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HORMONES REGULATING ENERGY HOMEOSTASIS IN BREASTFEEDING VERSUS FORMULA FEEDING MOTHERS

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To meet energy demands for lactogenesis and to sustain homeostatic conditions post-partum, the organism of breastfeeding mother undergoes combined endocrine and metabolic regulation. The main objective of this study was to determine basal serum concentrations of hormones involved in the maintenance and defense of energy balance in breastfeeding (BF) and formula feeding (FF) mothers. Twenty healthy exclusively breastfeeding mothers at 3rd month of lactation (EBF3), 17 healthy partially breastfeeding at 6th month of lactation (PB6) and 17 healthy FF mothers participated in this study. Fasting serum prolactin (PRL), acylated ghrelin (aGhr), total ghrelin (tGhr), leptin, adiponectin, insulin, and cortisol were determined for all study participants and correlations between studied parameters were calculated for BF women. We found significantly lower basal insulin ($p = 0.0048$) and cortisol ($p = 0.0002$) and significantly elevated basal prolactin ($p = 0.0020$) and leptin ($p = 0.0416$) in BF when compared with FF women. The differences were not associated with the duration of lactation (3 vs. 6 months), except for PRL, which was highest in EBF3. Levels of Ghr and adiponectin did not differ between study groups. In the BF group, the negative correlations were found between: aGhr and insulin, aGhr and adiponectin, leptin and cortisol, leptin and adiponectin, insulin and adiponectin, cortisol and adiponectin. Positive associations were noted between: insulin and leptin, leptin and aGhr, PRL and leptin, PRL and aGhr. Leptin and insulin correlated positively, whereas adiponectin negatively with BMI. These data may suggest that EBF3 and PB6 as compared with FF mothers, exhibit hormonal regulation which tends to be more advantageous for their metabolic profile and is not related to the duration of breastfeeding within the first 6 months of lactation.

Key words: *lactation ,breastfeeding, formula feeding mothers, maternal hypothalamic-pituitary-adrenal axis, prolactin, insulin, leptin, adiponectin, ghrelin, cortisol*

INTRODUCTION

Lactation is the time of considerable changes, during which the nature prepares a woman for extra energy expenditure and exposure to postpartum stressors. In exclusively breastfeeding mothers energy output for milk production accounts for about 20 – 30% of the resting energy requirement (1). Therefore, mothers' physiological metabolic shift is crucial for ensuring the optimal conditions for offspring and a lactating woman. Prolactin, leptin, adiponectin, ghrelin, insulin and cortisol are the hormones involved in energy homeostasis and potentially may play role in this process.

Prolactin secretion is regulated *via* an inhibitory activity of dopamine-releasing neurons, suckling stimulus and variety of species specific modulatory factors, including those involved in a reproductive function, i.e. GnRH, estrogens, or neurokinin B (2, 3). This pivotal lactogenic hormone exhibits pleiotropic activity in the body and affects maternal metabolism through mobilization of energy from fat and regulation of food intake (4, 5). It may possibly affect hypothalamic neurons regulating appetite, but its major effect promoting food intake is mediated *via* attenuation of central leptin sensitivity (6, 7). Prolactin may drive the body into a state of positive energy balance, however, this effect is evident in pathological hyperprolactinemia or in pregnant women, not during lactation (7).

Adipocyte-derived leptin affects Ob receptors and acts as a satiety factor suppressing orexigenic NPY/AgRP and stimulating anorectic pro-opiomelanocortin (POMC) neurons in the hypothalamus (8). Plasma leptin concentrations in humans correlate closely and positively with the amount of energy stored within adipose tissue until the leptin-resistant state develops (8). During pregnancy, serum leptin rises significantly to ensure transfer of nutrients to the growing fetus and placenta, and drops markedly within 24 hours after delivery. Then, serum leptin surges again and remains elevated during the first six months postpartum (9).

Adiponectin is another adipokine regulating energy metabolism, and its major peripheral effects are associated with

protection against insulin resistance and diabetes, obesity, atherosclerosis and inflammation (10, 11). During late pregnancy and postpartum, serum adiponectin declines, and a high level of prolactin significantly contributes to the suppression of adiponectin release (12, 13).

Ghrelin (Ghr) as an orexigenic hormone primarily synthesized by the stomach, facilitates the storage of energy substrates during limited energy supply (14, 15). High basal concentrations of Ghr are found in lean and anorectic people, while low in obese subjects (16), suggesting the body's response to negative or positive energy balance. A slight progressive elevation of circulating Ghr in lactating women within the first two weeks of lactation suggests a greater need for nutrients delivery with an increasing volume of produced milk (17).

Insulin, which provides a substrate for intracellular energy, is secreted in high amounts during late pregnancy. Gestational hyperinsulinemia is normal and expected physiological condition until it is transient and disappears following delivery (18). Breastfeeding may restore the whole-body insulin sensitivity protecting from metabolic effects of pregnancy, including insulin resistance, excessive weight gain, or altered lipid metabolism, which are associated with elevated risk of metabolic dysfunction (19).

Physiologically, metabolic effects of cortisol include increased rate of gluconeogenesis, enhanced protein catabolism, and stimulated lipolysis. Basal cortisol levels are elevated during late pregnancy around threefold non-pregnant levels and may downregulate hypothalamic production of corticotropin-releasing hormone (CRH). This, in turn, may affect the regulation of the maternal hypothalamic-pituitary-adrenal (HPA) axis during postpartum (20). In addition, lactation and high PRL are known to inhibit cortisol secretion in animals (21), and centrally released PRL may contribute to the blunted activity of HPA axis at that time (22). However, a number of authors suggest that breastfeeding does not affect basal cortisol level and HPA axis activity in humans. Instead, short-lasting and caused by suckling inhibition of the cortisol response to stress is present (23).

A growing body of evidence suggests that woman's nursing history potentially influences her metabolic health (24). Interestingly, little is known about the basal profile of hormones regulating maternal ingestion of food, according to the mode of feeding an infant. Therefore, the goal of the present study was to extend the knowledge about the physiology of lactation, by investigating the circulating maternal insulin, leptin, ghrelin, adiponectin, and cortisol according to the particular mode of feeding.

MATERIALS AND METHODS

Study population

The protocol was approved by the local Ethics Committee (Ethics Committee of Poznan University of Medical Sciences; Ref. KB-1096/16, annexed by Ref. KB-246/19) and written consent for participation was taken from all individuals entering the study. The study was performed in accordance with the Declaration of Helsinki annex Ref. KB- 889/18.

In 2017, a total of 100 postpartum women who delivered vaginally without any complications, were recruited from the outpatient clinics. Of these, 75 met the following inclusion criteria: healthy exclusively breastfeeding mothers at 3rd month of lactation (EBF3), healthy partially breastfeeding mothers at 6th month of lactation (PB6), completely formula feeding mothers between 3rd and 6th month post-partum without any known medical reason for use of breast-milk substitutes (FF). Exclusive breastfeeding was defined as a feeding an infant with only breast milk and no supplementary food, water, or other fluids since birth. Partially breastfeeding was defined as a feeding with breast milk with complementary foods provided at 6th month of infant's life in addition to breast milk. Complete formula feeding was defined as feeding an infant prepared formula instead of breastfeeding since birth.

Cesarean section, medical reason for formula feeding, chronic diseases (diabetes, hypertension, dyslipidemia, inflammation, malignancy), drug treatment, and acute infection were the factors disqualifying from participating in this study. *Fig. 1.* presents the recruitment procedure in detail.

FF mothers represented the control group for the breastfeeding women (BF), and BF mothers were divided into two subgroups according to mode and time of nursing. All mothers at the 3rd month of lactation (EBF3) were exclusive breastfeeders, while all breastfeeding mothers at the 6th month of lactation (PBF6) introduced supplementary products to their infants.

Study participants were asked to maintain their ordinary dietary and physical activity behaviour. Before sample collection, all subjects underwent a medical examination and completed an assessment survey on general health.

Anthropometrics

Anthropometric parameters were taken during routine postnatal pediatric visits. Weight was measured to the nearest 10 g (electronic personal scale, Mensor WE150P1, JAWAG, Morawica, Poland). Height was measured to the nearest 5 mm, using a wall-mounted stadiometer (Comed S.C., Koszalin, Poland). Body mass index (BMI; kg/m^2) was calculated as weight (kg)/height (m)^2.

Blood draws and hormone analysis

Blood samples were drawn in the morning (08:00 – 08:30 am) after 10-h overnight fast. Serum was separated and stored at –70°C.

Serum prolactin, leptin, adiponectin, insulin and cortisol were determined using enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle (DRG Instruments GmbH, Marburg, Germany, No. cat. EIA-1291; DRG Instruments GmbH, Marburg, Germany, No. cat. EIA-2395; DRG Instruments GmbH, Marburg, Germany, No cat. EIA-4177; DRG Instruments GmbH, Marburg, Germany, No cat. EIA-2935; IBL International GmbH, Hamburg, Germany No. cat. RE52061, respectively). Standards, samples and controls were added to microtitter wells coated with an enzyme conjugate at room temperature and then rinsed with wash solution following the manufacturer's instructions. After a washing step, a substrate solution was added and incubated at room temperature. The absorbance (optical density, OD) of each well was determined at 450 ± 10 nm with the DRG Instruments Microtiter Plate Reader (Marburg, Germany). The intensity of colour developed was proportional to the concentration of tested hormone. The intra-assay coefficients of variation were: for prolactin 3.5%, for leptin 4.9%, for adiponectin 4.2%, for insulin 4.0%, and for cortisol 8.0%.

Acylated ghrelin (aGhr) and total ghrelin (tGhr) were measured using a radioimmunoassay. The RIAs were carried out using for tGhr detection a rabbit polyclonal antibodies that recognize octanoylated and non-octanoylated ghrelin and [^{125}I] ghrelin as the tracer (Linco Research, St. Charles, MO, USA, No. cat. GHRT-89HK), and for aGhr detection using antibodies raised in guinea pigs that recognize octanoylated ghrelin and ^{125}I-octanoylated ghrelin as the tracer molecule (Linco Research, St. Charles, MO, USA, No. cat. GHRA - 88HK). All procedures were preformed according to their protocols. The intra-assay coefficients of variation were 5% for tGhr and 8% for aGhr.

To keep the high specificity and sensitivity of the tests, the protocols provided by manufacturers were not modified.

Statistical analysis

Descriptive statistics of all hormones, as well as, characteristics of the examined groups were presented as mean values with standard deviations (SD), standard errors, and confidence intervals (CI). The normal distribution was confirmed with the Shapiro-Wilk's test, while the variance homogeneity was confirmed by Levene's test. Therefore, t-tests or one-way variance analysis with post-hoc Tukey's multiple comparison tests were applied to examine the statistically significant differences between means at alpha significance level set at 0.05. Pearson's linear correlation coefficients were calculated to assess associations between variables, $p < 0.05$ was considered statistically significant. All calculations were carried out with the use of Statistica 13.1 Software (TIBCO Software Inc.).

RESULTS

Clinical characteristics and anthropometric measurements of study participants, as well as hormonal assessments, are summarized in *Table 1*. Multiple comparisons of circulating hormones, body weight, and BMI between FF, EBF3, and PB6 groups calculated with post-hoc Tukey test are presented in *Table 2*. There was a significant difference in circulating insulin, leptin, cortisol, and prolactin between FF and BF women ($p = 0.0048$, $p = 0.0416$, $p = 0.0002$, $p = 0.0020$, respectively), and results are shown in *Fig. 2*. Other tested parameters such as aGhr, tGhr, adiponectin, body weight, BMI, and blood pressure did not differ significantly, when compared BF and FF mothers. Statistically significant correlations in BF women were found for insulin (with: leptin, $p < 0.0001$; aGhr, $p = 0.020$; adiponectin, $p = 0.005$; body weight, $p < 0.0001$ and BMI, $p < 0.0001$), leptin (with: insulin, $p < 0.0001$; aGhr, $p < 0.0001$; cortisol, $p = 0.019$; PRL, $p = 0.001$; adiponectin, $p = 0.019$; body weight, $p < 0.0001$ and BMI, $p < 0.0001$), aGhr (with: insulin, $p = 0.020$; leptin, $p < 0.0001$; PRL, $p = 0.002$; adiponectin, $p = 0.002$; body weight, $p = 0.015$), cortisol (with: leptin, $p = 0.019$; adiponectin, $p = 0.013$); prolactin (with: leptin, $p = 0.001$; aGhr, $p = 0.002$; body weight, $p = 0.040$). Data regarding correlation coefficient (r) values are summarized in *Table 3*.

DISSCUSION

The present study demonstrates for the first time basal (after 10 hours fast) circulating levels of the hormones regulating food intake and energy homeostasis in postpartum women according to a different mode of feeding an offspring. Furthermore, we assessed the relationship between baseline serum concentrations of tested hormones in breastfeeding mothers, which complements the knowledge on the physiology of lactation.

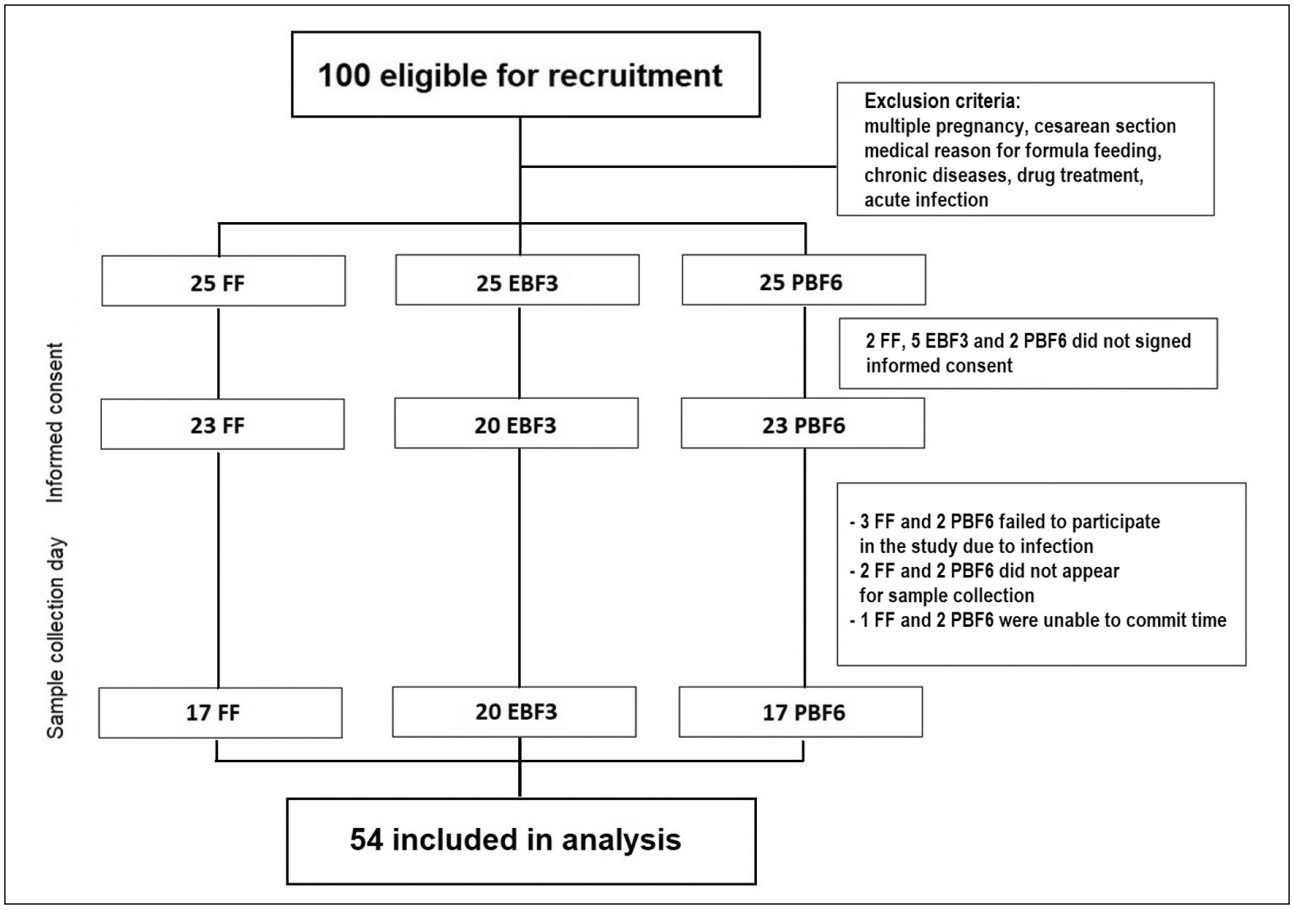

Fig. 1. The recruitment of participants procedure and their clinical exclusion criteria.
Abbreviations: FF, formula feeding mothers; EBF3, exclusively breastfeeding mothers at 3rd month of lactation; PB6, partially breastfeeding mothers at 6th month of lactation.

Table 1. Descriptive characteristics of study participants.

Mode of nursing	Mean value	SD	Standard error	CI	
				–95%	+95 %
Age [years]					
FF	27	4	1	24	29
EBF3	27	4	1	25	29
PB6	29	4	1	27	31
Pregnancy					
FF	2	1	0	1	2
EBF3	2	1	0	1	2
PB6	2	1	0	2	2
	Body weight [kg]				
FF	64.00	7.40	1.80	60.10	67.80
EBF3	66.70	10.10	2.30	62.00	71.40
PB6	63.10	9.10	2.20	58.40	67.80
BMI [kg/m^2]					
FF	23.60	2.91	0.71	22.10	25.10
EBF3	24.98	3.30	0.74	23.43	26.52
PB6	22.87	3.40	0.82	21.12	24.61
SP [mmHg]					
FF	119	12	3	113	125
EBF3	117	9	2	113	121
PB6	121	12	3	115	126
DP [mmHg]					
FF	71	6	1	68	74
EBF3	73	8	2	70	77
PB6	74	9	2	69	78
Insulin [μIU/ml]					
FF	14.81	3.35	0.81	13.09	16.53
EBF3	11.43	4.31	0.96	9.41	13.45
PB6	11.33	4.24	1.03	9.15	13.51
Leptin [ng/ml]					
FF	8.42	3.00	0.73	6.87	9.96
EBF3	13.05	7.27	1.63	9.65	16.45
PB6	11.87	8.51	2.06	7.49	16.24
Acylated Ghrelin [pg/ml]					
FF	28.36	8.03	1.95	24.24	32.49
EBF3	27.82	11.60	2.59	22.39	33.25
PB6	28.98	11.16	2.71	23.24	34.72
Total Ghrelin [pg/ml]					
FF	902.54	368.15	89.29	713.26	1091.83
EBF3	962.98	507.80	113.55	725.32	1200.64
PB6	646.81	213.52	51.79	537.03	756.60
Cortisol [ng/ml]					
FF	254.58	90.62	21.98	207.98	301.17
EBF3	149.62	70.12	15.68	116.80	182.44
PB6	173.46	76.64	18.59	134.06	212.87
Prolactin [ng/ml]					
FF	18.10	6.57	1.59	14.72	21.48
EBF3	52.54	31.28	7.00	37.90	67.18
PB6	29.99	24.12	5.85	17.59	42.39
Adiponectin [μg/ml]					
FF	10.17	3.58	0.87	8.33	12.02
EBF3	11.68	3.09	0.69	10.23	13.13
PB6	11.81	3.06	0.74	10.23	13.38

Abbreviations: FF, formula feeding mothers; EBF3, exclusively breastfeeding mothers at 3rd month of lactation; PB6, partially breastfeeding mothers at 6th month of lactation;
Note: Data were normally distributed and presented as means ± SD; $p < 0.05$ was considered significant.

Table 2. Multiple comparisons of circulating hormones, body weight and BMI between FF, EBF3 and PB6 groups (post-hoc Tukey's tests).

	Mode of nursing			p value		
	FF (n = 17)	**EBF3** (n = 20)	**PB6** (n = 17)	**FF** vs. **EBF3**	**FF** vs. **PB6**	**EBF3** vs. **PB6**
Insulin [µIU/ml]	14.81	11.43	11.33	0.045	0.038	ns
Leptin [ng/ml]	8.42	13.05	11.87	ns	ns	ns
aGhr [pg/ml]	28.36	27.82	28.98	ns	ns	ns
tGhr [pg/ml]	902.54	962.98	646.81	ns	ns	ns
Cortisol [ng/ml]	254.58	149.62	173.46	0.000	0.012	ns
Prolactin [ng/ml]	18.10	52.54	29.99	0.000	ns	0.021
Adiponectin [µg/ml]	10.17	11.68	11.81	ns	ns	ns
Body weight [kg]	64.00	66.70	63.10	ns	ns	ns
BMI [kg/m^2]	23.60	24,98	22.87	ns	ns	ns

Abbreviations: FF, formula feeding mothers, EBF3, exclusively breastfeeding mothers at 3rd month of lactation; PB6 partially breastfeeding mothers at 6th month of lactation; aGhr, acylated ghrelin; tGhr, total ghrelin; ns, not significant.
Note: $p < 0.05$ was considered significant.

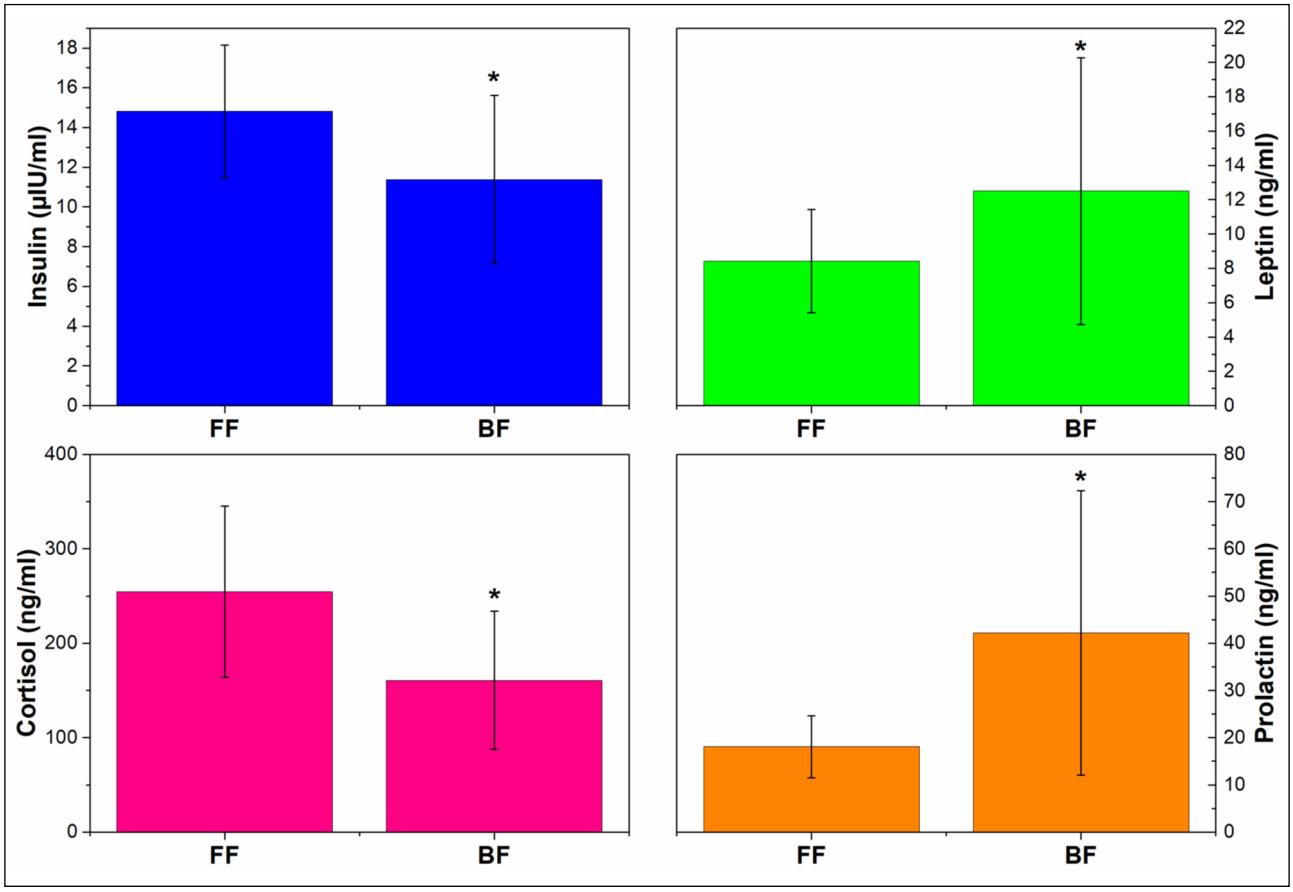

Fig. 2. Significant differences in circulating insulin, leptin, cortisol and prolactin between FF and BF women.
Abbreviations: FF, formula feeding mothers; BF, breastfeeding mothers. $p < 0.05$ was considered significant.

Prolactin

In the present study basal PRL concentration at the 3rd month of breastfeeding greatly exceeded values obtained for non-lactating women (FF), but at the 6th month of lactation, the difference did not reach statistical significance. Most authors suggest that basal levels of PRL at 6 months of lactation are still higher than those observed for non-lactating women (25). This apparent discrepancy may be explained by the presumably reduced frequency of breastfeeding when the solid food was introduced to the infant, since all PBF6 mothers in the present study discontinued exclusive breastfeeding and infants aged 6

Table 3. Baseline correlations of insulin, leptin, acylated ghrelin, total ghrelin, cortisol, prolactin, adiponectin, body weight and BMI in breastfeeding women.

	Insulin	Leptin	aGhr	tGhr	Cortisol	Prolactin	Adiponectin	Body weight	BMI
Insulin		0.540**	–0.315*	–0.058	–0.044	–0.024	–0.377*	0.510**	0.474**
Leptin			0.460**	–0.179	–0.319*	0.455*	–0.318*	0.614**	0.547**
aGhr				–0.172	0.049	0.410*	–0.408*	–0.330*	0.246
tGhr					–0.034	–0.145	–0.053	0.022	0.109
Cortisol						–0.130	–0.334*	–0.085	–0.117
Prolactin							–0.099	0.281*	0.245
Adiponectin								-0.458**	–0.416*
Body weight									0.916**

*p < 0.05; **p < 0.001; aGhr, acylated ghrelin; tGhr, total ghrelin.

months received maternal milk and extended diet with complementary food. In BF mothers, we observed a significant average correlation of circulating PRL with leptin and aGhr. Although the association between PRL and aGhr can suggest but not prove causality, it is supported by reports demonstrating that ghrelin may serve as an additional stimulatory factor for PRL secretion in healthy non-pregnant women (26). Whether this effect is present and significant during lactation requires further investigation. Also, leptin is known to stimulate PRL secretion, which is discussed later in this manuscript.

Insulin

It is well known that lowered circulating glucose levels during lactation may reduce the need for insulin secretion (27). Therefore, when compared with FF women, basal insulin in lactating BF mothers remains low even in the presence of elevated prolactin, which typically stimulates insulin secretion (28). While there are studies linking hyperprolactinemia with hyperinsulinemia and insulin resistance, it should be noted that this association usually refers to pathological conditions (29, 30). In our study, comparing with FF women, basal serum insulin was significantly decreased in breastfeeders, and this effect was not related to the time of lactation (EBF3 vs. PBF6 months) and, thus, presumably frequency of suckling, which affects PRL secretion. Analyzing correlations between PRL and insulin, we found no associations, which may suggest that the rise in PRL during lactation is not necessarily involved in the release of insulin. As we expected, insulin strongly and positively correlated with leptin in BF women, which is in accordance with previous studies (31).

Leptin

There are very few published studies on postpartum maternal leptin according to the mode of neonate feeding, and the available data vary in conclusions. Some authors described no significant difference in serum leptin between nonlactating and lactating mothers at 3 and 6 months after delivery (32). In contrast to this work, we observed higher serum leptin in the whole group of BF mothers as compared with FF women. However, possibly due to the reduced sample size, the difference was not significant anymore when we confronted FF with two subgroups - EBF3 and PBF6. Furthermore, there were no differences in the body weight and BMI between the two studied groups (BF and FF), indicating that significantly higher leptin concentration in breastfeeders must be associated with lactation-specific factors, possibly an elevated PRL level. Several lines of evidence suggest that PRL plays a modulatory role in the control of food intake and mediates metabolic adaptations of the maternal body (5, 6). Observed by us higher leptin level in BF mothers might potentially counteract prolactin-driven hyperphagia and represent an up-regulation effect to maintain leptin activity at the central level during lactation. This could be indirectly associated with normal BMI index of all tested BF women. Animal studies have shown mutual effects of PRL and leptin on their secretion. Prolactin directly stimulates leptin secretion from white adipose tissue and potentiates insulin-stimulated leptin expression and release from differentiated brown adipocytes (33, 34). On the other hand, leptin stimulates pituitary prolactin release *in vitro* and *in vivo*, suggesting its role in the relationship between nutrition and the reproductive functions (35, 36). Based on the above studies and considering our results (positive association between PRL and leptin), it is intriguing to speculate that high prolactin in breastfeeding mothers acts in accordance with leptin, to control energy homeostasis and maintain maternal adiposity within the normal range.

Adiponectin

Adiponectin's circulating concentrations are inversely related to adiposity and BMI (37). In agreement with this report, the negative association between adiponectin and body weight, and between adiponectin and BMI was confirmed in the present study.

Although it has been shown that high level of prolactin significantly reduces adiponectin release (12, 13), in our study, fasting concentrations of adiponectin did not differ between BF and FF women. We suppose that higher levels of cortisol and insulin in FF women compared with BF mothers may play a role, since both glucocorticoids and insulin potently inhibit adiponectin gene expression (13). Considering associations of adiponectin with other studied hormones, we found several correlations in breastfeeding mothers. Adiponectin, as an insulin-sensitizing hormone, was negatively associated with fasting insulin, and this observation is in line with studies demonstrating inverse correlations between adiponectin and insulin resistance or reporting that lower adiponectin levels are related to an expanded incidence and progression of diabetes type 2, which is often characterised by elevated insulin level (38, 39).

Consistent with previous research (40), we confirmed a negative correlation between these two hormones. Evidence suggests that peripheral and central leptin exerts inhibitory effects on adiponectin secretion (41). However, the physiological significance of this paracrine/autocrine activity, remains uncertain.

We also noticed inverse association between adiponectin and aGhr confirming results of the previous studies on animals (42) and differentiated cultured adipocytes (43).

Ghrelin

Although lactation is associated with greater requirements for nutrients, our results did not show any significant difference in circulating total and acylated ghrelin between BF and FF women, and fasting concentrations of ghrelin remained relatively stable when compared EBF3 to PBF6. The main reason for this could be the fact that 1) we studied lactating mothers at 12 and 24 weeks postpartum, which is not the initial phase of lactation and milk volume is usually stabilized at that time, and 2) all studied groups represented similar and normal BMI value. Similar findings were reported by Vila *et al*., who found no differences in circulating Ghr between exclusively breastfeeding and non-lactating women at 3 – 6 months postpartum (44). Based on the above results, one may speculate that in BF mothers, as compared with FF women, different responsiveness to the appetite-stimulating effects of ghrelin may play a role, or ghrelin-independent mechanism participates in a preprandial appetite control adapting woman to lactation. Indeed, it was suggested that ghrelin is not critically required for the regulation of appetite and fat deposition (45).

Analyzing associations between Ghr and other studied hormones, we found significant positive correlations of aGhr, which is the primary active form of ghrelin, with leptin and PRL, and, as mentioned before, a negative with adiponectin. aGhr was also weakly correlated with insulin, but this relation disappeared when the BF group was divided into two subgroups (EBF3 and PBF6). Evidence suggests that circulating Ghr and insulin demonstrate a reciprocal relationship. The crosstalks between Ghr and insulin signaling are present at the central level and peripherally (46). Authors reported a negative correlation between insulin and aGhr, but in the multiple regression analysis, they found that insulin sensitivity and glucose, not insulin *per se*, significantly affected circulating aGhr level (47).

We observed a positive correlation between aGhr and leptin, the two opposing hormones regulating appetite at the central level. Given the well-established anorexigenic actions of leptin and orexigenic effects of ghrelin, it is possible that in response to elevated (but not over normal range) circulating leptin in BF women, basal ghrelin is secreted in a greater amount to establish a new balance and maintain body weight. However, several studies have shown conflicting results (48).

Cortisol

Since postpartum mothers experience abnormal sleep patterns, to investigate cortisol during lactation diurnal cortisol secretion analysis is needed. Although we did not investigate a diurnal decline (slope) of cortisol, we found significantly lower basal cortisol in BF mothers, in which PRL level was high as compared with FF, and this difference was not associated with the duration of lactation (3 vs. 6 months). Our findings are partially in line with those reported by Tu *et al*., who showed significantly higher morning levels of cortisol in multiparous exclusively bottle-feeding mothers when compared with exclusively breastfeeding women (49).

As we expected and as reported earlier by others (50), we found a negative correlation between cortisol and leptin. Studies have shown, that leptin exhibits a direct inhibitory effect on cortisol secretion, which is suggested to be a kind of self-potentiating mechanism in anorexigenic activity of this peptide since glucocorticoids can limit the central effects of leptin on food intake (50). It is possible that this mechanism could also benefit postpartum breastfeeding mothers by preventing excessive weight gain.

Evaluating associations between basal cortisol and adiponectin we found a significant inverse correlation between cortisol and adiponectin in BF mothers, which is in a good accordance with previous studies demonstrating that glucocorticoids may decrease the synthesis and secretion of adiponectin (51).

Several limitations of the current study need to be acknowledged. The number of study participants was relatively low, however to make results more comparable we aimed to obtain potentially uniform study group and it was hard to find women who matched our narrow inclusion criteria and 1) decided to feed their infants with formula without any specific medical reason, 2) were exclusive breastfeeds at the 3rd month of lactation, 3) were partially breastfeeding at the 6th month of lactation, and their children were developmentally ready for solid food introduction. We understand that the interplay between hormones involved in the metabolic control of the mother is a complex process and unmeasured variables, e.g. body composition, blood lipid profile, glucose concentration, or insulin sensitivity, as adaptive metabolic consequences of lactation, may account for the final observations. However, our intention was to study basal hormonal profiles in postpartum women regarding different modes of feeding a baby, and we believe that these results extend the knowledge on the physiology of lactation, serving as a good starting point for future experiments.

In conclusion, our results may indicate that for maintaining a healthy phenotype, basal levels of insulin, leptin, and cortisol tend to have more favourable values in lean EBF3 and PB6 when compared with FF mothers.

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Conflict of interests: None declared.

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