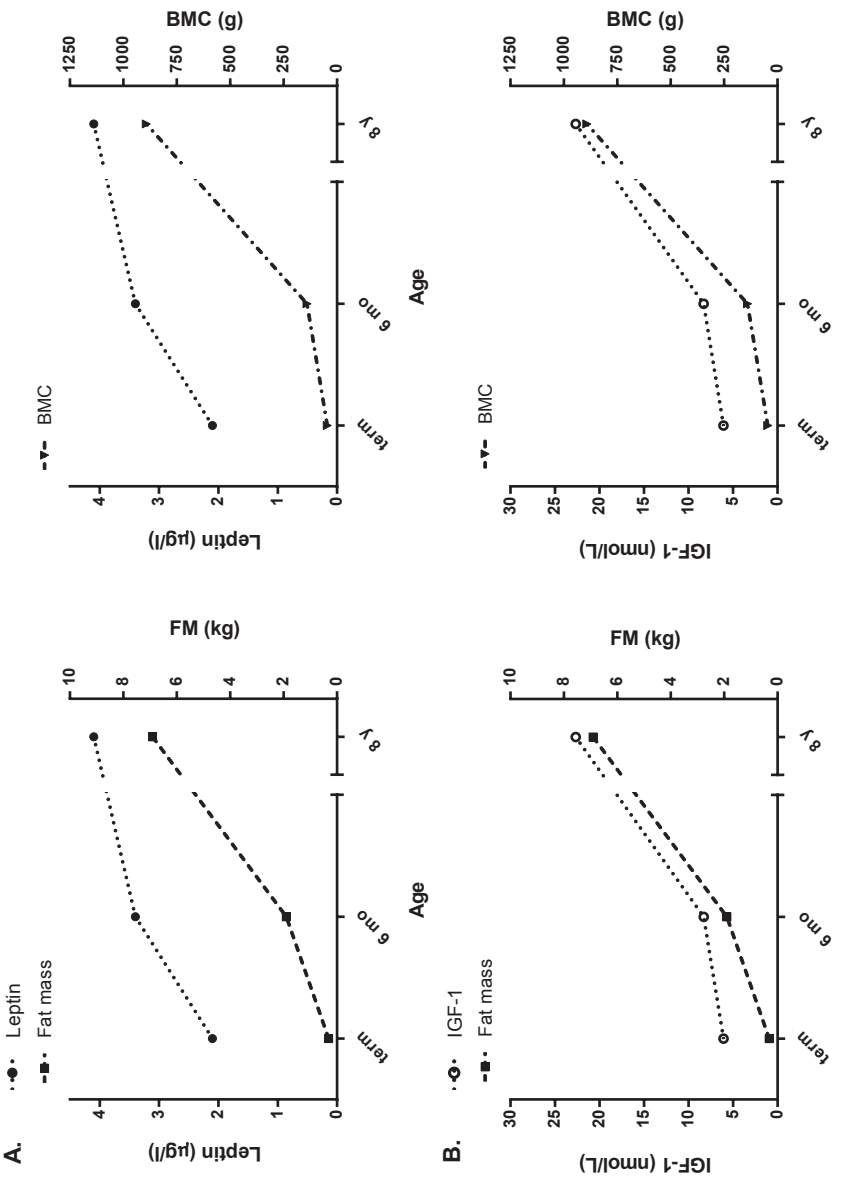

Figure 7.2. Leptin (A) and IGF-1 (B) with fat mass (FM) and bone mineral content (BMC) at term age, 6 months corrected age and 8 years.
Longitudinal analysis of these data showed significant associations over time ($P < 0.001$).

Table 7.3. Associations of IGF-1 with body composition and bone parameters from infancy to 8 years

	Term age	3 months CA	6 months CA	8 years
IGF-1, nmol/L	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
FM, kg				
Term age	0.04 (0.02; 0.07)**	–	–	–
6 months CA	0.00 (-0.11; 0.12)	0.04 (0.00; 0.08)**	0.06 (0.01; 0.11)**	–
8 years	0.19 (-0.19; 0.57)	0.00 (-0.15; 0.14)	-0.06 (-0.24; 0.12)	0.08 (0.00; 0.16)**
LM, kg				
Term age	0.08 (0.02; 0.14)**	–	–	–
6 months CA	0.06 (-0.04; 0.16)	0.05 (0.01; 0.08)**	0.04 (-0.01; 0.08)	–
8 years	0.09 (-0.37; 0.55)	0.12 (-0.05; 0.29)	0.09 (-0.12; 0.30)	0.18 (0.08; 0.27)***
BA, cm^2 [a]				
Term age	5.49 (1.87; 9.11)**	–	–	–
6 months CA	-0.93 (-8.80; 6.93)	0.61 (-2.42; 3.63)	2.08 (-1.52; 5.68)	–
8 years	-2.33 (-9.52; 4.85)	-1.66 (-4.37; 1.04)	0.16 (-3.20; 3.52)	-0.92 (-2.88; 1.04)
BMC, g[b]				
Term age	0.26 (-0.56; 1.09)	–	–	–
6 months CA	1.92 (0.11; 3.74)**	0.03 (-0.70; 0.76)	0.28 (-0.62; 1.19)	–
8 years	7.55 (-0.11; 15.21)	2.26 (-1.02; 5.55)	1.36 (-2.64; 5.36)	1.70 (-0.66; 4.06)
BMD, g/cm^2				
Term age	0.003 (0.001; 0.006)**	–	–	–
6 months CA	0.004 (0.000; 0.008)	0.001 (-0.001; 0.002)	0.001 (0.000; 0.003)	–
8 years	0.005 (-0.002; 0.013)	0.002 (-0.001; 0.005)	0.001 (-0.003; 0.005)	0.003 (0.001; 0.005)**

Linear regression analyses adjusted for sex and type of feeding from term age to 6 months CA. [a] Additionally adjusted for length/height; [b] Additionally adjusted for bone area and length/height. BA, bone area; BMC, bone mineral content; BMD, bone mineral density; CA, corrected age; CI, confidence interval; FM, fat mass; IGF-1, insulin-like growth factor 1; LM, lean mass.

* $P < 0.001$, ** $P < 0.05$, *** $P = 0.001$.

In longitudinal analyses, IGF-1 was associated with FM (β 0.10, 95% CI 0.08; 0.13, $P < 0.001$, **Figure 7.2B**) and LM (β 0.21, 95% CI 0.15; 0.26, $P < 0.001$). At 8 years, IGF-1 was associated with LM (R^2_{adj} 19%; **Table 7.3**).

At term age, 6 months CA and 8 years, IGF-1 was positively associated with both simultaneously measured BMC (data not shown) and BMD (R^2_{adj} 8%–17%; **Table 7.3**). After adjusting the BMC analyses for BA and length/height, in addition to sex and type of feeding, the associations between IGF-1 and BMC were no longer significant (**Table 7.3**). However, there was an increase in the explained variation in BMC (i.e., an increase in R^2_{adj}): at term age from 14% to 79%, at 6 months CA from 10% to 82% and at 8 years from 17% to 74%.

In longitudinal analyses, IGF-1 was associated with BMC (β 4.23, 95% CI 2.78; 5.68, $P < 0.001$; **Figure 7.2B**) and BMD (β 0.01, 95% CI 0.01; 0.01, $P < 0.001$) over time.

Body composition and bone

Body composition and bone parameters were associated with one another at most of the time points (**Table 7.4**).

This was confirmed in longitudinal analyses in which FM and LM were strongly associated with BMC and BMD over time (data not shown).

Adjusting the analyses with BMC for BA and length/height, in addition to sex and type of feeding, resulted in weaker associations between FM or LM and BMC, but an increase in the explained variation in BMC (i.e., an increase in R^2_{adj}): at term age from 60% to 83%/54% to 80%, at 6 months CA from 64% to 84%/27% to 82%, and at 8 years from 20% to 79%/62% to 85%, for FM and LM, respectively.

DISCUSSION

In children born preterm, we showed that leptin and IGF-1 were associated with FM during infancy and childhood, and that the association between leptin and bone in infancy was no longer present in childhood. Bone area and height were found to be more important for bone mineralization than were the investigated endocrine parameters. There appeared to be no predictive value of endocrine parameters measured in infancy on body composition and bone at age 8 years.

Studies on leptin in the neonatal period have described relations with several perinatal characteristics, including positive associations with fetal growth, gestational age and birth weight.[27,28] Likewise, the effects of IGF-1 during early life of preterm infants have

Table 7.4. Association between body composition and bone parameters from infancy to 8 years

	Term age	6 months CA	8 years
Fat mass, kg	β (95% CI)	β (95% CI)	β (95% CI)
BMC, g[a]			
Term age	15.04 (7.09; 22.99)*	–	–
6 months CA	12.27 (-2.56; 27.10)	9.40 (3.49; 15.31)**	–
8 years	46.34 (-19.92; 112.61)	-1.33 (-22.84; 20.17)	8.07 (2.52; 13.62)**
BMD, g/cm^2			
Term age	0.059 (0.042; 0.077)*	–	–
6 months CA	0.036 (0.009; 0.063)**	0.019 (0.012; 0.026)*	–
8 years	0.050 (-0.013; 0.114)	0.012 (-0.007; 0.031)	0.007 (0.002; 0.011)**
	Term age	6 months CA	8 years
Lean mass, kg	β (95% CI)	β (95% CI)	β (95% CI)
BMC, g[a]			
Term age	-4.83 (-10.49; 0.84)	–	–
6 months CA	5.73 (-4.19; 15.64)	-4.61 (-10.93; 1.71)	–
8 years	17.13 (-16.56; 50.83)	20.36 (-7.72; 48.43)	19.46 (13.21; 25.72)*
BMD, g/cm^2			
Term age	0.019 (0.010; 0.029)*	–	–
6 months CA	0.023 (0.010; 0.035)*	0.007 (-0.002; 0.016)	–
8 years	0.035 (0.005; 0.064)**	0.039 (0.018; 0.059)*	0.012 (0.010; 0.015)*

Linear regression analyses adjusted for sex and type of feeding from term age to 6 months CA. [a] Additionally adjusted for bone area and length/height.
BMC, bone mineral content; BMD, bone mineral density; CA, corrected age; CI, confidence interval.
* $P < 0.001$, ** $P < 0.05$, *** $P = 0.001$.

been extensively described.[29,30] Decreased levels of IGF-1 in early postnatal life have been associated with neonatal morbidities such as bronchopulmonary dysplasia and retinopathy of prematurity, as well as with impaired growth, adverse neurodevelopment and unfavorable body composition.[29,30] We previously showed that leptin was associated with FM and weight gain between term age and 6 months CA,[15] and that IGF-1, together with insulin, was suggested to be involved in the regulation of growth during infancy.[24]

Beyond infancy, data on the long-term effects of leptin and IGF-1 in preterm infants are limited. When comparing our findings with studies in healthy, term-born children of similar age, there are resemblances as well as contrasts. Leptin and IGF-1 have been positively associated with FM index in children aged 8–11 years.[13] We demonstrated a similar relation between leptin, IGF-1 and FM, although the latter was not adjusted for height.

In contrast to our results, a study in 7–8 year-old term-born children showed positive associations between IGF-1 and total body BMC, and between leptin and lumbar spine BMD.[14] However, consistent with our results, the same study found no independent effect of leptin on total body BMC and concluded that hormones only explained a relatively small proportion of variation in body composition or bone parameters. In addition, they found that LM and FM largely explained variation in BMC,[14] unlike our results that suggest a substantial contribution of BA and height. The latter finding may be supported by studies suggesting that increased leptin concentrations contribute to growth velocity and bone maturation.[31,32] Then again, others showed that neither leptin and IGF-1, nor FM were associated with BMC adjusted for height and BA.[33]

Assessing the actual effect of each of the discussed parameters separately, in particular since leptin and FM are strongly related, is difficult and this makes it hard to draw firm conclusions. It has also been suggested that the effect of leptin on bone in early and later life involves other components of bone metabolism,[17] and this might be reflected in the differences we found between infancy and childhood. Furthermore, considering the theory of a central inhibitory and peripheral stimulating effect of leptin on bone, we hypothesize that in preterm infants it may take some time before a balance between these two pathways is reached. In addition, an inverse association between IGF-1 in infancy and at 17 years has been described in healthy term-born children,[34] which was exactly opposite to the positive association that we found. We might speculate that we found this association as part of an ongoing change of the IGF-1 axis towards an eventually inverse association. In summary, it is probably fair to assume that a complicated interaction of multiple (counteractive) pathways, most likely also changing over time, is involved in the regulation of body composition and bone mineralization of preterm infants.

Strengths and limitations

The main strength of this study was the follow-up of preterm-born children from birth until age 8 years with repeated measurements of endocrine parameters and body composition. This study also has several limitations. First, the analyses performed are post hoc analyses of a nutritional RCT that did not include a term-born control group. A direct comparison with healthy peers can, therefore, not be made and only associations, not causal pathways, can be investigated. Second, attrition at follow-up, which is practically inevitable in long-term follow-up studies,[35] limited our sample size. In addition, refusal by participants or failure of obtaining blood further reduced our sample size, at least regarding the analyses with blood parameters. As a consequence, subgroups were too small to perform analyses for boys and girls separately, in spite of differences between sexes in hormone concentrations and body

composition. Instead, we adjusted our analyses for sex. Third, blood was obtained from term age, while the period between birth and term age is probably more important for the future settings of hormonal systems.[36] Fourth, even though we found strong significant associations, as a consequence of the design of our study we could not prove causality. Furthermore, with regard to clinical relevance, it is important to take regression coefficients (beta's), as a proxy for effect sizes, into account as well. Additionally reporting the adjusted R^2 gives insight in the proportion of the variance in the outcome variables that is explained by the independent variables, and may facilitate interpretation of the associations that we found. From that it could be concluded that sex, BA and height are important contributors to variation in bone mineralization, specifically at 8 years. What the exact role of leptin is in bone metabolism of preterm-born children remains uncertain. Follow-up beyond childhood is necessary to investigate the long-term effects of leptin and IGF-1 on body composition and bone parameters in children born preterm.

CONCLUSION

Leptin and IGF-1 were associated with body composition and bone parameters, although the latter only in infancy and not in childhood in our cohort of preterm-born children. This may suggest that during childhood other characteristics (such as sex, BA and height), become more important for bone mineralization.

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