Predictive value of appetite regulating hormone levels in early life for later fat mass trajectories

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ABSTRACT

Background The first 1000 days, from conception to age 2 years, are an important window for infant development. Appetite regulating hormones(ARH) coordinate appetite and food intake, and might contribute to adiposity programming. Studies examining ARH trajectories and early fat mass(FM) development do not exist.

Objective To investigate associations between ARH at 3, 6 months and 2 years and FM parameters at 2 years, and associations between ARH and FM trajectories until 2 years.

Methods Birth cohort of 174 healthy infants (Sophia Pluto Cohort). Ghrelin (acylated), peptide YY (PYY), adiponectin and leptin measured at 3, 6 months and 2 years. FM% measured by PEAPOD and DXA scan, abdominal FM by ultrasonography.

Results Ghrelin increased and PYY decreased from 3 months to 2 years ($p<0.001$), leading to increasing ghrelin/PYY ratio ($p<0.001$). Adiponectin and leptin decreased ($p<0.001$). Ghrelin, PYY and ghrelin/PYY ratio at 3 and 6 months did not correlate with FM parameters at 2 years. Adiponectin at 3 and 6 months correlated with FM% at 2 years ($R\geq0.282, p=0.001$) while the adiponectin decline correlated inversely. Leptin at 3 and 6 months correlated with FM% at 2 years, leptin at 2 years correlated strongly with FM% at same age ($R=0.426, p<0.001$). Ghrelin and ghrelin/PYY ratio trajectories from 3-6 months did not associate with FM% trajectory during that period, but infants with greater increase in ghrelin and ghrelin/PYY ratio had less increase in visceral FM ($p\leq0.009$). Leptin, not adiponectin, trajectories associated with FM% trajectories until 2 years.

Conclusions Our data show that ghrelin and ghrelin/PYY ratio increased during the first 2 years of life, while PYY, adiponectin and leptin decreased. Ghrelin and ghrelin/PYY ratio had no predictive value for later FM%, but early life adiponectin levels might predict FM% at 2 years, suggesting that adiponectin is involved in early adiposity programming. Leptin levels mainly reflected current FM%, but also had some predictive value for later FM%.

INTRODUCTION

Appetite regulating hormones (ARH) are involved in the regulatory system of appetite, food intake and satiety (1). The hypothalamus plays an important role in controlling glucose and energy homeostasis (1, 2). ARH are secreted from the gastrointestinal tract (ghrelin, PYY) and adipose tissue (leptin, adiponectin) (1, 3). Ghrelin stimulates food intake, which could influence infant growth and body composition, while leptin and PYY reduce food intake and increase metabolic rate. Adiponectin increases fatty acid and carbohydrate uptake (2, 3). Ghrelin/PYY ratio is of interest as an orexigenic drive marker, rather than ghrelin and PYY levels separately (4, 5).

The first 1000 days of life, from conception to age 2 years, is an important period for infant development (6, 7). Within this period, we previously found that the change in fat mass percentage (FM%) during the first 6 months, in contrast to the 6- to 12-month period, is associated with higher FM% and abdominal subcutaneous fat mass (FM) at age 2 years (8). These data support a critical window for adiposity programming in early life (9, 10).

Most studies used cord blood (11-16) or newborn blood spots (17, 18) to investigate ARH at birth and later growth and body composition, or included infants born premature or small-for-gestational age (19, 20). Two studies investigated only leptin and adiponectin levels at birth, 2, 5 and 9 years in a small group of infants or at 6 months, 5.5 and 8 years (3, 21). However, insights in ARH trajectories in association with FM parameters during the first 6 months and the period thereafter until 2 years in term-born infants are still lacking.

Our primary objective was to investigate longitudinal ghrelin, PYY, adiponectin and leptin levels during the first 2 years of life, at 3, 6 months and 2 years, and associate these levels with FM parameters at age 2 years to determine potential predictors for FM development in the total group and in exclusively breastfed versus formula fed infants. Additionally, we investigated associations of ARH trajectories until 6 months and from 6 months to 2 years with trajectories of FM parameters during the same periods. We hypothesized that ghrelin and ghrelin/PYY ratio at 3 and 6 months would predict FM% at 2 years, while adiponectin and leptin would reflect current FM%.

METHODS

Participants

The study population consisted of healthy, term-born infants, participating in the Sophia Pluto Birth Cohort in Rotterdam area(The Netherlands). All participants were term-born (≥37

weeks of gestation), age <28 days, uncomplicated neonatal period without signs of severe asphyxia (5-minutes Apgar score <3). Infants were excluded if they had known congenital or postnatal diseases, confirmed intrauterine infection, maternal use of corticosteroids during pregnancy or a significant maternal medical condition that could interfere with study results. For present study, we included 174 infants born singleton with blood sampling at 3, 6 months and 2 years. The Sophia Pluto Study obtained approval by the Medical Ethics Committee of Erasmus Medical Center and parental written informed consent for participants.

Data Collection and Measures

Outpatient clinic visits were scheduled at 1, 3, 6 months and 2 years (Table 1). Birth data were obtained from hospital records and anthropometric data were collected (22, 23).

Blood samples

Blood samples at 3, 6 months and 2 years were collected in EDTA tubes by toe prick after infants had fasted ≥2 hours. DPP4-inhibitor, Serine-Protease-inhibitor and Protease-inhibitor (Merck Chemicals Netherlands) were added for stabilizing ARH. Blood was centrifuged at 4°C to prepare plasma, which was quickly frozen and stored at −80°C until analyses. Ghrelin (acylated), PYY and leptin were determined by MILLIPLEX MAP Human Metabolic Hormone Magnetic Bead Panel (HMHEMAG-34K) and adiponectin by MILLIPLEX MAP Human Adipokine Magnetic Bead Panel 1 (HADK1MAG-61K, Millipore Corporation, Billerica, MA), using commercial protocol. Intra-assay and inter-assay CV were 10% and 15%, respectively (both panels). Median (IQR) fasting time (blood collection time minus last feeding time) was ≥2:20 (1:45-2:50) hours at all ages.

Fat mass

Until 6 months, FM was assessed by air-displacement plethysmography (PEA POD, COSMED, Italy) as described in detail elsewhere (22, 24). At 2 years, a Dual Energy X-ray Absorptiometry (DXA) scan was performed (Lunar Prodigy, GE Healthcare, UK, enCORE software version 14.1) (23).

Abdominal fat mass

Subcutaneous and visceral fat thickness (cm) were measured by ultrasound and described in detail elsewhere (22, 25).

Infant feeding

Exclusive breastfeeding (EBF; n=96) or formula feeding (EFF; n=45) was defined as infants receiving only EBF or EFF, respectively, and no mixed feeding for 3 months after birth.

Statistical Analysis

Clinical characteristics are expressed as median (interquartile range (IQR)) in Table 1. Longitudinal ARH levels during the first two years of life were analyzed using linear mixed model analysis. Sex was used as a covariate. Age was modeled as categorical variable indicating hospital visits at 3, 6 months and 2 years.

Spearman correlation coefficient was used to determine correlations between non-parametric parameters. We also investigated associations between ARH and FM parameters with adjustment for sex, but since the main results were similar, we present the unadjusted linear correlations. Data are presented for total group and for EBF and EFF only in case of different results compared to the total group.

Associations between ARH trajectories and trajectories of FM parameters were analyzed by linear mixed models with FM parameters (dependent variable) and sex, interaction of age with sex, change in ARH and interaction of age with change in ARH (covariates). SPSS statistical package version 25 (SPSS Inc. Chicago, Illinois) was used and p-values <0.05 were considered statistically significant.

Table 1. Clinical characteristics of 174 infants.

Age		Birth	1 month	3 months	6 months	2 years
Weight (kg)	M	3.38 [3.09 – 3.77]	4.37 [3.96 – 4.78]	6.24 [5.78 – 6.72]	7.96 [7.41 – 8.66]	13.31 [11.81 – 14.17]
	F	3.21 [2.80 – 3.51]	3.98 [3.52 – 4.33]	5.70 [5.09 – 6.21]	7.37 [6.83 – 7.89]	12.30 [11.44 – 13.10]
Length (cm)	M	51.0 [49.0 – 52.8]	55.0 [53.5 – 56.5]	62.4 [60.5 – 63.4]	69.0 [67.0 – 70.3]	89.9 [87.5 – 91.8]
	F	49.0 [48.0 – 50.5]	53.5 [51.4 – 55.1]	60.0 [58.5 – 61.2]	66.6 [65.2 – 68.1]	87.5 [85.5 – 89.7]
FM (%)	M	NA	15.7 [13.5 – 19.0]	22.8 [19.8 – 25.6]	24.5 [22.2 – 27.4]	17.7 [15.2 – 20.2]
	F	NA	15.1 [11.8 – 19.0]	24.4 [17.9 – 26.5]	25.6 [21.7 – 29.0]	17.8 [15.0 – 21.4]
Abdominal subcutaneous FM (cm)	M	NA	NA	0.41 [0.33 – 0.49]	0.41 [0.35 – 0.52]	0.35 [0.29 – 0.41]
	F	NA	NA	0.37 [0.31 – 0.50]	0.41 [0.33 – 0.51]	0.34 [0.27 – 0.40]
Visceral FM (cm)	M	NA	NA	2.41 [1.89 – 2.87]	2.08 [1.79 – 2.66]	2.02 [1.73 – 2.45]
	F	NA	NA	2.23 [1.94 – 2.70]	2.22 [1.72 – 2.64]	2.19 [1.84 – 2.63]

Data expressed as median [interquartile range] for boys (M) and girls (F). Birth length only available for 64 boys and 37 girls. FM: fat mass.

RESULTS

Clinical characteristics are presented in Table 1, 59.2% was male and 64.4% Caucasian. Median gestational age was 39.9 (39.0-40.9) weeks.

ARH trajectories during the first 2 years of life

Ghrelin (acylated) levels increased from 3 months to 2 years (p<0.001), while PYY levels decreased (p<0.001)(Figure 1), which resulted in an increasing ghrelin/PYY ratio (p<0.001). Ghrelin, PYY and ghrelin/PYY ratio were similar between boys and girls (all p≥0.30)(Table 2). Adiponectin levels decreased (p<0.001) and trajectories were similar between boys and girls. Leptin levels also decreased (p<0.001). Girls had higher leptin levels at 3 months compared to boys (p=0.002), but similar levels at 2 years.

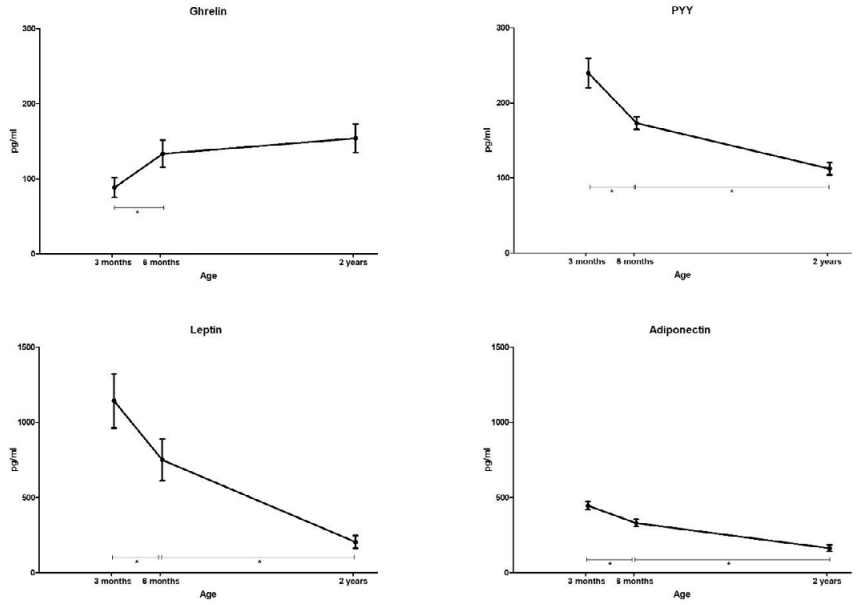

Figure 1. Appetite regulating hormone trajectories during the first 2 years of life.
Data are presented as estimated marginal means (95% confidence interval). * represent a significant change during that period. PYY: peptide YY.

Associations of ARH with FM parameters at 2 years

ARH during the first 6 months of life

First, we investigated if ARH levels at 3 and 6 months and change in ARH between these ages correlated with FM parameters at 2 years (Table 3) to determine potential predictive value. Ghrelin, PYY and ghrelin/PYY ratio at 3, 6 months and change from 3 to 6 months did not correlate with any FM parameter at 2 years.

Table 2. Levels of appetite regulating hormones at age 3 and 6 months and 2 years in boys and girls.

	Boys			Girls			
	3 months	**6 months**	**2 years**	**3 months**	**6 months**	**2 years**	**p-value ***
Ghrelin (pg/ml)	86.5 [68.6 – 104.3]	144.8 [121.5 – 168.2]	152.3 [127.6 – 177.1]	91.1 [71.4 – 110.8]	118.1 [90.9 – 145.4]	156.5 [126.2 – 186.8]	0.98
PYY (pg/ml)	245.2 [218.5 – 271.9]	176.6 [165.3 – 187.9]	110.8 [100.3 – 121.3]	233.4 [204.4 – 262.4]	167.9 [154.7 – 181.1]	114.5 [101.7 – 127.4]	0.44
Ghrelin/PYY ratio	0.43 [0.31 – 0.54]	0.90 [0.72 – 1.07]	1.75 [1.43 – 2.06]	0.47 [0.35 – 0.60]	0.81 [0.61 – 1.00]	1.63 [1.24 – 2.02]	0.51
Adiponectin (pg/ml)	436.4 [400.8 – 472.1]	329.3 [297.5 – 361.1]	166.9 [138.8 – 194.9]	457.5 [418.0 – 497.1]	333.2 [296.4 – 368.9]	158.2 [124.8 – 191.6]	0.30
Leptin (pg/ml)	864.7 [632.0 – 1097.4]	659.4 [476.7 – 842.0]	196.0 [142.2 – 249.8]	1471.0 [1218.3 – 1723.8]	869.7 [655.2 – 1084.1]	217.9 [151.9 – 283.9]	**0.002**

Data presented as estimated marginal means [95% confidence interval], based on mixed model analysis with samples at 3 months (n=102), 6 months (n=156) and 2 years (n=158). * p-value for the change in ARH from 3 months to 2 years in boys versus girls. PYY; peptide YY.

Table 3. Correlations between (change in) appetite regulating hormones and FM%, abdominal visceral and subcutaneous FM at the age of 2 years.

	FM% 2 years		**Visceral FM 2 years**		**Subcutaneous FM 2 years**	
	R	**p-value**	**R**	**p-value**	**R**	**p-value**
3 months						
Ghrelin	0.057	0.60	0.030	0.77	0.063	0.54
PYY	0.029	0.79	0.019	0.86	0.053	0.60
Ghrelin/PYY ratio	0.024	0.83	0.004	0.97	0.041	0.69
Adiponectin	**0.335**	**0.001**	0.013	0.90	0.144	0.15
Leptin	**0.214**	**0.047**	-0.025	0.81	0.177	0.08
6 months						
Ghrelin	0.095	0.27	0.096	0.24	-0.025	0.76
PYY	-0.004	0.96	0.002	0.98	0.074	0.37
Ghrelin/PYY ratio	0.076	0.38	0.098	0.24	-0.065	0.43
Adiponectin	**0.282**	**0.001**	0.021	0.80	0.081	0.33
Leptin	**0.286**	**0.001**	0.042	0.61	**0.305**	**<0.001**
2 years						
Ghrelin	-0.072	0.39	-0.076	0.35	0.026	0.75
PYY	-0.013	0.88	0.105	0.20	0.005	0.95
Ghrelin/PYY ratio	-0.077	0.37	-0.087	0.29	0.008	0.92
Adiponectin	0.124	0.15	0.038	0.65	0.019	0.82
Leptin	**0.426**	**<0.001**	0.024	0.77	**0.244**	**0.002**
Δ 3-6 months						
Ghrelin	-0.022	0.85	0.082	0.45	-0.105	0.32
PYY	-0.078	0.49	-0.080	0.45	-0.032	0.76
Ghrelin/PYY ratio	-0.022	0.85	0.125	0.24	-0.105	0.32
Adiponectin	**-0.271**	**0.015**	-0.072	0.50	-0.064	0.55
Leptin	-0.081	0.47	0.014	0.90	0.069	0.51
Δ 6 months – 2 years						
Ghrelin	-0.127	0.16	-0.144	0.09	0.061	0.48
PYY	0.050	0.58	0.041	0.64	-0.057	0.50
Ghrelin/PYY ratio	-0.153	0.087	-0.136	0.11	0.027	0.75
Adiponectin	**-0.218**	**0.016**	-0.073	0.41	-0.057	0.51
Leptin	-0.129	0.15	-0.038	0.66	**-0.214**	**0.012**

Data expressed as correlation coefficient (R) with corresponding p-values. FM%; fat mass percentage, FM; fat mass, PYY: peptide YY.

Adiponectin at 3 and 6 months correlated with FM% at 2 years (R=0.335 and R=0.282, p=0.001) and change in adiponectin from 3 to 6 months correlated inversely (R=-0.271, p<0.001), indicating that a greater decline in adiponectin correlated with higher FM% at 2 years. Adiponectin at 3 months correlated inversely with change in adiponectin from 3 to 6 months (R=-0.413, p<0.001). Adiponectin at 3 and 6 months and change in adiponectin did not correlate with abdominal visceral and subcutaneous FM at 2 years.
Leptin at 3 months and 6 months correlated with FM% at 2 years (R=0.214, p=0.047 and R=0.286, p=0.001,resp.), but change in leptin from 3 to 6 months did not. Leptin did not correlate with visceral FM, but leptin at 6 months correlated with abdominal subcutaneous FM at 2 years (R=0.244, p=0.002).

ARH from 6 months until 2 years
Secondly, we investigated if ARH levels at 2 years and change in ARH between 6 months and 2 years correlated with FM parameters at 2 years.
Neither ghrelin and ghrelin/PYY ratio at 2 years and change from 6 months to 2 years, nor adiponectin at 2 years correlated with FM parameters. Change in adiponectin from 6 months to 2 years, however, correlated inversely with FM% at 2 years (R=-0.218, p=0.016), indicating that a greater decline in adiponectin correlated with higher FM%, in line with the period from 3 to 6 months. Adiponectin at 6 months correlated inversely with change in adiponectin from 6 months to 2 years (R=-0.599, p<0.001). Leptin at 2 years correlated with FM% at 2 years (R=0.426, p<0.001), while the change from 6 months until 2 years did not. Leptin at 2 years and change in leptin from 6 months until 2 years did correlate with abdominal subcutaneous FM at 2 years (R=-0.214, p=0.012, R=0.244, p=0.002, resp.).

In EFF infants, ghrelin and ghrelin/PYY ratio at 2 years correlated inversely with visceral FM at 2 years (R=-0.519, -0.356, resp., p≤0.026), indicating that higher ghrelin and ghrelin/PYY ratio correlated with lower visceral FM. The increase in ghrelin and ghrelin/PYY ratio from 6 months to 2 years correlated also inversely with visceral FM at 2 years in EFF (R=-0.386, -0.436, resp., p≤0.026) and EBF infants (R=-0.228, -0.227, resp., p≤0.046).

Associations between ARH trajectories and FM trajectories during the first 2 years of life

In addition, we investigated if ARH trajectories were associated with the trajectories of FM parameters during the first 2 years of life. As FM% increases during the first 6 months of life and subsequently decreases until 2 years (23), we investigated trajectories of FM parameters from 3 to 6 months and from 6 months to 2 years separately (Table 4).

Table 4. Associations between appetite regulating hormone trajectories and trajectories of FM%, abdominal visceral and subcutaneous FM from 3 to 6 months and from 6 months to 2 years.

	Change in FM%		Change in visceral FM		Change in subcutaneous FM	
	Estimate	**p-value**	**Estimate**	**p-value**	**Estimate**	**p-value**
3 – 6 months						
Δ Ghrelin (pg/ml)	0.078	0.82	**-0.191**	**0.009**	-0.000	0.98
Δ PYY (pg/ml)	0.380	0.32	0.107	0.17	**-0.028**	**0.024**
Δ Ghrelin/PYY ratio	0.172	0.71	**-0.276**	**0.003**	0.008	0.61
Δ Adiponectin (pg/ml)	0.093	0.80	0.004	0.96	-0.008	0.54
Δ Leptin (pg/ml)	**0.121**	**0.001**	-0.006	0.45	**0.003**	**0.009**

	Change in FM%		Change in visceral FM		Change in subcutaneous FM	
	Estimate	**p-value**	**Estimate**	**p-value**	**Estimate**	**p-value**
6 months – 2 years						
Δ Ghrelin (pg/ml)	-0.307	0.28	-0.082	0.08	0.006	0.38
Δ PYY (pg/ml)	0.539	0.35	0.055	0.56	-0.007	0.63
Δ Ghrelin/PYY ratio	-0.395	0.12	-0.035	0.39	-0.003	0.62
Δ Adiponectin (pg/ml)	-0.009	0.98	-0.042	0.41	0.004	0.60
Δ Leptin (pg/ml)	**0.165**	**<0.001**	0.002	0.79	-0.000	0.80

Associations presented as Estimate per 100pg/ml change in ARH (except for ghrelin/PYY ratio), with p-value. FM%; fat mass percentage, FM; fat mass, PYY; peptide YY.

Trajectories during the first 6 months of life

The trajectories of ghrelin, PYY and ghrelin/PYY ratio from 3 to 6 months did not associate with the FM% trajectory during the same period. However, the ghrelin and ghrelin/PYY ratio trajectories from 3 to 6 months were inversely associated with the visceral FM trajectory during the same period (p=0.009 and 0.003, resp.), indicating that infants with greater increase in ghrelin and ghrelin/PYY ratio had less increase in visceral FM. The ghrelin and ghrelin/PYY ratio trajectories did not associate with the abdominal subcutaneous FM trajectory. The PYY trajectory from 3 to 6 months was inversely associated with the abdominal subcutaneous FM trajectory during the same period (p=0.024), indicating that infants with greater decline in PYY had a greater increase in abdominal subcutaneous FM.

The adiponectin trajectory from 3 to 6 months did not associate with FM%, visceral and subcutaneous FM trajectories during this period. The leptin trajectory from 3 to 6 months did associate with FM% and subcutaneous FM trajectories during the same period (p=0.001 and 0.009, resp.), indicating that infants with less decline in leptin had greater increase in FM% and abdominal subcutaneous FM.

Trajectories from 6 months until 2 years

In contrast to the period of 3 to 6 months, there were no associations between ghrelin, PYY and ghrelin/PYY ratio trajectories from 6 months to 2 years and any trajectory of FM parameters. The adiponectin trajectory did also not associate with any trajectories of FM parameters.

The leptin trajectory from 6 months to 2 years, however, associated with the FM% and abdominal subcutaneous FM trajectories during the same period ($p<0.001$), indicating that infants with less decline in leptin had less decline in FM%.

DISCUSSION

We present for the first time ARH trajectories during the first 2 years of life and associations with longitudinally measured FM parameters in healthy infants. Ghrelin and ghrelin/PYY ratio, representing orexigenic drive, increased from 3 months until 2 years, while PYY, adiponectin and leptin decreased. When investigating the potential predictive value of ARH for adiposity development, we observed that adiponectin at 3 and 6 months, a greater decline in adiponectin during the first 2 years and leptin at all ages correlated with higher FM% at 2 years. When investigating ARH trajectories, ghrelin and ghrelin/PYY ratio trajectories from 3-6 months only associated with the visceral FM trajectory during the same period. The leptin trajectory associated with the FM% trajectory until 2 years. Our findings suggest that ghrelin and ghrelvin/PYY ratio in early life are, in contrast to our hypothesis, no predictors for later FM%, but adiponectin during the first 6 months, and not thereafter, might predict FM% at 2 years. Leptin mainly reflects current FM%, but has also some predictive value for later FM%.

The first 2 years of life are an important period for infant development (6, 7), but early life longitudinal studies with multiple ARH measurements are very limited. Most studies used cord blood to investigate ARH at birth (12, 15, 16) or one sample during infancy (26, 27). One study showed lower adiponectin and leptin at 2 years compared to levels at birth, but had no samples between those ages (3). We now show that ghrelin increased during the first 2 years of life, while PYY, adiponectin and leptin decreased. The changes in ghrelin and PYY until 2 years result in an increase in ghrelin/PYY ratio over time. As this ratio is considered a marker for orexigenic drive (4, 5), the increase in ghrelin/PYY ratio in infants might indicate increasing orexigenic drive, leading to more appetite stimulation over time.

ARH trajectories were similar in boys and girls, but girls had higher leptin levels compared to boys until 2 years. This difference started with significantly higher leptin levels in girls at 3 months, which is in line with literature (13, 28, 29).

The first 6 months of life are considered a critical window for adiposity programming (9, 10). FM% increases during this period (23, 30, 31) and decreases thereafter (23). We have previously shown that only the change in FM% until 6 months and not in the 6 months thereafter associated with greater adiposity at 2 years (8). We, therefore, investigated whether ARH levels during a critical window for adiposity programming and thereafter, from 6 months until 2 years, were associated with FM parameters at 2 years. In addition, we investigated if ARH trajectories from 3 to 6 months and from 6 months to 2 years associated with trajectories of FM parameters in the same periods.

In contrast to our hypothesis, ghrelin and ghrelin/PYY ratio at 3 and 6 months were not associated with any FM parameter at 2 years, thus not predictive for FM% at 2 years. Ghrelin and ghrelin/PYY ratio trajectories during the first 6 months of life did also not associate with the FM% trajectory during the same period. However, greater increase in ghrelin and ghrelin/PYY ratio during the first 6 months associated with less increase in visceral FM during the same period. These findings are in line with literature describing that visceral FM inversely correlated with ghrelin levels (32) and studies reporting lower ghrelin levels in obese children and adults (33, 34). In subjects with Prader-Willi syndrome, characterized by hyperphagia and excessive weight, higher ghrelin levels (34, 35) and reduced visceral adiposity compared to obese controls have been described (35, 36).

In EFF infants, a higher ghrelin and ghrelin/PYY ratio at 2 years correlated with lower visceral FM at 2 years, while a greater increase in ghrelin and ghrelin/PYY ratio from 6 months to 2 years correlated with lower visceral FM at 2 years in both EFF and EBF infants. Our data, therefore, suggest that ghrelin and ghrelin/PYY ratio trajectories during a critical window for adiposity programming might contribute specifically to visceral FM development instead of total FM development. This could be an important finding as specifically increased visceral FM has been associated with unfavorable metabolic health profiles in childhood and later life (37, 38). Future studies with longer-term follow-up are required for confirmation.

A greater decline in PYY during the first 6 months was only associated with greater increase in subcutaneous FM in the same period, which is in line with mice studies showing that PYY-deficient mice had increased subcutaneous adiposity (39).

Higher adiponectin at 3 and 6 months and greater decline in adiponectin until 2 years resulted in higher FM% at 2 years. This suggests that, in contrast to our hypothesis, the actual adiponectin levels during a critical window for adiposity programming might predict FM% at 2 years, while also the decline in adiponectin between 3 months and 2 years associated with FM% at 2 years. The latter finding could be explained by the fact that infants with greater decline in adiponectin from 3 months to 2 years had higher adiponectin levels at 3

and 6 months. Our findings are in line with literature showing lower adiponectin in adults with overweight or obesity (40-42) and complement a study investigating adiponectin and leptin at birth, 2, 5 and 9 years (3). That study, however, did not investigate adiponectin between birth and 2 years. Factors affecting adiponectin and consequences of its changes during early infancy were therefore not investigated (3). We now show that specifically the adiponectin levels during the first 6 months as well as the decline in adiponectin from 3 months to 2 years might be involved in adiposity programming.

Leptin at 3 and 6 months correlated with FM% at 2 years, but leptin at 2 years showed the strongest correlation with FM% and abdominal subcutaneous FM at the same age. The leptin trajectory from 3 months to 2 years also associated with FM% and abdominal subcutaneous FM trajectories during this period. We have previously also shown that leptin at 3 and 6 months correlated with FM% at the same ages (43). Altogether, these findings indicate that leptin mainly reflects current FM%. This is in line with the abovementioned study showing that leptin closely reflects body size (3). We now add that also the leptin trajectory during the first 2 years of life corresponds with the FM% trajectory during the same period and that early leptin levels have some predictive value for later FM%.

We had expected that ARH during the period from 6 months until 2 years would have shown more associations with FM parameters at 2 years, since this period is closer to the study endpoint. Our findings, however, show that mainly ARH levels during the period from 3 to 6 months associated with FM parameters at 2 years, thereby supporting that the first 6 months of life are a critical window for adiposity programming in which particularly adiponectin and leptin could play a role in FM development.

Conclusions

In conclusion, ghrelin levels increase during the first 2 years of life and PYY levels decrease, resulting in an increase in ghrelin/PYY ratio which represents an increase in orexigenic drive. Leptin and adiponectin levels decrease. Our data show that ghrelin and ghrelin/PYY ratio in early life had, in contrast to our hypothesis, no predictive value for later FM%, but early life adiponectin levels and its trajectories might predict FM% at 2 years, suggesting that adiponectin is involved in early adiposity programming. Leptin levels mainly reflect current FM%, but have also some predictive value for later FM%. Our findings can potentially be used for personalized screening tools for obesity prevention starting at early age.

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