Serum Leptin, Adiponectin and Vaspin Concentrations in Early Infancy: Relation to Feeding Practices and Indices of Growth and Adiposity

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Abstract: Early infant feeding practices can be considered as a target for later obesity prevention. Investigating whether some adipokines are involved is particularly interesting. The aim of this study was to investigate leptin, adiponectin and vaspin concentrations under different feeding practices in early infancy and their relation with growth and adiposity indices. Sixty healthy term infants, 33 males and 27 females, aged 2-5 months were grouped according to feeding practices into 29 exclusively breastfed, 14 exclusively formula-fed and 17 mixed-fed, and their leptin, adiponectin and vaspin were quantified and correlated to infant weight-for-age, length-for-age, weight-for-length, body mass index (BMI)-for-age and head circumference-for-age WHO *Z*-scores. Neither anthropometric Z-scores of males and females nor leptin, adiponectin or vaspin were affected by feeding practice. These adipokines were also not affected by age. In contrast to adiponectin and vaspin, leptin exhibited marked gender differences ($p<0.05$) in breastfed, formula-fed and mixed-fed infants. When all infants were analyzed together, leptin correlated positively ($p<0.0001$) with weight-for-age ($r=0.68$), length-for-age ($r=0.62$), weight-for-length ($r = 0.44$), BMI-for-age ($r=0.53$) and head circumference-for-age ($r=0.25$) Z-scores. With the exception of the latter, concordant correlations for leptin were obtained when study groups were processed separately according to feeding practices. Both adiponectin and vaspin did not correlate with any anthropometric Z-scores**.** In conclusions, the results of our study do not support that variation of infant feeding practice has an important impact on leptin, adiponectin and vaspin concentrations in early infancy. Leptin seems to be gender-dependent and reflects growth and adiposity patterns in early infancy, independent of feeding practice.
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1. Introduction

Obesity, a low inflammatory grade condition, is an epidemic disease and a risk factor for several chronic disorders [1]. Worldwide, at least 2.8 million people die each year as a result of being overweight or obese [2]. Obesity is also a critical health problem in infants, affecting an increasing number of people in later stages of life [3]. Given the several health risks of obesity [1], its prevention is becoming a major challenge, highlighting the need to identify obesity related factors from an early life [4].

Although several studies have reported that breastfed infants exhibit different growth patterns compared to formula-fed infants [5-9], the evidence is not consistent [10-13]. The exact reason for varied growth patterns in breastfed and formula-fed infants is not known, but differences in endocrine responses to feeding practices may be involved [14].

White adipose tissue is a dynamic endocrine organ able to produce and release several bioactive adipokines, such as leptin, adiponectin and vaspin, a visceral adipose tissue- derived serine protease inhibitor [15]. In adult humans, adipokines have a potential clinical relevance as biomarkers for food intake, energy metabolism, fat mass, fat distribution, insulin sensitivity and subclinical inflammation associated with increased risk for obesity, type 2 diabetes mellitus and cardiovascular disease [1, 15]. The role of these adipokines in infants is not clearly understood [16].

Although breastfeeding is shown to protect against later obesity, the mechanism of action underlying this effect is not yet clear. Compared to formula-feeding, breastfeeding is reported to relate with lower obesity in a dose-response manner [7, 17, 18]. This effect is attributed to the quantitative and qualitative compositional differences between human milk and formulas. Human milk contains several bioactive compounds such as leptin, adiponectin and ghrelin which regulate infant metabolism [19]. It is postulated that leptin positively controls satiety in early infancy, a matter which possibly affects metabolic programming of energy balance regulation in later life [20].

The evidence that links serum leptin with early infant feeding practices and growth and adiposity is

inconsistent [11-16, 20-22]. Plasma leptin levels are not shown to be higher in breastfed than in formula-fed infants, but they are shown to be affected by sex and adiposity at the age of 1-4 months [11]. During the first 4 months of life, breastfed infants exhibit higher serum leptin concentrations compared to breast-fed infants with no differences in their anthropometric measures [12, 21]. Similar serum leptin concentrations and growth and adiposity indices in exclusively breastfed, exclusively formula-fed and mixed-fed infants aged 4 months are documented [16]. On the other hand, higher leptin concentrations in formula-fed neonates compared to breastfed neonates are reported [22].

There is scarcity of studies that link serum adiponectin and vaspin with nutrition mode in early infancy, or those relate these adipokines to growth and adiposity of infants. However, breast milk adiponectin is reported to decline throughout lactation and to correlate with serum adiponectin playing a role in the early growth and development of breastfed infants [23, 24]. Vaspin, a serpin produced by visceral adipocytes [1, 15], is demonstrated in the fetus and neonate [25]. Serum vaspin and leptin are recently reported to associate with obesity and obesity related health problems, such as insulin resistance and dyslipidemia in prepubertal children [26].

The objectives of this study were to determine serum concentrations of leptin, adiponectin and vaspin in healthy term exclusively breast-fed, exclusively formula-fed and mixed-fed infants and to examine possible associations between these adipokines and age- and gender- controlled anthropometric Z-scores of growth and adiposity.

2. Material and Methods

Subjects

The study sample consisted of 60 healthy term infants, 33 males and 27 females, who were attending the maternal and child health clinic at Nour Al-Hussein medical center, Amman, Jordan for the routine general checkup monthly visit, between September 2013 and January 2014. Inclusion criteria: age 2-5 months, normal birth weight and growth rate, free of nutritional, congenital and chronic diseases, and apparent disabilities or current illnesses affecting growth. Infants taking any medications or supplements or who being introduced to solid foods were excluded. Infants were exclusively breastfed (n= 29), exclusively formula-fed infants (n= 14) or mixed-fed (n= 17). Exclusively breastfed infants were fed exclusively breast milk. Exclusively formula-fed infants were exclusively fed formula. Mixed-fed infants were fed both breast milk and formula or those who switched between methods of feeding. Infants were also divided into three age groups: 2.0-2.9 months (n=32), 3.0-3.9 months (n=12) and 4.0-4.9 months (n=16). Ethical approval of the study was obtained from The University of Jordan and Nour Al-Hussein medical center. Parents gave written consent for the inclusion of their infants in the study.

Anthropometric measurements

Weight, length and head circumference of infants enrolled in the study were measured by the researcher in duplicates following standard methods of anthropometry [27]. Infants were weighed to the nearest 0.01kg without clothes using a pan-type pediatric scale. Length was measured to the nearest 0.1cm using a recumbent infant board. Head circumference was measured with a flexible, non-stretchable measuring tape to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg)/length (m^2).

Anthropometric Z-scores

Anthropometric measurements were applied to the WHO growth standards using the anthropometric software for personal computers program (version 3.2.2, January 2011). The software is set for the 2006 WHO Growth Standards: Weight-for-age, length-for-age, weight-for-length, BMI-for-age and head circumference-for-age [28]. The Z-scores were then used to assess anthropometric measures for the purpose of evaluating the growth and adiposity of infants [29].

Biochemical assays

Venous blood samples (3ml) were obtained from infants at least 1.5 hours postfeeding. Samples were centrifuged at 4300 rpm for 5 minutes. The obtained serum was stored frozen at -20˚C. Concentrations of serum adipokines were determined in duplicates using enzyme-linked immunosorbent assay (ELISA) technique following the procedures of the standard biochemical kits: Leptin (LDN, GmbH and Co. KG, Germany), adiponectin (E. Bioscience Inc., USA) and vaspin (Cusabio Inc., USA).

Statistical analysis

Statistical analyses were performed using the SPSS version 19 (SPSS Inc., Chicago, USA). Z-scores of weight-for-length, BMI-for-age and head circumference-for-age were assessed by ANOVA and Z-scores of weight-for-age and length-for-age were assessed by Welch ANOVA after applying Lavene's test for homogenization, Shapiro-Wilk test for normality and the outlier labeling rule test for outliers' detection. Serum levels of leptin, adiponectin and vaspin were statistically analyzed using both

parametric, two-way ANOVA and non-parametric, Kruskal-Wallis tests. Correlation analyses between serum adipokines and growth and adiposity indicators were performed using both parametric Pearson's and non-parametric Spearman's tests. Results are expressed as means ± SEM. Statistical significance was set at $p < 0.05$ and a trend was considered at $p < 0.08$.

3. Results

Anthropometric measures

The mean ± SEM of age and anthropometric measures of the study sample (n=60) were: age (3.1±0.11months), weight (6.2±0.12 kg), length (p>0.05) weight, length, BMI, head circumference and birth weight (Table 1). These anthropometric characters were not also influenced by gender and age, with the exception of infants aged 4-4.9 months who weighed more than the other two age groups.

Anthropometric Z-scores

There were no significant differences (p>0.05) in gender-controlled Z-scores of weight-for-age, length-for-age, weight-for-length, BMI-for-age and head circumference-for-age among breastfed, formula-fed and mixed-fed infants (Table 2). The majority of infants enrolled in this study exhibited normal anthropometric gender-controlled Z-scores.

Serum adipokines

Serum concentrations of leptin, adiponectin and vaspin of the entire study sample (n=60) were 4.39±0.41 ng/ml, 22.00±0.36 µg/ml and 55.71±3.33 pg/ml respectively. The concentrations of these adipokines were not statistically (p>0.05) different among the breastfed, formula-fed and mixed-fed infants (Table 3). Serum concentrations of leptin, adiponectin and vaspin were also not significantly different between infants of various age groups. Females had significantly (p<0.05) higher serum leptin concentrations than males (5.79±0.66 vs. 3.24±0.44 ng/ml), whereas both females and males had statistically (p>0.05) similar serum levels of adiponectin (22.45±0.53 vs. 21.58±0.48 µg/ml) and vaspin (59.27±5.16 vs. 52.80±4.35 pg/ml).

Females of breastfed and mixed-fed groups (5.00 and 7.06 ng/ml) had significantly (p<0.05) higher leptin concentrations than their counterpart males (2.88 and 3.62 ng/ml), as processed by using kruskal-Wallis and two-way ANOVA tests (Table 4). Non-parametric analysis indicated that formula-fed females tended (p<0.08) to have higher leptin concentrations than formula-fed males (5.81vs. 3.53 ng/ml). There were no significant differences (p> 0.05) in serum concentrations of adiponectin and vaspin between males and females of the various feeding practice groups, as revealed by parametric and non-parametric analyses.

Table1. Anthropometric characteristics of the study groups according to feeding practice, age and gender

Character	Breastfed (n=29)	Formula-fed (n=14)	Mixed-fed (n=17)	2-2.9 months (n=32)	3-3.9 months (n=12)	4-4.9 months (n=16)	Males (n=33)	Females (n=27)
Weight (kg)	6.2 ±0.17^{a}	6.2 ± 0.34^{b}	6.3 ± 0.21^{c}	5.8 ± 0.15^{a}	6.2 ± 0.23^{b}	7.0 ± 0.23^{ab}	6.2 ± 0.19^{a}	6.2 ± 0.16^{b}
Length (cm)	59.6 ± 0.50^{a}	60.3 ± 1.03^{b}	60.2 ± 0.65^{c}	58.5 ± 0.36^{a}	59.5 ± 0.61^{b}	63.0 ± 0.70^{c}	59.9 ± 0.52^{a}	60.0 ± 0.56^{b}
BMI* (kg/m^{2})	17.2 ± 0.30^{a}	16.8 ± 0.55^{b}	17.4 ± 0.38^{c}	16.9 ± 0.32^{a}	17.3 ± 0.45^{b}	17.5 ± 0.39^{c}	17.2 ± 0.35^{a}	17.1 ± 0.25^{b}
HC$^{\#}$ (cm)	40.0 ± 0.21^{a}	39.6 ± 0.44^{b}	39.9 ± 0.45^{c}	39.2 ± 0.19^{a}	39.8 ± 0.29^{b}	41.4 ± 0.29^{c}	40.0 ± 0.29^{a}	39.8 ± 0.26^{b}
Birth wt (kg)	3.3 ±0.06^{a}	2.9 ± 0.07^{b}	3.2 ± 0.08^{c}	3.3 ± 0.06^{a}	3.0 ± 0.12^{b}	3.2 ± 0.06^{c}	3.2 ± 0.07^{a}	3.2 ± 0.06^{b}

Data are given as; mean ± SEM.; BMI*: body mass index; HC$^{\#}$: head circumference; Values in rows sharing the same superscripts are significantly different ($p < 0.05$) at a given feeding practice, age or gender (Both kruskal-Wallis and two-way ANOVA tests)

Table 2. Z-Scores of the study groups according to feeding practice and gender

Indicator	Breastfed infants		Formula-fed infants		Mixed-fed infants	
	Males (n=16)	Females (n=13)	Males (n=8)	Females (n=6)	Males (n=9)	Females (n=8)
Weight-for-age$^{\#}$	-0.57± 0.32^{a}	0.17 ± 0.20^{b}	-0.83 ± 0.70^{c}	-0.18 ± 0.38^{d}	0.11 ± 0.28^{e}	0.58 ± 0.45^{f}
Length-for- age$^{\#}$	-0.61 ± 0.22^{a}	-0.19 ± 0.23^{b}	-1.14± 0.56^{c}	-0.54 ± 0.39^{d}	-0.19 ± 0.29^{e}	-0.10 ± 0.55^{f}
Weight-for- length*	0.63 ± 0.30^{a}	0.50 ± 0.18^{b}	0.12 ± 0.53^{c}	0.43 ± 0.49^{d}	0.38 ± 0.45^{e}	0.83 ± 0.16^{f}
BMI^{1}-for-age*	0.39 ± 0.34^{a}	0.38± 0.19^{b}	-0.26 ± 0.59^{c}	0.17 ± 0.51^{d}	0.31 ± 0.41^{e}	0.73 ± 0.24^{f}
HC^{2}-for-age*	-0.32 ± 0.21^{a}	0.11 ± 0.15^{b}	-0.93 ± 0.51^{c}	-0.89 ± 0.21^{d}	-0.07 ± 0.38^{e}	0.13 ± 0.32^{f}

Data are given as mean ± SEM; BMI^{1}: Body mass index; HC^{2}: Head circumference; Values in rows sharing the same superscripts are significantly different ($p < 0.05$); * ANOVA test; $^{\#}$ Welch ANOVA test.

Table 3. Serum concentrations of adipokines of infants according to feeding practice, age and gender

Serum adipokine	Breastfed (n=29)	Formula-fed (n=14)	Mixed-fed (n=17)	2-2.9 months (n=32)	3-3.9 months (n=12)	4-4.9 months (n=16)	Males (n=33)	Females (n=27)
Leptin (ng/ml)	3.83 ± 0.55^{a}	4.51 ± 0.89^{b}	5.24 ± 0.86^{c}	4.51 ± 0.56^{a}	4.25 ± 1.05^{b}	4.24 ± 0.79^{c}	3.24 ±0.44^{a}	5.79 ± 0.66^{a}
Adiponectin (µg/ml)	21.78 ± 0.61^{a}	21.77 ± 0.63^{b}	22.47 ± 0.50^{c}	22.61 ± 0.46^{a}	22.40 ± 0.64^{b}	20.38 ± 0.73^{c}	21.58 ± 0.48^{a}	22.45 ± 0.53^{b}
Vaspin (pg/ml)	56.18 ± 5.02^{a}	51.34 ± 6.13^{b}	58.51 ± 6.50^{c}	57.43 ± 4.56^{a}	58.80 ± 8.39^{b}	49.96 ± 6.01^{c}	52.80 ± 4.35^{a}	59.27 ± 5.16^{b}

Data are given as; mean ± SEM; Values in rows sharing the same superscripts are significantly different ($p < 0.05$) at a given feeding type, age or gender (Both kruskal-Wallis and two-way ANOVA tests).

Table 4. Serum concentrations of adipokines of the study groups according to feeding practice and gender

Serum adipokine	Breastfed infants		Formula-fed infants		Mixed-fed infants	
	Males (n=16)	Females (n=13)	Males (n=8)	Females (n=6)	Males (n=9)	Females (n=8)
Leptin (ng/ml)	2.88 ± 0.51$^{ab}{}_{\alpha\beta}$	5.00 ± 0.98$^{c}{}_{\alpha}{}^{*}$	3.53 ± 1.39$^{d}{}_{\gamma}$	5.81 ± 0.79$^{ae}{}_{\varepsilon}{}^{*}$	3.62 ± 0.66$^{f}{}_{\zeta}$	7.06 ± 1.45$^{bf}{}_{\beta\zeta}$
Adiponectin (µg/ml)	21.13 ± 0.81$^{a}{}_{\alpha}$	22.57 ± 0.93$^{b}{}_{\beta}$	21.42 ± 1.04$^{c}{}_{\gamma}$	22.25 ± 0.61$^{d}{}_{\delta}$	22.53 ± 0.49$^{e}{}_{\varepsilon}$	22.39 ± 0.96$^{f}{}_{\zeta}$
Vaspin (pg/ml)	52.73 ± 6.68$^{a}{}_{\alpha}$	60.44 ± 7.75$^{b}{}_{\beta}$	46.69 ± 7.03$^{c}{}_{\gamma}$	57.53 ± 11.07$^{d}{}_{\delta}$	58.35 ± 9.05$^{e}{}_{\varepsilon}$	58.69 ± 10.01$^{f}{}_{\zeta}$

Data are given as; mean ± SEM; Values in rows sharing the same English superscripts are significantly different ($p < 0.05$) (kruskal-Wallis test); Values in rows sharing the same Greek subscripts are significantly different ($p < 0.05$) (Two-way ANOVA test); * $p<0.08$ (trend): breastfed males vs. breastfed females and formula-fed males vs. formula-fed females (Kruskal-Wallis test).

Correlation analysis

Considering the whole study sample (n=60), significant correlations (p<0.0001) were obtained between serum leptin and weight-for-age (r=0.68), length-for-age (r=0.62), weight-for-length (r=0.44), BMI-for-age (r=0.53) and head circumference-for-age (r=0.25) Z-scores (Table 5). With the exception of head circumference-for-age, almost similar pattern of correlations between leptin and the afore-mentioned anthropometric Z-scores were obtained when study infants were processed separately according to feeding practices. No correlations were found between leptin and the other studied adipokines. There were no significant correlations ($p>0.05$) between both adiponectin and vaspin, and any of the anthropometric Z-scores (Tables 6 and 7).

Table 5. Associations of leptin with growth and adiposity indices and other adipokines

Indicator	Breastfed infants (n=29)		Formula-fed infants (n=14)		Mixed-fed infants (n=17)		Overall infants (n=60)	
	r^{α}	r^{β}	r^{α}	r^{β}	r^{α}	r^{β}	r^{α}	r^{β}
Weight-for-age	0.59^{a}	0.63^{a}	0.80^{a}	0.76^{a}	0.81^{a}	0.71^{a}	0.68^{a}	0.68^{a}
Length-for-age	0.59^{a}	0.65^{a}	0.78^{a}	0.69^{b}	0.56^{c}	0.39	0.62^{a}	0.56^{a}
Weight-for-length	0.33^{d}	0.34^{d}	0.54^{c}	0.59^{c}	0.53^{c}	0.59^{c}	0.44^{a}	0.44^{a}
BMI*-for- age	0.43^{c}	0.41^{c}	0.63	0.67^{b}	0.68^{b}	0.66^{b}	0.53^{a}	0.54^{a}
HC$^{\#}$-for-age	0.08	0.12	0.49^{d}	0.40	0.30	0.28	0.25^{c}	0.20
Serum adiponectin	0.18	0.03	-0.09	-0.10	0.09	0.30	0.09	0.09
Serum vaspin	0.29	0.22	0.07	0.02	-0.23	-0.22	0.08	0.12

$p <0.0001^{a}$; $p <0.01^{b}$; $p <0.05^{c}$; $p<0.08^{d}$ (trend); r^{α} refers to Pearson and r^{β} refers to Spearman correlation coefficients; BMI*: body mass index; HC$^{\#}$: head circumference.

Table 6. Associations[1] of adiponectin with growth and adiposity indices and other adipokines

Indicator	Breastfed infants (n=29)		Formula-fed infants (n=14)		Mixed-fed infants (n=17)		Overall infants (n=60)	
	r^{α}	r^{β}	r^{α}	r^{β}	r^{α}	r^{β}	r^{α}	r^{β}
Weight-for-age	0.22	0.19	-0.30	-0.44	0.08	0.31	-0.03	0.06
Length-for-age	0.07	0.05	-0.19	-0.41	0.16	0.19	0.03	-0.32
Weight-for-length	0.26	0.36	-0.23	-0.16	-0.11	0.40	0.06	0.22
BMI*-for-age	0.26	0.30	-0.31	-0.20	-0.04	0.36	-0.06	0.18
HC$^{\#}$-for-age	0.10	0.12	-0.23	-0.48	-0.21	0.01	-0.13	-0.05
Serum leptin	0.18	0.03	-0.09	-0.10	0.09	0.30	0.09	0.09
Serum vaspin	0.09	0.13	0.04	0.04	0.15	-0.10	0.09	0.09

[1]All associations are at $p>0.05$; r^{α} refers to Pearson and r^{β} refers to Spearman correlation coefficients. BMI*: body mass index; HC$^{\#}$: head circumference.

Table 7. Associations[1] of vaspin with growth and adiposity indices and other adipokines

Indicator	Breastfed infants (n=29)		Formula-fed infants (n=14)		Mixed-fed infants (n=17)		Overall infants (n=60)	
	r^{α}	r^{β}	r^{α}	r^{β}	r^{α}	r^{β}	r^{α}	r^{β}
Weight-for-age	0.26	0.28	-0.11	-0.00	-0.29	-0.28	0.04	0.09
Length-for-age	0.32	0.33	-0.13	-0.04	-0.10	-0.12	0.09	0.15
Weight-for-length	0.09	0.18	-0.11	-0.01	-0.32	-0.25	-0.06	0.02
BMI*-for-age	0.15	0.24	-0.07	0.01	-0.33	-0.23	-0.00	0.07
HC#-for-age	0.21	0.15	-0.14	-0.11	-0.40	-0.39	-0.02	0.01
Serum leptin	0.29	0.22	0.07	0.02	-0.23	-0.22	0.08	0.12
Serum vaspin	0.09	0.13	0.04	0.04	0.15	-0.10	0.09	0.09

[1]All associations are at $p>0.05$; r^{α} refers to Pearson and r^{β} refers to Spearman correlation coefficients; BMI*: body mass index; HC#: head circumference.

4. Discussion:

We used WHO gender-controlled Z-scores of weight-for-age, length-for-age, weight-for-length, BMI-for -age and head circumference-for-age to assess growth and adiposity in infants. These scores are based on the 2006 WHO growth standards for infants that are relevant to developed and developing countries. They reflect the reference distribution of the study sample adding a profound value for detecting under and over nutrition due to their standardized measures [29].

There were no significant differences in the anthropometric Z-scores between breastfed, formula-fed and mixed-fed infants, or between males and females, or between infants with different ages. These results may indicate that infants under examination follow close growth patterns regardless of feeding practice and gender. Similar to our findings, no differences among breastfed, formula-fed and mixed-fed infants aged 4 months according to gender and anthropometric parameters have been found [16]. Insignificant differences in these variables between breastfed and formula-fed infants at birth and at 3 months of age have been also found [13]. Further, similar anthropometric patterns between breastfed and formula-fed infants, and between males and females <12 months old have been reported [21]. On other hand, it has been documented that breastfed infants exhibit different growth trends from that of formula-fed infants [7, 11]. Weight gain of breastfed infants has been shown to be higher [6, 11] or lower [7, 30] than that of formula-fed infants at ages <12 months. Previous studies have used anthropometric measures but not their standardized Z-scores, a matter which may partly explain the apparent differences in these parameters between the results of our study and those of others [6, 7, 11, 30].

The presently recorded serum leptin levels were 4.39, 3.24 and 5.79 ng/ml for the whole study infants, males and females respectively. These concentrations were close [11, 12], lower [21] or higher [13, 16, 31] than the reported values for infants at 1-8 months of age. Infants' age and body composition, as well as methodological, population or sampling differences most probably explain these variations.

No significant differences were found in serum leptin concentrations between breastfed, formula-fed and mixed-fed infants aged 2-5 months (3.83, 4.51 and 5.24 ng/ml respectively). The effect of feeding practice in early infancy on serum leptin levels has been a controversial subject for years. Letpin levels of 92 breastfed infants have not been found to be different from those of 101 formula-fed infants at 1and 4 months of age, though the latter exhibited slightly yet higher levels than the former at 6 months of age [11]. No differences in letpin concentrations between 28 breastfed, 15 formula-fed and 21 mixed-fed 4 months old infants have been also found [16]. However, higher leptin concentrations have been shown in 13 breastfed than in 22 formula-fed infants aged <12 months [21], and in 25 breastfed than in 26 formula-fed infants aged 1-3 months [12]. Results from a longitudinal study involving 237 infants revealed that breastfed infants had higher leptin concentrations than formula-fed infants ([14]. Contrary to these findings, higher leptin levels in formula-fed neonates compared to breastfed neonates have been reported [22].

To explain the reported higher serum leptin concentrations in breastfed than formula-fed infants, many investigators have proposed that breast milk leptin contributes significantly to breastfed infants' serum leptin concentrations [12, 14, 21]. It is known that leptin is present in human milk, and infant formula contains no leptin [9]. However, the possibility of the infants to absorb the ingested breast milk leptin in physiological amounts has been questioned [32]. Thus, it is doubtful that breast milk leptin is absorbed in a quantity that would influence leptin concentrations in breastfed infants. This provides a reasonable explanation for the results of the present study.

Age of the infants has been considered a confounding factor affecting the results of leptin concentrations between infants of different feeding practices, though clear analysis of its possible effect has not been given [12, 14, 21]. There were no differences in this variable among the study infants at 2-2.9, 3-3.9 and 4-4.9 months of age (4.51, 4.25 and 4.24 ng/ml respectively). Some studies have not found differences in leptin concentrations between infants at 1 and 4 months [11], or at 1 and 6 months [31]. These results are in close agreement with the findings of the present study.

There is a general agreement regarding the influence of gender on serum leptin concentrations, a matter that is consistent with the results of the current study. It has been repeatedly documented that breastfed or formula-fed female infants, whether at birth or at various age groups of 1-12 months, have significantly higher leptin concentrations than male infants [11-13], although not consistently so [21]. Gender differences in leptin concentrations are apparent during infancy, yet they are not as great as what have been seen in adults [33]. These differences have been shown in human fetuses at 37 weeks of gestation and in newborns [34], as well as in umbilical cord plasma at 15 days of age [35]. Thus, gender differences in leptin concentrations start very early in life. In males, leptin concentrations have been reported to rise between the ages of 5-10 years, drop from 10-15 years and then rise again modestly in adult men [36]. The rise in leptin concentrations during prepubertal and pubertal stages in females have been found to associate with rises in serum follicle-stimulating hormone, luteinizing hormone, and estradiol [36]. These findings highlight the role of male steroids which are known to decrease leptin concentrations and female steroids that increase them; especially since male and female infants do not differ with respect to body fat mass and fat distribution [33].

The marked positive correlations shown in this study between serum leptin concentrations and standardized anthropometric gender-controlled Z-scores suggest that leptin is highly related to adiposity and growth measures at 2-5 months of age, independently of infants' feeding practices. These correlations were obtained in the whole study sample and in the separate groups of breastfed, formula-fed and mixed-fed infants, such relationships have not been previously reported. However, few studies, with considerable controversy, have utilized non-standardized anthropometric measures and related them to leptin concentrations in infants. Positive correlations between leptin concentrations and BMI have been documented in <12 months old infants irrespective to feeding practice [11, 31]. The former have been also shown to correlate with infants' head circumference [31]. No correlations between leptin concentrations and any anthropometric parameters have been documented in infants aged <12 months [12, 21] and 4 months [16].

Serum adiponectin concentrations were not affected by age, gender and feeding practice in the study infants; they were also not correlated with any of the growth or adiposity Z-scores in breastfed, formula-fed and mixed-fed infants or in the entire sample. These results do not provide support for the effect of feeding practices on adiponectin levels in early life. Studies that link serum adiponectin with feeding practice in early infancy are scarce, and those available have mostly dealt with infants according to their gestational age [37, 38]. This limits the comparison of the current results with those of the other studies. Breastfed small-for-gestational age infants have been shown to have lower adiponectin levels than breastfed appropriate-for-gestational age infants, but higher than those of formula-fed small-for-gestational age infants at 4 months of age [38]. Infants exposed to high breast milk adiponectin have been shown to experience increased Z-scores of weight-for-age and weight-for-length in contrast to those exposed to low breast milk adiponectin [37]. Breast milk adiponectin has been also shown to correlate with infants' and maternal serum adiponectin concentrations. The authors have claimed that breast milk adiponectin may be physiologically active during the time of breastfeeding, a matter which has been disputed elsewhere [32]. Although adiponectin is present in breast milk, it is unlikely to affect serum adiponectin concentrations in breastfed infants and, in turn, cause the claimed effects. This reasoning well justifies the findings of the present study.

Furthermore, no differences in adiponectin concentrations have been documented between appropriate-for-gestational age and small- for-gestational age infants at birth, though the latter weighed less than the former [31, 39]. This indicates that adiponectin does not correlate with adiposity in infancy, and goes in line with the results of the present study. But the progressive decrease in this variable that has been reported during the first year of life supports the relation between growth-adiposity and adiponectin concentrations with increasing age [31].

To the best of our knowledge, this study is perhaps the first to link serum vaspin with measures of anthropometric growth and adiposity and feeding practice in early infancy. Serum vaspin levels were not affected by age, gender and feeding practices, and were not correlated with any of the growth and adiposity Z-scores in breastfed, formula-fed and

mixed-fed infants or in the entire study sample. These findings may suggest that serum vaspin is not altered by growth and adiposity similar to what have been seen in older persons [40, 41]. They also allow questioning the role of vaspin in predicting later obesity development. However, overweight 9 years old children have recently been shown to have higher vaspin concentrations than children of normal weight [26]. This may indicate that the possible relation between serum vaspin and later obesity is still not determined. Feeding practices did not affect serum vaspin levels in the present study, a matter which has not been investigated by others. Lack of gender differences in vaspin levels have been documented in small-, appropriate- and large-for-gestational age infants at birth [42]. Recently, gender differences in vaspin concentrations have been shown to develop during pubertal progression in females [43]. Higher serum vaspin concentrations in women than men have been reported [44]. Consistent with the present study, the available evidence indicates that vaspin levels during early infancy are gender-independent.

In conclusion, our results provide evidence that unlike adiponectin and vaspin, leptin is gender-dependent and strongly correlates with the anthropometric Z-scores of growth and adiposity in early infancy independently of whether infants are breastfed, formula-fed or both. However, the significance of the results of the present study demands further investigations.

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