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SHORT COMMUNICATION

Leptin, acylcarnitine metabolites and development of adiposity in the Rhea mother–child cohort in Crete, Greece

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Summary

Objective

This study aims to investigate relations of serum leptin at age 4 with development of adiposity and linear growth during 3 years of follow-up among 75 Greek children and to identify serum metabolites associated with leptin at age 4 and to characterize their associations with adiposity gain and linear growth.

Methods

Linear regression models that accounted for maternal age, education and gestational weight gain and child's age and sex were used to examine associations of leptin and leptin-associated metabolites measured at age 4 with indicators of adiposity and linear growth at age 7.

Results

Each 1-unit increment in natural log-(ln)-transformed leptin corresponded with 0.33 (95% CI: 0.10, 0.55) units greater body mass index-for-age z-score gain during follow-up. Likewise, higher levels of the leptin-associated metabolites methylmalonyl-carnitine and glutaconyl-carnitine corresponded with 0.14 (95% CI: 0.01, 0.27) and 0.07 (95% CI: −0.01, 0.16) units higher body mass index-for-age z-score gain, respectively. These relationships did not differ by sex or baseline weight status and were independent of linear growth.

Conclusions

These findings suggest that leptin, methylmalonyl-carnitine and possibly glutaconyl-carnitine are associated with weight gain during early childhood. Future studies are warranted to confirm these findings in other populations.

Keywords: metabolites, adiposity, children, leptin.

Abbreviations

BMIZ	body mass index-for-age z-score
HAZ	height-for-age z-score
SS	subscapular skinfold
TR	triceps skinfold

Introduction

Leptin, an adipocyte-derived hormone, is an important regulator of weight and metabolism. Although high circulating leptin is traditionally regarded as a consequence of excess fat mass, elevated leptin can also precede weight gain. In adults, high leptin in conjunction with overweight/obesity status suggests hypothalamic resistance to leptin's hunger-inhibiting effects (1), which could lead to additional weight gain over time. Several studies have also observed a direct relationship between circulating leptin and subsequent weight gain after adjusting for body size at baseline (2–4), suggesting that leptin resistance can occur independently of weight status. This relationship is less clear in children. Studies in

paediatric populations indicate that both low (5,6) and high (7–9) leptin are associated with weight gain over time. Potential explanations for the discrepancies include the possibility that young children may not yet have developed leptin resistance (10) and the fact that leptin has pleiotropic effects on bone growth (11), which could confound the relationship between leptin and adiposity gain.

The overarching aim of this study was to investigate relations of serum leptin at age 4 years with adiposity gain and linear growth during 3 years of follow-up among 75 children. To improve understanding of biochemical pathways linking leptin to weight gain, a secondary aim was to identify metabolites associated with leptin at baseline and to explore their relations with adiposity and linear growth during follow-up.

Our hypothesis was that higher leptin, as well as any leptin-related metabolites, is associated with greater gains in adiposity, as indicated by change in age-standardized and sex-standardized body mass index (BMI), the sum of the triceps and subscapular skinfold thicknesses, % fat mass and waist circumference, independently of linear growth. Results from this work will enhance understanding of etiological pathways underlying the accrual of excess fat mass during early life.

Methods

This study includes participants of the Rhea cohort in Crete, Greece (12). The present analysis included 75 children with data on anthropometry, serum leptin and targeted metabolomics data at the 4-year research visit (baseline) and anthropometry at the 7-year visit (follow-up). All procedures were approved by the ethics committee of the University Hospital in Heraklion, Crete, Greece. All participants provided written informed consent.

Anthropometry

At the 4-year visit, research assistants (RAs) measured the children's weight, height, waist circumference and the subscapular (SS) and triceps (TR) skinfold thicknesses (13). Body mass index-for-age (BMIZ) and height-for-age (HAZ) z-scores were derived using the World Health Organization growth standard (14–16). Indicators of adiposity included BMIZ, SS + TR, % fat calculated from skinfolds using Slaughter's equation (17) and waist circumference. At the 7-year visit, RAs measured anthropometry and calculated the same anthropometric indices. At this time, RAs also conducted bioelectrical impedance assessments (BIA) of fat mass and % fat. Outcomes of interest were change in each adiposity indicator (BMIZ, SS + TR, Slaughter's % fat and waist circumference) and linear growth (HAZ) between the 4- and 7-year visits. Attained BIA fat mass and % fat at 7 years were also assessed, although it was not possible to calculate change as BIA was only carried out at follow-up.

Serum leptin and targeted metabolomics

At the 4-year visit, RAs collected blood from the children, from which serum leptin (ng mL^{-1}) was quantified (18). Targeted metabolomics analyses were carried out on serum to quantify amino acid and acylcarnitine species previously associated with childhood obesity and leptin (19) using a mass spectrometry-based approach (Absolute IDQ p180 kit, Biocrates Life Sciences AG, Austria). Metabolite concentrations were adjusted for batch effects and normalized as a z-score centred on the median and scaled by the mean absolute deviation from the median.

Data analysis

The first step of the analysis was to examine associations of leptin with background characteristics in order to identify potential confounders. Owing to non-normal distributions, leptin was natural log-(ln)-transformed prior to regression analysis. The next step was to estimate change in each adiposity indicator and HAZ with respect to quartiles of leptin using linear regression. The associations were linear, so leptin was evaluated continuously. In multivariable analysis, the models accounted for maternal age, education and gestational weight gain (20) and child's age at the 4- and 7-year assessments and sex. In all models for the adiposity outcomes, HAZ change was also included as a covariate.

To identify metabolites associated with leptin, linear regression models with leptin as the independent variable, age and sex as covariates and individual metabolite z-scores as the outcome were used. Metabolites were retained for further analyses if the *P*-value for leptin's β was <0.05. Next, relations of each leptin-associated metabolite with change in the anthropometric outcomes were assessed using the same approach as for leptin.

In sensitivity analyses, maternal pre-pregnancy BMI, birthweight-for-gestational-age and breastfeeding duration were included in the models; accounting for these covariates did not change the results, thus they were not included in the final models. Formal tests for statistical interactions with sex and baseline weight status were carried out for all associations. All analyses were performed using SAS 9.3 (Cary, NC, USA).

Results

Median age at baseline was 4.2 years (range 4.0–4.5); 57.3% were boys. Table 1 shows associations of leptin

Table 1 Distribution of serum leptin concentrations at baseline (4 years) according to characteristics of 75 Rhea cohort mother–child pairs

	N*	Mean ± SD Leptin (ng mL^{-1})	P†
Overall	75	2.34 ± 1.49	
Family and perinatal characteristics			
Mother's age at enrollment (years)			0.23
20–24	4	2.08 ± 1.06	
25–34	55	2.20 ± 1.43	
35–43	14	2.73 ± 1.60	
Mother's education			0.82
Less than university	33	2.41 ± 1.80	
University education	40	2.33 ± 1.22	
Parity			0.91
Primiparous	30	2.33 ± 1.27	
Muliparous	37	2.29 ± 1.61	
Mother's pre-pregnancy BMI (kg m^{-2})			0.16
Normal weight (18.5–24.9)	51	2.05 ± 1.11	
Overweight (25–29.9)	15	3.35 ± 2.18	
Obese (≥30)	6	1.89 ± 0.31	
Gestational weight gain‡			0.13
Inadequate	16	2.43 ± 1.61	
Adequate	33	2.60 ± 1.59	
Excessive	23	1.81 ± 0.99	
Birth size§			0.94
SGA (<10th %tile)	6	2.36 ± 2.59	
AGA (10–≤90th %tile)	63	2.33 ± 1.40	
LGA (>90th %tile)	6	2.43 ± 1.33	
Mother smoking habits			0.38
Never	49	2.47 ± 1.63	
Former	12	1.84 ± 0.86	
Smoked during pregnancy	8	2.18 ± 1.11	
Duration of breastfeeding (months)			0.82
<6	55	1.27 ± 1.42	
≥6	20	2.28 ± 1.60	
Child characteristics at the 4-year research visit			
Sex			0.03
Female	32	2.77 ± 1.79	
Male	43	2.02 ± 1.14	
BMI-for-age z-score¶			0.0002
≤1	46	1.89 ± 1.14	
>1 and ≤2	15	2.72 ± 1.57	
>2	14	3.41 ± 1.85	
SS + TR (mm)			<0.0001
Tertile 1 (median = 12.5)	23	1.38 ± 0.68	
Tertile 2 (median = 16.5)	24	2.30 ± 1.06	
Tertile 3 (median = 21.4)	24	3.39 ± 1.84	
Slaughter's % fat			<0.0001
Tertile 1 (median = 13.5)	23	1.33 ± 0.64	
Tertile 2 (median = 19.0)	24	2.34 ± 1.04	
Tertile 3 (median = 24.6)	24	3.39 ± 1.84	
Waist circumference (cm)			<0.0001
Tertile 1 (median = 50.0)	24	1.40 ± 0.72	
Tertile 2 (median = 53.5)	24	2.35 ± 1.19	
Tertile 3 (median = 57.2)	26	3.25 ± 1.74	
Height-for-age z-score¶			0.007
≤1	59	2.17 ± 1.30	
>1 and ≤2	13	2.60 ± 1.38	
>2	3	4.69 ± 3.46	

*Totals may not add up to 75 because of missing values.
†Represents a test for linear trend with the exception of sex, smoking habits and breastfeeding duration (Wald test).
‡According to the IOM 2009 gestational weight gain guidelines (12).
§Based on internally standardized percentile values as previously described (4).
¶According to the World Health Organization growth reference (6–8). For both BMI and height, the '<1 z-score' category includes one child with z-score <−2.
AGA, appropriate for gestational age; BMI, body mass index; IOM, Institute of Medicine; LGA, large for gestational age; SD, standard deviation; SGA, small for gestational age; SS, subscapular; TR, triceps.

with background characteristics. All adiposity indicators and HAZ were positively related to leptin at baseline.

In multivariable analysis, each increment in ln-leptin corresponded with 0.33 (95% CI: 0.10, 0.55) units greater BMIZ gain during follow-up. Similar associations were observed for the other adiposity indicators, although the confidence intervals contained the null. Leptin at 4 years was positively associated with BIA fat mass and % fat at 7 years (Table 2).

Of the 21 amino acids and 20 acylcarnitines quantified from serum at the 4-year visit, two were associated with leptin: glutanconyl-carnitine and methylmalonyl-carnitine (Table S1); both were positively associated with BMIZ gain as well as BIA fat mass and % fat at 7 years (Table 2). Neither leptin nor the metabolites were related to linear growth. Adjustment for HAZ change in models for the adiposity outcomes attenuated effect sizes, although the relations with BMIZ remained apparent. There was no evidence of effect modification by sex or baseline weight status.

Discussion

In this study of Greek children, higher leptin at age 4 was associated with greater BMIZ gain during 3 years of follow-up. Higher concentrations of the leptin-associated metabolites methylmalonyl-carnitine and glutaconyl-carnitine at baseline also corresponded with greater BMIZ gain. These relations did not differ by sex or baseline weight status and were independent of linear growth.

Our results add to current evidence in several ways. First, most paediatric investigations of leptin and weight gain were conducted in older children (>6 years at

Table 2 Associations of leptin and two leptin-associated metabolites with adiposity and linear growth during follow-up

		β (95% CI) for change in each anthropometric indicator		
	Mean ± SD	Leptin Per unit*	Methylmalonyl-carnitine Per z-score†	Glutaconyl-carnitine Per z-score†
Δ Adiposity (7 years-4 years)				
Δ BMI z-score	0.75 ± 0.92			
Model 1		0.33 (0.10, 0.55)	0.14 (0.01, 0.27)	0.07 (-0.01, 0.16)
Model 2		0.25 (0.04, 0.45)	0.11 (0.00, 0.23)	0.09 (0.01, 0.16)
Δ SS + TR (mm)	1.19 ± 6.59			
Model 1		1.41 (−0.33, 3.17)	0.49 (−0.53, 1.51)	0.08 (−0.59, 0.74)
Model 2		1.14 (−0.59, 2.88)	0.50 (−0.50, 1.49)	0.18 (−0.47, 0.83)
Δ Slaughter's % fat	−2.17 ± 5.15			
Model 1		0.28 (−1.14, 1.71)	0.19 (−0.64, 1.01)	0.04 (−0.49, 0.57)
Model 2		0.09 (−1.34, 1.51)	0.19 (−0.62, 1.00)	0.11 (−0.42, 0.63)
Δ Waist circumference (cm)	4.88 ± 4.87			
Model 1		0.72 (−0.54, 1.99)	−0.04 (−0.78, 0.70)	−0.23 (−0.71, 0.24)
Model 2		0.39 (−0.77, 1.55)	−0.05 (−0.71, 0.62)	−0.12 (−0.56, 0.31)
Linear growth (7 years-4 years)				
Δ Height z-score	0.29 ± 0.37	0.06 (−0.02, 0.14)	0.02 (−0.03, 0.07)	−0.01 (−0.04, 0.02)
BIA measurements at 7 years				
Fat mass (kg)	6.0 ± 3.0			
Model 1		1.60 (0.85, 2.36)	0.52 (0.06, 0.98)	0.28 (−0.02, 0.59)
Model 2		1.45 (0.71, 2.18)	0.47 (0.02, 0.91)	0.32 (0.03, 0.61)
% Fat	23.0 ± 6.6			
Model 1		2.84 (1.09, 4.58)	0.48 (−0.20, 1.17)	0.99 (−0.04, 2.02)
Model 2		2.66 (0.91, 4.41)	0.92 (−0.10, 1.94)	0.53 (−0.15, 1.20)

Model 1: Adjusted for mother's age, education and gestational weight gain (IOM 2009 categories) and child's age and sex.
Model 2: Model 1 + change in height z-score.
*Natural log-(ln)-transformed for regression analysis.
†Each metabolite was centred at the median and scaled by the mean absolute distance from the median.
BIA, bioelectrical impedance assessments; BMI, body mass index; CI, confidence interval; IOM, Institute of Medicine; SS, subscapular; TR, triceps.

baseline) and have yielded both positive (7–10) and inverse associations (5,6). The discrepant literature could be due to the influence of pubertal onset on the relation between leptin and body composition (21). The positive association observed between leptin and BMIZ gain aligns with results from similarly aged children in the USA (10) and suggests that leptin resistance may begin early in the life course. Identification of specific timeframes and opportunities to mitigate leptin resistance is an area of ongoing human (10) and animal research (22,23).

Second, the relation between leptin and BMIZ gain was independent of HAZ change, suggesting that leptin stimulates fat accretion rather than linear growth during early childhood. However, because leptin was not associated with change in the other adiposity indicators, the relation with BMIZ may also reflect gains in lean mass that was not accounted for by height change. Future studies assessing change in directly measured body composition (e.g. dual x-ray absorptiometry) are warranted to confirm our findings.

Finally, despite the established link between leptin and obesity (1), little is known of the underlying biochemical pathways. Two leptin-associated metabolites were identified in the analysis, methylmalonyl-carnitine and glutaconyl-carnitine, both of which also correlated with BMIZ gain. Although the literature on glutaconyl-carnitine is scant, elevated methylmalonyl-carnitine is a hallmark of methylmalonic acidemia, a genetic defect in metabolism of certain fats and proteins (24). Because leptin is involved in hepatic lipid oxidation (25), the relationships among leptin, methylmalonyl-carnitine and adiposity gain may revolve around altered fatty acid metabolism. Specifically, leptin resistance may lead to defective hepatic lipid oxidation, which can lead to dysregulated satiety (26) and weight gain (27). In this scenario, methylmalonyl-carnitine is a marker of defective fatty acid metabolism rather than a causal mechanism; whether methylmalonyl-carnitine exerts independent pro-adipogenic effects deserves further investigation.

Strengths of this study include the prospective design, availability of blood specimens during early childhood

and added mechanistic insight via targeted metabolomics data. Limitations include the non-fasting nature of the blood draws and the relatively small sample size.

In conclusion, higher leptin, methylmalonyl-carnitine and glutaconyl-carnitine at age 4 corresponded with greater BMIZ gain between ages 4 and 7 years and higher BIA fat mass at 7 years. The magnitude of associations, particularly for leptin (0.33 higher BMIZ per unit ln-leptin), may have implications at the population level. In a large retrospective study of Danish adults, each unit of BMIZ at age 7 corresponded with 10% and 7% higher risk of fatal cardiovascular disease in men and women, respectively (28). The risk increased linearly with age such that it was twice as high by 13 years (28), emphasizing the importance of identifying determinants of excess weight gain as early as possible. Additional work is required to confirm our findings and to identify modifiable determinants of leptin during early life.

Conflicts of Interest Statement

No conflict of interest was declared.

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Supporting Information

Table S1 Associations of serum amino acid and acylcarnitine metabolites with leptin among 75 Rhea children[a]