Leptin and Tumor necrosis factor -alpha in breast –fed and formula –fed infants

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Abstract

Leptin is an adipocyte –secreted hormone that regulates weight centrally and regulates food intake and energy metabolism which is present in breast milk and thus may be involved in body composition and differences between breast feeding (BF) and formula-fed (FF) infant. Also there's independent associated between activation of the tumor necrosis factor – alpha (TNF-α) and leptin levels that suggest that TNF- α can directly induce leptin gene expression in humans.

Subjects and Methods: this study included 47 infants aged (2-24 months) they were 28 male and 19 females infant, exclusive BF was present in 27 of them and the rest were FF, beside full clinical examination and anthropometric measurements (weight (wt), length, head circumference (HC), body mass index (BMI), surface area, skin fold thickness) and full blood count, cholesterol, triglyceride, High density lipoprotein (HDL-C), and low density lipoprotein (LDL-c) , .also serum leptin and TNF- α are measured.

Results: BF infant showed significantly higher length /age ratio than FF. Also BF infants showed significantly higher level of serum leptin than FF (2.96ng/ml ±2.8, 1. versus 45ng/ml±1.29), in the other hand there's significant decrease in the mean level of TNF- α in the BF than FF (15.8pg/ml±1.8 versus 17.18pg/ml ±1.75). From these results, it could be concluded that the presence of cytokines and leptin in human milk suggest that the proinflammatory cytokines (TNF- α)and leptin may mediate putative differential anorectic responses of BF and FF infants.

Introduction

Considering that overweight and obesity derive from a condition of altered energy balance, one of the major interests of nutritional researches is to provide breakthroughs in the understanding of hormonal patterns involved in energy balance regulation. Epidemiological surveys indicate that breast feeding is protective against obesity in later life, even if the precise magnitude of this association remains not well defined. A lot of studies have shown the detection of hormones in breast milk, which have a role in energy balance regulation **(Savino et al 2008).**

The growth pattern of formula-fed infants has been shown to be different from that of breast-fed infants **(Dewey et al; 1992 ,and Dewey et al 1995)** . Most studies showed that weight gain of the former group is lower than that of the latter, although not consistently so weight-for-length scores are significantly higher in formula-fed than in breast-fed infants from 7 to 18 month of age and the sum of skinfold thicknesses was significantly higher in formula-fed infants from 5 month of age, suggesting that breast-fed infants are leaner **(Dewey ;et al 1993).** The reasons for the observed differences in body composition are not known, although it has been speculated that differences in the volume of milk or formula ingested the amount of

complementary foods eaten, or the hormonal responses to diet may be involved **(Dewey et al; 1995).** Differences in the endocrine response to feeding may contribute to the differences in body composition between formula fed and breast-fed infant **(Lucas et al; 1980)**.

Leptin , the 16 KD a non – glycosylated protein product of the ob gene , and it is a hormone synthesized mainly in adipose cell to regulate weight control in a central manner , via its cognate receptor in the hypothalamus **(Margalet et al ; 2003).** Plasma concentration of leptin reflect body fat mass in adults and children and are markedly elevated in obese individuals suggest that most obese persons are insensitive to endogenous leptin production. **Smith – Kirwis et al;1998** reported that leptin in human milk appears to be associated with milk fat globules, Leptin has been demonstrated to modulate monocyte - machrophage function and to regulate Proinflammatory response, However the proinflammatory cytokines (tumour necrosis factor-alpha (TNF-α)and interleukin –1 (IL-1) can raise mouse leptin levels in vitro resulting in anorexia and weight loss .That suggest that TNF- α can directly induce leptin gene expression in humans in vitro resulting in anorexia and weight loss .that suggest that TNF-α can directly induce leptin gene expression in humans**(Margalet et al ; 2003).**

Breast fed (BF) and (FF) infants differ in the amount and type of polyunsaturated fatty acids consumed, and are accompanied by changes in monocytes , cytokines production and hence a modification of immunological response . Therefore the presence of cytokines and leptin in human milk suggest that the pro-inflammatory cytokines IL-1 ,TNF- α and leptin may mediate putative anorectic responses of BF and FF infants **(Granto et al ;2000).**

The aims of the work: is to evaluate predictors of circulating leptin and TNF- α in health BF and FF and to explore their relationship with anthroprometeric measurements and to evaluate whether leptin or TNF- α level are regulated by BF or FF.

Subjects and Methods

Forty seven healthy infants who admitted to pediatric out patients Clinic for their routine health control at the pediatric Department of Assuit University Hospital.

Inclusion criteria were: age ranging from birth to 12 month birth weight ranging from (2-24 month) and absence of neonatal disease, chronic illness or current pathology compromising growth .Infants was exclusively breastfed (BF) or formula-fed (FF).All infants are subjected to the following:

1-Anthroprometric measurements include:

1-Weight (Kg), length in centimeter (cm) Head Circumference in centimeter (cm),

2-Body mass index (BMI) calculated by the equation

3- Body surface area.

4- Skin fold thickness (Tricipital and subscapular skin fold thickness) were assessed with skin fold caliper.

2- Routine investigations include: complete blood picture, lipid profile (Triglycerides, high density lipoproteins cholesterol (HDL-C), and low density lipoproteins cholesterol (LDL-C).

3-BLood samples were collected from all subjects and then centrifuged, and then the resulting serum was stored at -70C until analyzed for **estimation of:**

a- **Leptin** levels were measured in duplicate using a sandwich enzyme immunoassay for the quantitive measurement supplied by BioVendor Laboratory Medicine Inc.

b- Tumour necrosis factor-alpha was measured with quantitative sandwich enzyme-linked immunosorbent assay (ELISA, BioSource International, Inc, Camarillo, California, USA).

Statistical analysis:

Data were analyzed using Prism Software program, graph-Pad Version 3.0. Values were expressed as mean ± SD. Statistical differences between different groups of subjects were calculated using unpaired student t-test and one way analysis of variance. Pearson's correlation coefficient was performed for evaluating the correlation, values. $P<0.05$ was considered a significant difference.

Results

Table (1) shows that there significance difference between BF and FF in (hemoglobin Hb , white blood cell (WBC) ,red blood cell(RBC), mean corpuscular volume (MCV) , serum triglycerides) **.**

Anthroprometeric measurements :

There were no significant differences in anthroprometeric measurements including (weight / age , body surface area /age ,BMI /age , HC/age) but there significane difference in length/age (P=0. 003) (table 2).

Leptin and TNF - α:

Considering the whole samples, serum leptin values were statistically significantly higher in BF than FF (P=0.030)(table 1) .The ratios between (leptin /
Weight and leptin/ BMI were significantly higher in BF than FF infants.

Parameters	BF (n=27)	FF (n=20)	P. Value
Age(Month)	9.62 ± 5.86	9.85 ± 5.14	N.S
Sex **Male** **Female**	 18(66.7%) 9(33.3%)	 10(50%) 10(50%)	N.S
HB (g/dl)	9.003 ± 1.07	7.63 ± 1.10	0.000*
WBC ($\times10^9$/L)	11.70 ± 3.07	9.56 ± 1.54	0.000*
RBC ($\times10^{12}$/L)	4.07 ± 0.69	3.28 ± 0.79	0.001*
MCV (fL)	54.71 ± 24.51	75.61 ± 9.55	0.001*
MCH (Pg)	22.90 ± 4.20	23.39 ± 3.02	N.S
MCHC (g/dl)	31.72 ± 3.89	31.14 ± 3.89	N.S
Platelet count (cell/mm^3)	350.72 ± 126.64	400.70 ±168.71	N.S
Total cholesterol (mg/dl)	119.70 ± 26.13	107.25 ± 32.58	N.S
S. triglycerides (mg/dl)	131 ± 11 ± 52.15	238.15 ± 112.15	0.000*
HDL C (mg/dl)	23.44 ± 8.67	26.70 ±1.78	N.S
LDL C (mg/dl)	70.29 ± 8.67	26.70 ± 1.78	N.S
Leptin (ng/ml)	2.96 ± 2.80	1.45 ± 1.29	0.030*
TNF-α (pg/ml)	15.80 ± 1.80	17.18 ± 1.75	0.012*

Table (1): Shows the clinical and labratory studied parameters between Breast Fed (BF) and Formula-Fed (FF) (mean ±SD).

*Significant (P<0.05) NS: Non Significant

Parameters	BF (n=27)	FF (n=20)	P. Value
Weight/ Age (kg/M)	1154.18 ± 1071.26	1596.63 ± 2330.9	N.S
Length/ Age (cm/M)	8.41 ± 4.24	5.38 ± 1.25	0.003*
Skin fold/ Age (mm/M)	0.811 ± 0.68	1.13 ± 1.25	N.S
Body surface area /Age (m^2/M)	0.059 ± 0.042	0.05 ± 0.05	N.S
Body mass index/Age (Kg/ m^2/M)	3.019 ± 2.76	3.95 ± 4.64	N.S
Head circumference/ Age (cm/M)	6.51 ± 4.58	7.57 ± 7.45	N.S
Leptin /wt (kg/M)	0.358 ± 0.05	0.118 ± 0.05	0.003
Leptin/BMI	0.156 ± 0.025	0.071 ± 0.029	0.003

Table(2): Shows the anthropometrics measurement between Breast Fed (BF) and Formula-Fed (FF) (mean ±SD).

	BF		FF	
	r-Coefficient	P-Value	r-coefficient	P-Value
HB (g/dl)	-0.238	0.23	-0.042	N.S
Wt (kg)	0.154	0.44	0.070	N.S
BMI (Kg/ m^2)	-0.059	0.76	-0.279	N.S
B.S.A ($m\ m^2$)	0.16	0.400	0.137	N.S
Skin fold thickness (mm)	-0.398	0.04	-0.491	0.028*
Leptin (ng/ml)	0.023	0.91	-0.437	N.S
Length (cm)	-0.103	0.61	0.248	N.S
HC (cm)	-0.37	0.05	0.202	N.S
Age (Month)	0.299	0.130	-0.002	N.S
TC (mg/dl)	0.20	0.31	0.223	N.S
Triglycerides (mg/dl)	0.25	0.19	0.132	N.S
HDL-C (mg/dl)	-0.10	0.60	-0.295	N.S
LDL-C (mg/dl)	0.176	0.38	-0.059	N.S

Table(3): Correlation coefficients between selected variables and serum TNF- α concentration in (BF) and (FF) infants

	BF		FF	
	r-Coefficient	P-Value	r-coefficient	P-Value
HB (g/dl)	-0.19	0.32	0.45	0.046*
Wt (kg)	-0.10	0.060	-0.14	N.S
BMI (Kg/m^2)	-0.12	0.52	0.88	0.000*
B.S.A ($m\ m^2$)	0.257	0.19	-0.322	N.S
Skin fold thickness (mm)	0.17	0.38	0.39	N.S
TNF-α (pg/ml)	0.023	0.91	-0.437	0.05
Length (cm)	-0.14	0.47	-0.62	0.003*
HC (cm)	-0.297	0.13	-0.43	0.05
Age (Month)	-0.13	0.94	-0.482	0.032*
TC (mg/dl)	0.09	0.637	-0.18	N.S
Triglycerides (mg/dl)	0.299	0.13	-0.22	N.S
HDL-C (mg/dl)	-0.33	0.08	-0.144	N.S
LDL-C (mg/dl)	0.119	0.55	-0.02	N.S

Table(4): Correlation coefficients between selected variables and serum leptin concentration in (BF) and (FF) infants

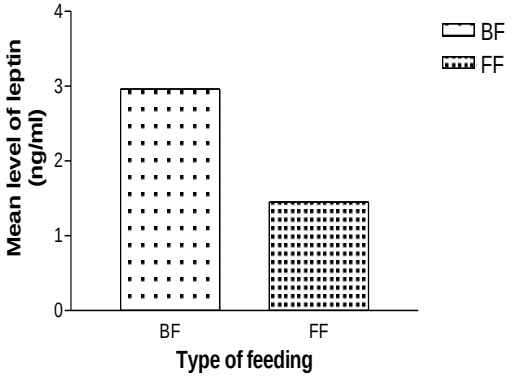

Fig.(1): The mean level of serum leptin between Bf and FF

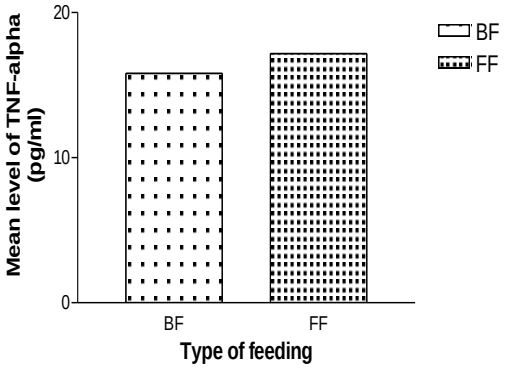

Fig.(2): The mean level of serum TNF-α between Bf and FF

Discussions

The discovery of leptin (from the Greek work leptos: i.e. thin) the product of ob gene has greatly advanced our understanding of energy balance regulation. This hormone produced mainly by adipose tissues carries to the brain information about the size of energy stores and activates hypothalamic centers that regulate energy intake and expenditure the realization that adipose tissues is not simply a storage depot but it is an important endocrine gland has created opportunities for the investigations of body weight, length and probably fold thickness parameters in young infants and children **(Christos et al; et al 1998**).

In humans a fat mass increment causes increased levels of leptin accession leading to an increased metabolic rate and a reduction in food intake. Leptin seems to be involved in many processes. It potentially plays a role in infant growth. The leptin receptor is expressed in a variety of routines, not only in the central nervous system (The main target of its action), but also in hematopoietic tissues **(Auwerx and Staels 1998** ,and **Materese 2000).** In the present study a significant positive correlation was encountered between hemoglobin level and serum leptin level (p=0.046, table 4).

Savino et al., (2002) reported that leptin is present in breast milk and may be involved in some of the differences between breast fed and artificially fed infants. **Smith –Kirwis et al;1998** reported that Leptin in human milk appears to be associated with milk fat globules therefore, the hormone concentration is higher in whole than in skimmed milk because whey proteins added to formula are isolated from skimmed **(Savino et al ; 2008).**It is hypothesized that artificial formulae do not contain leptin because whey protein added and bovine milk , and leptin associated with milk fat globules would be removed during the skimming process **(Resto et al ; 2001).** In the present study significantly increase in the levels of serum leptin was encountered in BF than FF infants (table1, figure1). Also significant reduction of energy intake suppresses leptin levels too much greater extent then would be expected based on the changes in adipose tissue mass. In the present study no correlation however was encountered between leptin levels and skin fold thickness whether breast fed or formula fed. On the other hand significant negative correlation was found of leptin level with length, significant negative correlation was found in FF infant ($r= -0.621$ $P < 0.05$). Furthermore positive significant correlation between length /age and

leptin level (r = 0.292, P =< 0.046). A similar results was reported by **Savino et el., (2002)** that reduction al energy intake suppresses leptin level to a much greater extent than would be expected based on the changes in adipose tissue mass reflecting a relative leptin deficiency for a given fat mass. They added that leptin levels were associated with body mass index. In the present study there was no correlation encountered between wt/ age ratio and leptin level. In fact wt/age ratio did not differ significantly between breast fed and artificially fed infants, neither did BMI/ age ratio. in the present study leptin /wt ratio was significantly higher in breast fed than artificially fed infants, (P =< 0.003) similarly leptin / BMI was highly significantly higher in breast fed then artificially fed infants (P< 0.003, table 2).

Montoroz and Moschos ., (1998) concluded that leptin levels were consistently associated with body adiposity and BMI since body weight regulated by complex mechanisms including numerous metabolic and hormonal signals, elucidation of leptin mode of action as well as its interactions with other molecular regulating energy homeostasis is badly needed.

Bennett et al; 1996 stated that although leptin appears to stimulate erythropiosis, it is not currently known whether its effect are mainly endocrine or principally paracrine. The exact mechanism of leptin role in hematopoiesis as well as in the immunological responses is not fully understood. In the present study both hemoglobin level and white cell count were significantly higher in BF than FF infants (p=0.000 table1), suggesting that leptin is secreted in human milk and can pass the infant gastrointestinal tract to the blood thus in addition to neonatal leptin, maternal leptin milk may play a role in regulating food intake and /or growth. It remains controversial whether leptin regulates energy balance by controlling food intake and /or by increasing total energy expenditure in children.

Leptin is present in breast milk, although cows milk does not contain leptin it is possible that the specific nutrient content differs and antigenicity of its constituents may alter leptin secretion and circulating levels. This could explain the observed difference between the present study and previous studies.

In the present study, breast fed infants showed significantly higher leptin level than formula fed ones. In previous studies leptin level have been reported to be positively associated with adiposity and with infant length in formula fed infants in the neonatal period. In the present study of postneonatal artificially fed infants, leptin showed a significantly negative correlation with infants length (r = -0.625 and P =< 0.05).

Montozoros and Moschos (1998) stated that cytokines regulate leptin expression and circulating levels in humans, they reported an independent association between activation of the TNF alpha system and leptin levels, suggesting that TNF can directly induce leptin gene expression in humans. In the present study TNF alpha levels, showed significant negative correlation with leptin levelsin FF (r=0.43 , p= 0.07). Furthermore skin fold thickness showed significant negative correlation with TNF alpha levels in FF(r=0.49, P =0.028 table 3). In this respect our results are in keeping with those **Montozoras and Mocchos (1998).**

In the present study breast fed infants showed significantly higher length age ratio then artificially fed ones. The reasons for growth and body composition differences between

breasts fed and artificially fed infant are not completely known. Differences in body composition between breast bed infants and formula fed infants may be due to a different endocrine response to feeding. This different response may be influenced by leptin. The present finding of significant difference between breast fed and artificial fed infants in length /age ratio may suggests that the significantly higher leptin concentration observed in breast fed infants in the present study may be contribute to the differences between the two groups not only in adipose tissue production but also to the action of leptin.

The functional significance of cytokines in breast milk in vivo is under investigation. Milk TNF- is secreted by milk macrophages and by mammary epithelium **(Buescher and Malinowska 1996).** Besides mediating proinflammatory events, TNF- is a physiologically significant regulator of mammary gland development, stimulating growth and branching morphogenesis of mammary epithelial cells and modulating functional differentiation **(Shea-Eaton et al 2001)**. TNF- in colostrums and milk bridges the defective TNF- production by the neonate. Presence of its soluble receptors in human milk may suppress local inflammatory effects **(Meki et al ;2003).**

In conclusion the discovery of leptin is present in human milk, but not in formula, because the hormone is destroyed by pasteurization. Further more, the leptin concentration in milk correlates with serum leptin concentrations in both infant and mother. In the other hand pro inflammatory cytokines in breast milk exhibit biological variability at difference periods of human lactation.

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